TOPICS IN
MEDICINAL CHEMISTRY

01

Volume Editor R. H. Bradbury

Cancer

Springer

1
Topics in Medicinal Chemistry

Cancer

Volume Editor: Robert H. Bradbury

With contributions by
P. Angibaud · J. Arts · J. F. Blake · J. S. de Bono · D. H. Boschelli
R. H. Bradbury · K. Van Emelen · D. W. End · P. C. Fong
R. A. Galemmo Jr · C. Garcia-Echeverria · J. Hoffmann · P. ten Holte
M. Janicot · D. L. Johnson · H. Koblish · E. R. Laird · J. Lyssikatos
L. Mevellec · K. J. Moriarty · K. Paz · A. Sommer · T. K. Sawyer
E. M. Wallace · T. C. Yeh · Z. Zhu

Springer

Drug research requires interdisciplinary team-work at the interface between chemistry, biology and medicine. Therefore, the new topic-related series should cover all relevant aspects of drug research, e.g. pathobiochemistry of diseases, identification and validation of (emerging) drug targets, structural biology, drugability of targets, drug design approaches, chemogenomics, synthetic chemistry including combinatorial methods, bioorganic chemistry, natural compounds, high-throughput screening, pharmacological in vitro and in vivo investigations, drug-receptor interactions on the molecular level, structure-activity relationships, drug absorption, distribution, metabolism, elimination, toxicology and pharmacogenomics.
In references *Topics in Medicinal Chemistry* is abbreviated *Top Med Chem* and is cited as a journal.

Springer WWW home page: springer.com
Visit the TIMC content at springerlink.com

Library of Congress Control Number: 2006935870

ISSN 1862-2461
ISBN-10 3-540-33119-0 Springer Berlin Heidelberg New York
ISBN-13 978-3-540-33119-3 Springer Berlin Heidelberg New York
DOI 10.1007/978-3-540-33120-9

Springer is a part of Springer Science+Business Media

springer.com

Cover design: KünkelLopka GmbH, Heidelberg
Typesetting and Production: LE-TEX Jelonek, Schmidt & Vöckler GbR, Leipzig

Printed on acid-free paper 02/3100 YL – 5 4 3 2 1 0

Volume Editor

Dr. Robert H. Bradbury

AstraZeneca Pharmaceuticals
Mereside 3S111, SK 10 4TG
Macclesfield, Cheshire
United Kingdom

Editorial Board

Topics in Medicinal Chemistry
Also Available Electronically

For all customers who have a standing order to Topics in Medicinal Chemistry, we offer the electronic version via SpringerLink free of charge. Please contact your librarian who can receive a password or free access to the full articles by registering at:

springerlink.com

If you do not have a subscription, you can still view the tables of contents of the volumes and the abstract of each article by going to the SpringerLink Homepage, clicking on "Browse by Online Libraries", then "Chemical Sciences", and finally choose Topics in Medicinal Chemistry.

You will find information about the

- Editorial Board
- Aims and Scope
- Instructions for Authors
- Sample Contribution

at springer.com using the search function.

Preface to the Series

Medicinal chemistry is both science and art. The science of medicinal chemistry offers mankind one of its best hopes for improving the quality of life. The art of medicinal chemistry continues to challenge its practitioners with the need for both intuition and experience to discover new drugs. Hence sharing the experience of drug discovery is uniquely beneficial to the field of medicinal chemistry.

The series *Topics in Medicinal Chemistry* is designed to help both novice and experienced medicinal chemists share insights from the drug discovery process. For the novice, the introductory chapter to each volume provides background and valuable perspective on a field of medicinal chemistry not available elsewhere. Succeeding chapters then provide examples of successful drug discovery efforts that describe the most up-to-date work from this field.

The editors have chosen topics from both important therapeutic areas and from work that advances the discipline of medicinal chemistry. For example, cancer, metabolic syndrome and Alzheimer's disease are fields in which academia and industry are heavily invested to discover new drugs because of their considerable unmet medical need. The editors have therefore prioritized covering new developments in medicinal chemistry in these fields. In addition, important advances in the discipline, such as fragment-based drug design and other aspects of new lead-seeking approaches, are also planned for early volumes in this series. Each volume thus offers a unique opportunity to capture the most up-to-date perspective in an area of medicinal chemistry.

Dr. Peter R. Bernstein
Prof. Dr. Armin Buschauer
Prof. Dr. Ulrik Gether
Dr. John Lowe
Dr. Hans Ulrich Stilz

Preface to Volume 1

With supreme irony, the beginnings of modern cancer chemotherapy originated in chemical warfare. Autopsy findings from soldiers killed in the First World War by exposure to sulphur mustard gas led to the proposal in the 1940s that low doses of nitrogen mustard might cause regression of human lymphatic tumors. The pioneering success of this idea, albeit only equating to brief remission of disease, established the principle that rapidly growing tumors could be more susceptible to cytotoxic agents than normal tissues.

During the next half-century, through the endeavours of government institutions, academia and the pharmaceutical industry, a variety of potent cytotoxic drugs were discovered, such as antifolates, anthracyclins and platins. Although there have been successes, most notably in treatment of testicular cancer by platinum-based drugs, chemotherapy can currently still offer only a modest increase in survival time in the majority of advanced disease cases. An optimistic view, however, is that in the coming decades advances in prevention, detection and treatment will finally see cancer become considered not a fatal but chronic disease.

During the 1970s, recognition that tumors in the breast and prostate are subject to hormonal regulation had provided the first opportunity for a more targeted approach to cancer chemotherapy. The pioneering antiestrogenic agent tamoxifen originated from fertility research in the 1960s and later became the first anticancer drug approved for preventative use by the US Federal Drug Administration. Progress in the treatment of hormone-dependent prostate cancer followed advances in breast cancer, with the introduction of nonsteroidal androgen antagonist drugs like flutamide. The first chapter in this volume summarises more recent developments in the area of antihormonal chemotherapy.

Since the effects of cytotoxic agents on normal cells are responsible for many of the well-known side effects of these drugs, the emphasis has now moved predominantly to drug targets essential to tumor function but not to vital organs and tissues, an approach which should in principle give a better selectivity margin than seen for historical cytotoxic drugs. By the late 1980s, advances in molecular biology had begun to provide greatly increased understanding of regulatory and signaling networks in normal cells that control fundamental cellular processes such as vascularisation, growth and proliferation. The role

of many of these networks was found to be greatly enhanced in tumor cells, in response to factors such as genetic make up, age and exposure to environmental carcinogens. Interference with these key regulatory and signaling networks forms the content of much of this volume.

During the late 1990s humanised monoclonal antibodies, such as trastuzumab for treatment of breast cancer, provided the first clinical success using molecular targeted treatment. Advances in understanding of tumor biology also coincided with developments in chemical synthesis and in vitro screening technology, which increased the feasibility of finding small molecule leads with activity against the new targets, and for some targets structural knowledge also played an increasing role in optimisation of these leads.

In the opening years of the 21st century, regulatory approval followed for imatinib, gefitinib and erlotinib, the first small molecule signal transduction inhibitors. Like monoclonal antibodies, clinical studies with these drugs are providing tumor profiling data from which better understanding of the role of genetic factors in determining patient response is starting to emerge. Clinical experience is also beginning to fulfil the anticipation that these targeted agents could offer a more manageable side effect profile than cytotoxic therapy.

The last decade has thus seen clinical trials for a range of drugs that exploit fundamentally different cellular mechanisms from historical cytotoxic chemotherapy, and a number of these agents have now been granted regulatory approval, a landmark recently highlighted as the journal Nature's 24th Milestone in Cancer. Experience from these trials is providing growing insight into the role of factors such as patient selection, clinical trial design and drug resistance mechanisms. An estimated 500 chemotherapeutic agents were undergoing clinical trials in 2004, and this volume reviews the medicinal chemistry behind some key classes of anticancer agent encompassed within these numbers that have the potential to follow drugs like imatinib into clinical practice. The coming decades will reveal if the shift to personalised medicine widely envisaged through introduction of these agents becomes reality against the full diversity of human tumors, and provides a real breakthrough towards fulfilment of a therapeutic vision which began over half a century ago.

September 2006, Alderley Park, UK Robert H. Bradbury

Contents

Overview
R. H. Bradbury 1

Anti-hormone Therapy: Principles of Endocrine Therapy of Cancer
J. Hoffmann · A. Sommer 19

Inhibition of Growth Factor Signaling by Small-Molecule Inhibitors of ErbB, Raf, and MEK
E. M. Wallace · T. C. Yeh · E. R. Laird · J. F. Blake · J. Lyssikatos 83

Farnesyl Protein Transferase Inhibitors: Medicinal Chemistry, Molecular Mechanisms, and Progress in the Clinic
D. W. End · L. Mevellec · P. Angibaud 133

Survival Signaling
C. Garcia-Echeverria 169

Progress in the Development of Agents to Control the Cell Cycle
K. J. Moriarty · H. Koblish · D. L. Johnson · R. A. Galemmo Jr 207

HDAC Inhibition in Cancer Therapy: An Increasingly Intriguing Tale of Chemistry, Biology and Clinical Benefit
P. ten Holte · K. Van Emelen · M. Janicot · P. C. Fong
J. S. de Bono · J. Arts 293

Development of Angiogenesis Inhibitors to Vascular Endothelial Growth Factor Receptor 2 for Cancer Therapy
K. Paz · Z. Zhu 333

Novel Small-Molecule Inhibitors of Src Kinase for Cancer Therapy
T. K. Sawyer 383

Bcr-Abl Kinase Inhibitors
D. H. Boschelli 407

Author Index Volume 1 445

Subject Index 447

Top Med Chem (2007) 1: 1–17
DOI 10.1007/7355_2006_001

Published online: 16 December 2006

Overview

Robert H. Bradbury

Cancer and Infection Research, AstraZeneca, Mereside, Alderley Park, Macclesfield SK10 4TG, UK
Rob.Bradbury@astrazeneca.com

1 Introduction 2

2 Historical Perspective 2

3 Cytotoxic Drugs 3

4 Anti-hormonal Drugs 5

5 Targeted Drugs 6

6 Structure of This Volume 12

7 Conclusion 14

References 14

Abstract After over half a century of chemotherapy research, cancer remains one of the most difficult life-threatening diseases to treat, a consequence of factors which include limitations of animal models, tumour diversity, drug resistance and side effects of therapy. This introductory overview gives a brief perspective on the discovery of historical cytotoxic and anti-hormonal drugs, and then highlights the shift in research emphasis over the last 15 years towards agents that aim to selectively target regulatory and signalling processes known to drive tumourigenesis. Experience with newer drugs like imatinib (GLEEVEC™) is providing growing insight into the role of patient selection, design of clinical trials and mechanisms of drug resistance, and is also beginning to fulfil the anticipation that such agents could offer a more manageable side-effect profile than cytotoxic therapy. For medicinal chemists, the aims of anti-cancer drug discovery programmes have come more into line with other areas of drug therapy, with emphasis moving more towards orally bioavailable drugs with pharmacokinetics suitable for dosing once or twice a day, and with a property profile that not only limits toxic effects on proliferating tissue such as bone marrow but also minimises risks due to effects such as cardiac arrhythmia potential, cytochrome P450 liability and variable absorption. This volume reviews medicinal chemistry approaches to small molecule inhibitors of some key cellular regulatory and signalling networks, which have the potential to follow drugs like imatinib into clinical practice.

Keywords Cancer · Chemotherapy · Medicinal · Chemistry · Tumour

Abbreviations
ALL Acute lymphoblastic leukemia
AML Acute myeloid leukemia

ATP Adenosine triphosphate
CML Chronic myelogenous leukemia
EGF Epidermal growth factor
FDA Food and Drug Administration
LHRH Luteinsing hormone-releasing hormone
RTK Receptor tyrosine kinase
VEGF Vascular endothelial growth factor

1 Introduction

After over half a century of chemotherapy research, cancer remains one of the most difficult life-threatening diseases to treat, a consequence of factors that include limitations of animal models, tumour diversity, drug resistance and the side effects of therapy. Although there have been successes, most notably in treatment of testicular cancer [1], chemotherapy can currently still offer only a modest increase in survival time in the majority of advanced disease cases [2]. The incidence of cancer is increasing due to ageing populations in most countries, and it has been estimated that in 20 years time there will be 20 million new cancer patients worldwide each year [3]. An optimistic view, however, is that in the coming decades advances in prevention, detection and treatment will see cancer becoming considered not as a fatal but as a chronic disease [3].

This volume aims to review for the non-specialist reader recent advances in cancer chemotherapy research, which have followed from increased understanding of fundamental processes of cancer biology acquired during the last three decades of the 20th century. Nine topics have been selected to reflect a range of current medicinal chemistry approaches. An emphasis is deliberately placed on small molecule drugs, while biopharmaceutical agents such as monoclonal antibodies are primarily highlighted only to illustrate proof of principle. Each chapter aims to cover drug target and biological rationale, chemotypes, clinical status and future prospects. This introductory overview gives a brief historical perspective [4–6] and then highlights the shift in research emphasis over the last 15 years towards drugs that aim to target selectively the biochemical processes now known to drive tumourigenesis.

2 Historical Perspective

With supreme irony, the beginnings of modern cancer chemotherapy had an origin in chemical warfare [7, 8]. Autopsy findings from the lymphatic glands of soldiers killed in the First World War by exposure to sulphur mustard gas

led to animal experiments that showed rapid shrinkage of murine tumours following dosing with nitrogen mustard, an agent also developed primarily as a war gas in the 1930s. These observations prompted pharmacologists Louis Goodman and Alfred Gilman to propose, in the early 1940s, that injection of low doses of a solution of the hydrochloride salt of nitrogen mustard might cause regression of lymphatic tumours. The pioneering success of this idea, albeit only equating to brief remission of disease, established the principle that rapidly growing tumours could be more susceptible to cytotoxic agents than normal tissue. As concluded in the closing paragraph of the initial clinical paper [8]:

"…the heuristic aspects of the actions of nitrogen mustard may eventually prove of greater importance than the clinical results obtained."

Subsequent studies demonstrated that the mechanism of action of nitrogen mustard involves formation of a covalent bond with DNA through alkylation of specific sites on purine bases, leading to cross-linking of DNA strands and cell death [9]. Stabilisation of the nitrogen mustard gave improved alkylating agents such as cyclophosphamide (CYTOXAN™), which could be administered orally for treatment of lymphomas, leukemias and, to a lesser extent, solid tumours [10].

3
Cytotoxic Drugs

In stark contrast, the second historically significant anti-cancer drug, methotrexate, originated from nutritional research. The observation that the vitamin folic acid stimulated proliferation of acute lymphoblastic leukemia (ALL) cells in children prompted synthesis of folate analogues. In the late 1940s methotrexate became the first drug to induce remissions in children with ALL [11].

Nearly a decade later, treatment with methotrexate provided the first demonstrable cure of a solid tumour [12] although, like nitrogen mustard, many years elapsed before the mechanism of action of the drug became fully understood [13]. From the earliest studies, however, it was apparent that tumours quickly became resistant to drugs such as cyclophosphamide and methotrexate, and that either combination with other drugs or use as adjuvant therapy after surgery gave a better prospect for long term remission or cure [14].

The chemical structures of cyclophosphamide, methotrexate and some other landmark cytotoxic drugs discovered in the second half of the last century are shown in Fig. 1. Table 1 summarises the clinical utility and principal mode of action of these agents. While the therapeutic origins of nitrogen mustard and methotrexate were rooted in pharmacology, the discovery in the 1950s of drugs such as 5-fluorouracil (ADRUCIL™) was based on biochemical

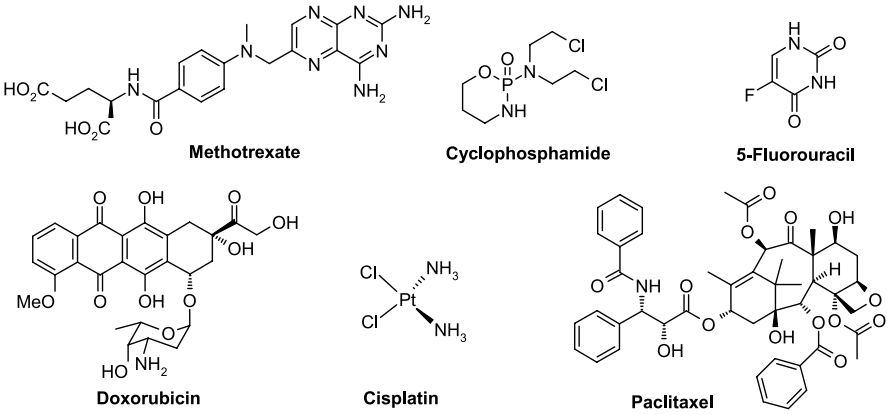


Fig. 1 Chemical structures of cytotoxic drugs

Table 1 Cytotoxic drugs

Drug	FDA approval[a]	Clinical utility[b,c]	Principal mode of action
Methotrexate	1953	ALL	Inhibitor of DNA precursor synthesis [13]
Cyclophosphamide	1959	Non-Hodgkin's lymphoma	DNA alkylating agent [10]
5-Fluorouracil	1962	Colorectal, gastric	Inhibitor of DNA precursor synthesis [15]
Doxorubicin	1974	Osteogenic sarcoma, Hodgkin's disease, CML, soft tissue sarcoma	Inhibitor of DNA replication, transcription, repair [20]
Cisplatin	1978	Ovarian, head and neck, lung, testicular	DNA coordinating agent [19]
Paclitaxel	1992	Ovarian, breast, small cell lung cancer	Inhibitor of microtubule assembly [21]

[a] Year of first FDA approval (http://www.fda.gov/cder/cancer/approved.htm)
[b] Not comprehensive
[c] Usually as part of combination therapy

reasoning around modification of nucleotide bases and consequent effects on steps preceding cellular RNA and DNA synthesis [15, 16].

In a classic case of serendipity, cisplatin (PLATINOL™), a key component of the combination therapy which revolutionised treatment of testicular cancer [1], was uncovered fortuitously in the 1960s during studies on the effect of an electric current on the growth of *E. coli* [17, 18]. Cell division was inhibited not by the electric current but by production of a platinum complex from the

electrodes, an effect later attributed to coordination of the platinum complex to purine bases in cellular DNA [19].

A number of major cytotoxic drugs introduced into clinical practice in the last decades of the 20th century originated in the 1950s and 1960s from screening of natural product extracts in mouse leukemia models. Drugs like the anthracycline doxorubicin (ADRIAMYCIN™, an inhibitor of a topoisomerase enzyme mediating DNA replication, transcription and repair [20]) and paclitaxel (TAXOL™, an inhibitor of cell division through effects on assembly of microtubules, which form an essential part of cell structure [21]) are nowadays increasingly used in combination with more recently discovered targeted agents.

Formidable chemical obstacles stood in the way of clinical development of these agents derived from natural products, notably in formulation of very poorly soluble compounds for intravenous infusion and in manufacture of bulk drug. For a time, production of Taxol from the bark of the Pacific Yew tree aroused public controversy, which became a story of "nature and politics in pursuit of an anti-cancer drug" [22].

The effects of cytotoxic agents on normal cells are responsible for many of the well-known side effects of these drugs; effects which can seriously compromise most organs of the body. Prophetically, drug resistance and therapy limiting effects of bone marrow toxicity were observed in the very first patient treated with nitrogen mustard in 1942 [8]. Despite the advent of a number of supportive measures to ameliorate bone marrow suppression, long term effects on lung, heart, kidney and reproductive organs remain formidable barriers to effective use of cytotoxic agents [23].

4 Anti-hormonal Drugs

Recognition that tumours in breast and prostate are subject to hormonal regulation [24] provided the first opportunity for a more targeted approach to cancer chemotherapy (Fig. 2, Table 2). The anti-hormonal drug tamoxifen (NOLVADEX™), initially viewed as "a most unlikely pioneering medicine", originated from fertility research in the 1960s and later became the first cancer drug approved by the FDA for preventative use [25]. In proof of a principle first highlighted as early as the 1930s [26], effects on breast tumour cells following administration of tamoxifen were shown to reflect high affinity for the estrogen receptor [27].

The increasing clinical importance of tamoxifen in the 1980s prompted development of drugs that indirectly target the estrogen receptor, for example the aromatase inhibitor anastrozole (ARIMIDEX™), a selective inhibitor of estrogen biosynthesis [28]. Progress in treatment of hormone-dependent prostate cancer followed advances in breast cancer, with demonstration that

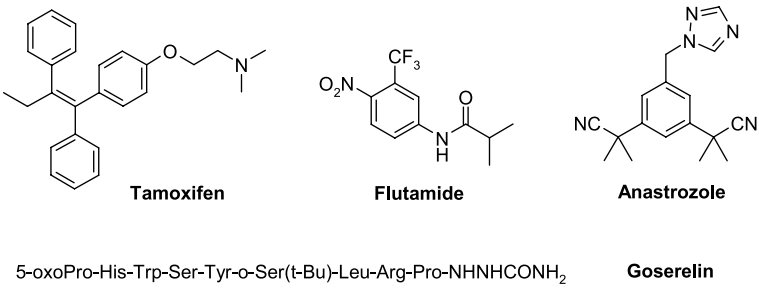


Fig. 2 Chemical structures of anti-hormonal drugs

Table 2 Anti-hormonal drugs

Drug	FDA approval [a]	Clinical utility [b, c]	Principal mode of action
Tamoxifen	1977	Breast	Estrogen antagonist [24]
Flutamide	1989	Prostate	Androgen antagonist [29]
Anastrozole	1995	Breast	Aromatase inhibitor [28]
Goserelin	1995	Breast, prostate	LHRH agonist [30]

[a–c] See Table 1

non-steroidal androgen antagonist drugs like flutamide (EULEXIN™) block binding of the endogenous ligand dihydrotestosterone to the androgen receptor in prostate [29].

Another major approach to treatment of hormone-dependent cancer is based on the finding that the pituitary gland becomes desensitised by prolonged stimulation with the peptide hormone LHRH. Sustained release depot formulations of highly potent LHRH agonists such as goserelin (ZOLADEX™) exert their effect through inhibition of testicular androgen synthesis in men and ovarian estrogen synthesis in women [30].

5 Targeted Drugs

While introduction of anti-hormonal therapy represented a major advance in treatment of breast and prostate tumours, Table 1 reflects relatively slow progress in developing treatments for a wider range of tumours. In part, this may be considered attributable to inability of historical mouse models based on rapidly dividing haematological tumours to identify agents active against slower growing solid tumours [31]. Human tumour cell lines and xenografts in immunodeficient mice provided alternative models, but these screening

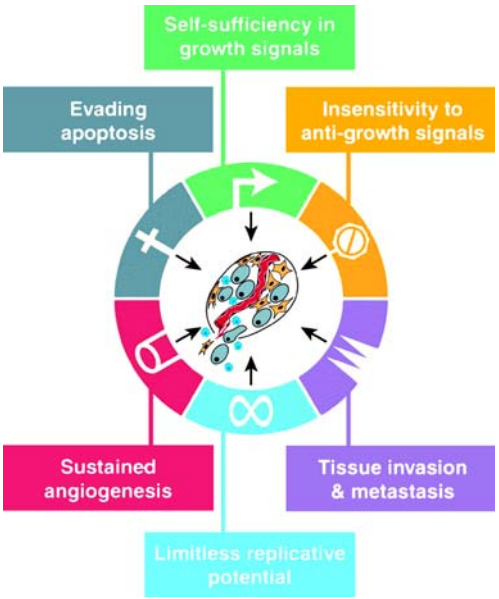


Fig. 3 Acquired capabilities of cancer. Reproduced from [34] with permission of Elsevier

strategies continued to prove of limited success in predicting the outcome of clinical trials [32].

By the late 1980s, however, advances in molecular biology had begun to provide greatly increased understanding of regulatory and signalling networks in normal cells that control fundamental cellular processes such as vascularisation, growth and proliferation. The role of many of these networks was found to be greatly enhanced in tumour cells, in response to factors such as genetic make up, age and exposure to environmental carcinogens [33].

In a seminal overview published in the first month of the year 2000 [34], Douglas Hanahan and Robert Weinberg outlined some fundamental principles underlying the complexities of signalling pathways responsible for transforming normal human cells into malignant cancers. As delineated in Fig. 3, human tumour cells are proposed to acquire a number of essential capabilities, "the hallmarks of cancer", which collectively promote malignant growth. Each of these changes represents the breaching of a fundamental anti-cancer defence mechanism. The multiplicity of defences may explain why cancer is not more common during the average human lifetime.

These findings have provided the basis for seeking inhibitors of macromolecular targets essential to the malignant tumour phenotype but not utilised in vital organs and tissues, an approach which in principle should lead to a better selectivity margin than seen for historical cytotoxic drugs [35]. Interference with these regulatory and signalling networks forms the content of much of this volume and is described in detail in specific chapters.

Advances in understanding of tumour biology coincided with developments in chemical synthesis and in vitro screening technology, which increased the feasibility of finding chemical leads with activity against the new targets [36]. For some targets, structural knowledge has also played an increasing role in optimisation of these leads [37, 38]. Prompted by these developments, by the mid 1990s the pharmaceutical industry, start-up companies and research institutes were dedicating a major increase in resources to discovery of anti-cancer drugs.

Receptor tyrosine kinases (RTKs), the protein kinases which catalyse phosphorylation of hydroxyl groups on tyrosine residues, predominantly following activation by an extracellular ligand, have proved to be a particularly tractable class of drug target involved in a wide range of cellular signalling pathways [39, 40]. Inhibitors of protein kinases most commonly target the ATP binding site of the activated kinase, although binding to an adjacent allosteric site or to an inactive form of the kinase has also been exploited [37, 41]. In contrast, progress has been less dramatic against other classes of comparably well-validated signalling targets, for example targets that involve inhibition of protein–protein interactions such as the p53 tumour suppression pathway [42].

A number of agents derived from this change in approach were introduced during the years 1997–2004 (Fig. 4, Table 3), and validation was first provided by humanised monoclonal antibodies to extracellular ligand binding domains [43]. Rituximab (RITUXAN™), approved by the FDA in 1997 for treatment of Non-Hodgkin's lymphoma, targets tumour-associated membrane proteins [44]. Trastuzumab (HERCEPTIN™) increases survival time in patients with metastatic breast tumours, which over-express erbB2, a member of the EGF family of growth factors [45]. The latter agent is now also giving encouraging results in clinical trials in patients with erbB2-positive

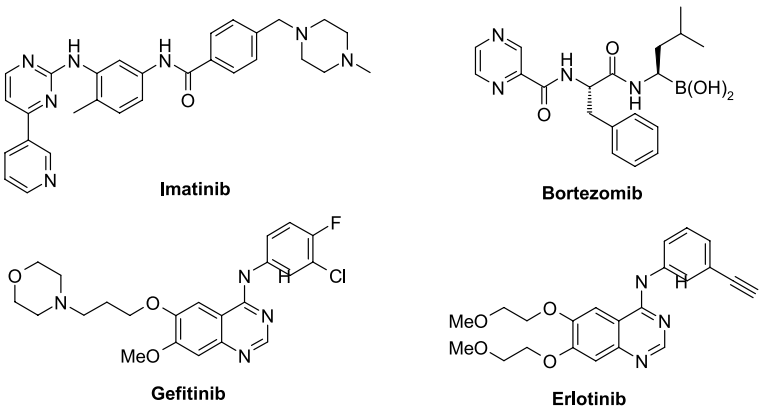


Fig. 4 Chemical structures of marketed targeted drugs

Table 3 Marketed targeted drugs

Drug	FDA approval[a]	Clinical utility[b,c]	Principal mode of action
Rituximab	1997	Non-Hodgkin's lymphoma	Monoclonal antibody (anti-CD20) [44]
Trastuzumab	1998	Breast	Monoclonal antibody (erbB2) [45]
Imatinib	2001	CML	Bcr–Abl kinase inhibitor [48]
Gefitinib	2002	Non-small cell lung cancer	EGFR kinase inhibitor [49]
Bortezomib	2003	Multiple myeloma	Proteasome inhibitor [51]
Bevacizumab	2004	Colorectal	Monoclonal antibody (VEGF) [47]
Erlotinib	2004	Non-small cell lung cancer, pancreatic	EGFR kinase inhibitor [50]

[a–c] See Table 1

early breast cancer [45], an outcome likely to herald a wider trend towards treatment of early disease in patients selected by diagnostic profiling of malignant tissues [46]. More recently, bevacizumab (AVASTIN™), an antibody to the vascular growth factor VEGF, has provided the first realisation of anti-angiogenic therapy [47].

Regulatory approval followed for imatinib (GLEEVEC™), gefitinib (IRESSA™) and erlotinib (TARCEVA™), the first small molecule signal transduction inhibitors [48–50], and for the proteasome inhibitor bortezomib (VELCADE™) [51]. Like monoclonal antibodies, clinical studies with these drugs are providing tumour profiling data from which better understanding of the role of genetic factors in determining patient response is starting to emerge [35]. Clinical experience is also beginning to fulfil the anticipation that these targeted agents could offer a more manageable side-effect profile than cytotoxic therapy [52–54].

In the case of gefitinib, an inhibitor of the EGF signalling pathway which regulates tumour cell growth and survival, objective responses were observed in phase II and III monotherapy in 10–20% of patients with advanced refractory non-small cell lung cancer, but no significant additive effects were seen in first line phase III trials in combination with chemotherapy [53, 55–57]. More recent work has, however, provided evidence that mutations in the EGF receptor appear to confer increased sensitivity to inhibition by gefitinib [57, 58], a hypothesis now supported by increased incidence of mutations in tumour samples taken from patients with a higher objective response rate to gefitinib [55, 59].

Tumour profiling during gefitinib clinical trials has also shown a longer median survival time in patients with a high EGF receptor gene copy num-

ber [58], a finding that opens up the prospect of use of a genetic marker to identify patients more likely to be susceptible to the drug. Instead of therapy based on histological classification, the stage nevertheless now looks set for molecular tumour profiling and patient selection to become more central to cancer therapy [60].

Clinical data for imatinib, an inhibitor of the tyrosine kinase Bcr-Abl, the fusion protein product of a chromosomal translocation involved in pathogenesis of CML, are casting light on the subtleties of genetic mutation in development of tumour cell resistance [61]. Mutations in the Bcr-Abl gene produce drug-resistant cells in which the kinase domain binds the drug poorly but remains catalytically active [62]. In some patients, drug-resistant cells have been detected before exposure to drug [63], a finding which shows that cancer cells have inherent capacity for resistance even prior to treatment. However, as seen with monoclonal antibodies [43], clinical trials have demonstrated synergy between imatinib and cytotoxic agents in suppressing the effects of drug resistance [61].

From the earliest clinical studies with targeted agents, it has been acknowledged that tumour shrinkage criteria traditionally used to evaluate cytotoxic agents in phase II clinical trials are likely to be less suited to demonstrating clinical efficacy with agents that are primarily cytostatic rather than cytotoxic [64, 65]. While effects on survival, tumour response or time to disease progression in randomised phase III studies will continue to be a long term regulatory requirement in advanced disease [66, 67], a number of newer agents have achieved FDA Fast Track designation on the basis of biomarker and safety data in phase I trials [35]. Thus, for example, in phase I studies with imatinib, blood levels of Bcr-Abl transcript mRNA were used as an early marker of patient response [68]. Advances in use of imaging techniques such as positron emission tomography are also beginning to offer the prospect of demonstrating the molecular effects of targeted agents by non-invasive methods [69, 70].

The beneficial side-effect profile of newer agents such as imatinib also highlights the potential of these agents in early disease, but raises challenges around evaluation in randomised trials against clinical endpoints other than survival [65, 71]. However, while imitanib provides an outstanding example of clinical development among newer drugs, experience with other classes of agent such as farnesyl transerase inhibitors has so far been less rewarding. This outcome may to some extent reflect over-reliance on clinical trial strategies more suited to evaluation of cytotoxic agents [65].

For cancer biologists and translational scientists, the advances of the last two decades have led to a more rational approach in which knowledge of the biology of the target has become central to drug discovery and development [72]. From the large number of potential drug targets emerging from sequencing of the human genome, molecular profiling can identify oncogenes that are over-expressed in tumours and encode for signalling

pathways mediating one or more of the acquired capabilities referred to earlier [73].

Protein production and in vitro assay development enables primary screening against a drug target within a signalling pathway. Secondary screening assays using cell lines derived from tumours driven by, for example, over-expression or mutation of the target protein can then be used to confirm mechanism of action and effect on malignant phenotype. Cellular mode of action screens can also form the basis of pharmacodynamic assays to demonstrate target effect in an animal model [73], while understanding of the signalling pathway can identify biomarkers to enable detection of effects in biopsy samples taken in early clinical trials [74]. Debate continues around the role of selectivity screening and the relative merits of selective versus multi-targeted drugs [75, 76], as clinical experience with signalling pathway inhibitors has already provided several examples where effects on mechanistically related targets contribute to clinical activity [72].

For medicinal chemists, the aims of anti-cancer drug discovery programmes have come more into line with other areas of drug therapy, with emphasis shifting more towards orally bioavailable drugs with pharmacokinetics suitable for dosing once or twice a day, and with a property profile that not only limits toxic effects on proliferating tissue such as bone marrow but also minimises risks due to effects such as cardiac arrhythmia potential [77], cytochrome P450 liability [78] and variable absorption [79].

By way of illustration, Fig. 5 summarises the discovery of the Bcr-Abl tyrosine kinase inhibitor imatinib from a chemical lead initially identified in a screen for inhibitors of protein kinase C, a serine-threonine kinase [48, 80]. During optimisation of the lead structure, it was found that introduction of an amide group at the 3-position in the phenyl ring gave improved activity against tyrosine kinases such as Bcr-Abl, and that substitution of a methyl group at the 6-position led to selectivity versus protein kinase C. Finally, addition of a polar *N*-methyl piperazine moiety gave improved water solubility and oral bioavailability commensurate with oral human dosing. Subsequent X-ray structural work showed that the piperazine ring not only improved physical properties but also made a key contact with the backbone of the inactive form of the kinase [81]. These structural studies provide insight into

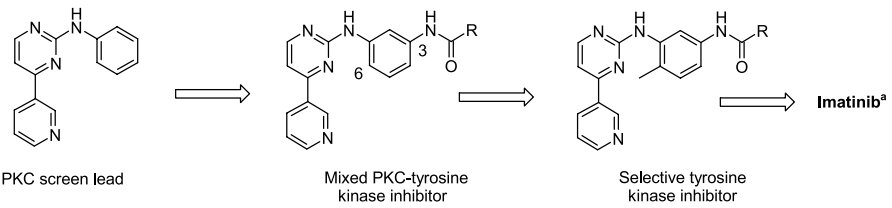


Fig. 5 Discovery of imatinib [48, 80]. See Fig. 4 for structure of imatinib

how mutations in the Bcr-Abl gene produce the resistant form of the kinase alluded to earlier [63], knowledge which offers the potential to design drugs to overcome resistance [82].

6 Structure of This Volume

The major part of this volume covers medicinal chemistry approaches to inhibition of regulatory and signalling networks, and individual chapters review a significant number of small molecule agents that have the potential to follow drugs like imatinib into clinical practice. Chemical structures of some drugs granted regulatory approval during the past year or currently in phase II/III trials are shown in Fig. 6, and the properties of these agents are summarised in Table 4.

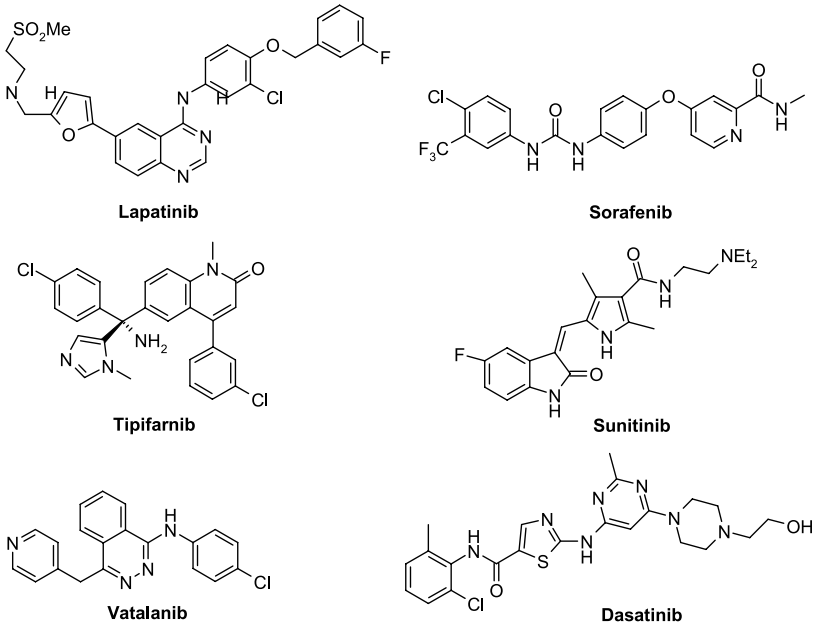


Fig. 6 Chemical structures of targeted drugs recently approved by FDA or in phase II/III clinical trials

In the opening chapter, **Drs. Jens Hoffmann** and **Anette Sommer** summarise recent advances in anti-hormonal research, a more longstanding area of therapy where significant opportunities still remain for novel or improved drugs. The following two chapters review inhibitors of cell growth and proliferation: **Drs. Eli Wallace**, **Ellen Laird**, **Tammie Yeh**, **James Blake** and **Joseph**

Table 4 Targeted drugs recently approved by FDA or in phase II/III clinical trials

Drug	Clinical utility[a,b]	Principal mode of action	Volume chapter
Lapatinib	Breast	EGFR/erbB2 kinase inhibitor	2
Sorafenib[c]	Renal cell	Raf/PDGFR/VEGFR/KIT kinase inhibitor	2
Tipifarnib	AML	Farnesyl transferase inhibitor	3
Sunitinib[d]	Renal cell, gastrointestinal	PDGFR/VEGFR/KIT/Flt3 kinase inhibitor	7
Vatalanib	Colorectal	VEGFR kinase inhibitor	7
Dasatinib[e]	ALL, CML	Bcr–Abl/Src kinase inhibitor	8, 9

[a] Most advanced clinical trial
[b] Investigational Drugs Database (http://www.iddb3.com)
[c] NEXAVAR™, approved by FDA, December 2005
[d] SUTENT™, approved by FDA, January 2006
[e] SPRYCEL™, approved by FDA, June 2006

Lyssikatos discuss compounds targeting the erbB pathway, agents which aim to follow the EGF kinase inhibitors gefitinib and erlotinib into clinical practice, while **Drs. Patrick Angibaud**, **David End** and **Laurence Mevellec** review farnesyl transferase inhibitors, a compound class which has given disappointing results in phase III clinical trials against solid tumours, but which still has potential for treatment of haematological malignancies and glioblastoma. **Dr. Carlos Garcia-Echeverria** then outlines some approaches to inhibition of survival signalling, an area of research at a much earlier stage where the first compounds have provided proof of concept in preclinical studies and have entered clinical trials. The two subsequent chapters review approaches to inhibition of the cell cycle: **Drs. Robert Galemmo**, **Dana Johnson**, **Holly Koblish** and **Kevin Moriarty** discuss intervention at a number of key steps in the cycle responsible for cell growth and arrest, while **Drs. Peter ten Holte**, **Kristof van Emelen**, **Michel Janicot**, **Peter Fong**, **Johann de Bono** and **Janine Arts** review inhibitors of histone deacetylases, enzymes which play a key role in expression of genes encoding for cell cycle arrest, differentiation and apoptosis. In both these areas, a number of compounds are now undergoing phase I/II clinical trials. In the next chapter, **Drs. Keren Paz** and **Zhenping Zhu** review inhibition of VEGF receptor-2, which represents the most clinically advanced small molecule approach to anti-angiogenic therapy, with several agents currently being evaluated in phase II/III trials. The final chapters focus on inhibitors of the related kinases Src and Bcr-Abl: **Dr. Tomi Sawyer** discusses Src as an anti-metastatic therapeutic target, and in the concluding chapter **Dr. Diane Boschelli** reviews new inhibitors of Bcr-Abl targeted at tumours resistant to imatinib.

Each chapter reviews published literature through to early 2006. Limitations of space have inevitably led to omission of a number of other ap-

proaches that have led to small molecule drugs progressing to clinical trials in recent years. Reviews are available elsewhere describing the clinical potential of proteasome inhibitors [83], endothelin antagonists [84], sensitising agents such as inhibitors of checkpoint kinase [85] and inhibitors of poly(ADP-ribose) polymerase [86], matrix metalloprotease inhibitors [87], inhibitors of mitotic kinesins [88] and inhibitors of urokinase plasminogen activator [89]. Research also continues on new cytotoxic agents, and reviews can again be found elsewhere [19, 90].

7 Conclusion

The last decade has seen clinical trials of a range of drugs that exploit fundamentally different cellular mechanisms from historical cytotoxic chemotherapy, and a number of these agents have now been granted regulatory approval. Experience from these trials is providing growing insight into the role of factors such as patient selection, design of clinical trials and mechanisms of drug resistance. An estimated 500 chemotherapeutic agents were undergoing clinical trials in 2004, a number predicted to rise by an order of magnitude by 2010 [2]. This volume reviews the medicinal chemistry behind a number of key classes of anti-cancer agents encompassed within these numbers. The coming decades will reveal whether the shift to "personalised medicine" widely envisaged through introduction of these agents becomes reality against the full diversity of human tumours, and provides a real breakthrough towards fulfilment of a therapeutic vision that began over half a century ago.

Acknowledgements Heartfelt thanks to all chapter authors for their contributions, to Dr Peter Bernstein for editorial advice, and to colleagues at AstraZeneca for critical reading of the manuscript.

References

1. Bosl GJ, Motzer RJ (1997) N Engl J Med 337:242
2. McVie G, Schipper H, Sikora K (2004) Exp Rev Anticancer Ther 4:S43
3. Sikora K, Timbs O (2004) Expert Rev Anticancer Ther 4:S11
4. Zubrod CG (1979) Semin Oncol 6:490
5. Pratt WB, Ruddon RW, Ensminger WD, Maybaum J (eds) (1994) The anticancer drugs, 2nd edn. Oxford University Press, New York, p 17
6. Chabner BA, Roberts TG Jr (2005) Nat Rev Cancer 5:65
7. Gilman A, Philips FS (1946) Science 103:409
8. Goodman LS, Wintrobe MM, Damashek W, Goodman MJ, Gilman A, McLennan MT (1946) JAMA 132:126
9. Hausheer FH, Singh UC, Saxe JD, Colvin OM (1989) Anti-Cancer Drug Des 4:281

10. Brock N (1989) Cancer Res 49:1
11. Farber S (1949) Blood 4:160
12. Li MC, Hertz R, Bergenstal DM (1958) N Engl J Med 259:66
13. Jolivet J, Cowan KH, Curt GA, Clendeninn NJ, Chabner BA (1983) N Engl J Med 309:1094
14. Frei E III (1985) Cancer Res 45:6523
15. Heidelberger C (1965) Fluorinated pyrimidines. In: Davidson JN, Cohn WE (eds) Progress in nucleic acid research and molecular biology, vol 4. Academic Press, New York, p 1
16. Elion GB, Hitchings GH (1965) Adv Chemotherapy 2:91
17. Rosenberg B, Van Camp L, Krigas T (1965) Nature 205:698
18. Rosenberg B, Van Camp L, Trosko JE, Mansour VH (1969) Nature 222:385
19. Ho YP, Au-Yeung SCF, To KKW (2003) Med Res Rev 23:633
20. Binaschi M, Bigioni M, Cipollone A, Rossi C, Goso C, Maggi CA, Capranico G, Animati F (2001) Curr Med Chem – Anti-Cancer Agents 1:113
21. Horowitz SB (1992) Trends Pharmacol Sci 13:134
22. Goodman J, Walsh V (2001) The story of Taxol. Cambridge University Press, Cambridge
23. Klener P (1999) Basic Clin Oncol 19:279
24. Jordan VC, Furr BJA (eds) (2002) Hormone therapy in breast and prostate cancer. Humana Press, Totowa, NJ, p 1
25. Jordan VC (2003) Nat Rev Drug Disc 2:205
26. Lacassagne A (1936) Am J Cancer 27:215
27. Jordan VC (1984) Pharmacol Rev 36:245
28. Cuzick J (2005) Drugs Today 41:227
29. Brogden RN, Clissold SP (1989) Drugs 38:185
30. Chrisp P, Goa KL (1991) Drugs 41:254
31. Takimoto CH (2003) Cancer Chemother Pharmacol 52(Suppl 1):29
32. Kamb A (2005) Nat Rev Drug Disc 4:161
33. Kufe DW, Pollock RE, Weichselbaum RR, Bast RC Jr, Gansler TS, Holland JF, Frei E III (eds) (2003) Cancer medicine-6, vol 1. BC Decker, Hamilton
34. Hanahan D, Weinberg RA (2000) Cell 100:57
35. Ross JS, Schenkein DP, Pietrusko R, Rolfe M, Linette GP, Stec J, Stagliano NE, Ginsburg GS, Symmans WF, Pusztai L, Hortobagyi GN (2004) Am J Clin Pathol 122:598
36. Lombardino JG, Lowe JA III (2004) Nat Rev Drug Disc 3:853
37. Cherry M, Williams DH (2004) Curr Med Chem 11:663
38. Williams SP, Kuyper LF, Pearce KH (2005) Curr Opin Chem Biol 9:371
39. Cohen P (2002) Nat Rev Drug Disc 1:309
40. Dancey J, Sausville EA (2003) Nat Rev Drug Disc 2:296
41. Ohren JF, Chen H, Pavlovsky A, Whitehead C, Zhang E, Kuffa P, Yan C, McConnell P, Spessard C, Banotai C, Mueller WT, Delaney A, Omer C, Sebolt-Leopold J, Dudley DT, Leung IK, Flamme C, Warmus J, Kaufman M, Barrett S, Tecle H, Hasemann CA (2004) Nat Struct Mol Biol 11:1192
42. Arkin M (2005) Curr Opin Chem Biol 9:317
43. Adams GP, Weiner LM (2005) Nat Biotech 23:1147
44. Maloney DG, Smith B, Rose A (2002) Semin Oncol 29(1, Suppl 2):2
45. Yeon CH, Pegram MD (2005) Invest New Drug 23:391
46. Manne U, Srivastava R-G, Srivastava S (2005) Drug Disc Today 10:965
47. Ferrara N, Hillan KJ, Gerber H-P, Novotny W (2004) Nat Rev Drug Disc 3:391
48. Lydon NB, Druker BJ (2004) Leukemia Res 28(Suppl 1):29

49. Wakeling AE (2005) Discovery and development of Iressa. In: Pinna LA, Cohen PTW (eds) Inhibitors of protein kinases and protein phosphatases. Handbook of experimental pharmacology, vol 167. Springer, Berlin Heidelberg New York, p 433
50. Brown ER, Shepherd FA (2005) Exp Rev Anticancer Ther 5:767
51. Albanell J, Adams J (2002) Drugs Future 27:1079
52. Deininger MWN, O'Brien SG, Ford JM, Druker BJ (2003) J Clin Oncol 21:1637
53. Giaccone G (2005) J Clin Oncol 23:3235
54. Adams J, Elliott PJ, Bouchard P (2004) Preclinical development of bortezomib (Velcade): Rationale for clinical studies. In: Adams J (ed) Proteasome inhibitors in cancer therapy. Humana Press, Totowa, NJ, p 233
55. Thatcher N, Chang A, Parikh P, Pereira JR, Ciuleanu T, von Pawel J, Thongprasert S, Tan EH, Pemberton K, Archer V, Carroll K (2005) Lancet 366:1527
56. Onn A, Herbst RS (2005) Lancet 366:1507
57. Pao W, Miller VA (2005) J Clin Oncol 23:2556
58. Giaccone G, Rodriguez JA (2005) Nat Clin Pract Oncol 2:554
59. Holloway B, Thatcher N, Chang A, Parikh P, Pereira JR, Ciuleanu T, von Pawel J, Flannery A, Ellison G, Donald E, Knight L, Watkins C (2005) AACR-NCI-EORTC international conference on molecular targets and cancer therapeutics: discovery, biology and clinical applications. Abstract A269
60. Roberts TG Jr, Chabner BA (2004) N Engl J Med 351:501
61. Weisberg E, Griffin JD (2003) Drug Res Updates 6:231
62. Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, Sawyers CL (2002) Cancer Cell 2:117
63. Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos VN, Lai JL, Philippe N, Facon T, Fenaux P, Preudhomme C (2002) Blood 100:1014
64. Rewinsky EK (2000) Drugs 60(Suppl 1):1
65. Nagahiro S, Tomohide T, Kazuto N (2003) Cancer Chemother Pharmacol 52(Suppl 1): S97
66. Johnson JR, Williams G, Pazdur R (2003) J Clin Oncol 21:1404
67. Dagher RN, Pazdur R (2004) The phase III clinical cancer trial. In: Teicher BA, Andrews PA (eds) Anticancer drug development guide, 2nd edn. Humana Press, Totowa, NJ, p 401
68. Crossman LC, O'Brien S (2004) Leukemia Res 28(Suppl 1):3
69. Dancey JE (2003) Cancer Biol Ther 2:601
70. Hammond LA, Cavanaugh SX, Thomas R (2004) Nat Biotech 22:677
71. Williams G, Pazdur R, Temple R (2004) J Biopharm Stat 14:5
72. Newell DR (2005) Eur J Cancer 41:676
73. Workman P (2003) Cancer Chemother Pharmacol 52(Suppl 1):S45
74. Hanke JH, Webster KR, Ronco LV (2004) Eur J Cancer Prev 13:297
75. Drevs J, Medinger M, Schmidt-Gersbach C, Weber R, Unger C (2003) Curr Drug Targets 4:113
76. Broxterman HJ, Georgopapadakou NH (2005) Drug Res Updates 8:183
77. Recanatini M, Poluzzi E, Masetti M, Cavalli A, de Ponti F (2005) Med Res Rev 25:133
78. Wienkers LC, Heath TG (2005) Nat Rev Drug Disc 4:825
79. Li S, He H, Parthiban LJ, Yin H, Serajuddin ATM (2005) J Pharm Sci 94:1396
80. Capdeville R, Buchdunger E, Zimmerman J, Matter A (2002) Nat Rev Drug Disc 1:493
81. Nagar B, Bornmann WG, Pellicena P, Schindler T, Veach DR, Miller WT, Clarkson B, Kuriyan J (2002) Cancer Res 62:4236
82. Burgess MR, Skaggs BJ, Shah P, Lee FY, Sawyers CL (2005) Proc Nat Acad Sci USA 102:3395

83. Daniel KG, Kuhn DJ, Kazi A, Dou QP (2005) Curr Cancer Drug Targets 5:529
84. Nelson J, Bagnato A, Battistini B, Nisen P (2003) Nat Rev Cancer 3:110
85. Collins I, Garrett MD (2005) Curr Opinion Pharmacol 5:366
86. Jagtap P, Szabo C (2005) Nat Rev Drug Disc 4:421
87. Vihinen P, Ala-aho R, Kaehaeri V-M (2005) Curr Cancer Drug Targets 5:203
88. Duhl DM, Renhowe PA (2005) Curr Opinion Drug Disc Dev 8:431
89. Rockway TW, Giranda VL (2003) Curr Pharm Des 9:1483
90. Cozzi P, Mongelli N, Suarato A (2004) Curr Med Chem – Anti-Cancer Agents 4:93

Top Med Chem (2007) 1: 19–82
DOI 10.1007/7355_2006_002

Published online: 11 November 2006

Anti-hormone Therapy: Principles of Endocrine Therapy of Cancer

Jens Hoffmann (✉) · Anette Sommer

Schering AG, Müllerstr. 172–178, 13342 Berlin, Germany
jens.hoffmann@schering.de

1 Introduction . . . 21
1.1 Anti-hormones as Targeted Drugs . . . 21
1.2 Steroid Hormone Receptors . . . 23
1.3 Steroid Hormone Receptors as Transcription Factors . . . 23
1.3.1 Estrogen Receptors ERα and ERβ . . . 24
1.3.2 Progesterone Receptors . . . 26
1.3.3 Androgen Receptor . . . 28
1.4 Co-factors . . . 29
1.5 Ligand-Independent Activation of Steroid Hormone Receptors and Non-genomic Effects of Steroids . . . 31

2 Chemistry and Pharmacology of Endocrine Therapy . . . 34
2.1 Hormone Deprivation – Inhibition of Steroid Hormone Biosynthesis . . . 34
2.1.1 Aromatase Inhibitors . . . 34
2.1.2 17β-Hydroxysteroid-dehydrogenase Inhibitors . . . 41
2.1.3 Steroid Sulfatase Inhibitors . . . 43
2.1.4 5α-Reductase Inhibitors . . . 46
2.1.5 CYP-17 Inhibitors . . . 50
2.2 Hormone Antagonists – Blockade of Steroid Hormone Receptors . . . 51
2.2.1 Anti-estrogens . . . 51
2.2.2 Progesterone Receptor Antagonists . . . 60
2.2.3 Anti-androgens . . . 64
2.3 Hormone Interference – Estrogens and Progestins . . . 69
2.3.1 Inhibitors of Releasing Hormones . . . 70

3 Conclusion and Outlook . . . 73

References . . . 74

Abstract Surgical oophorectomy and orchiectomy, first proposed over a century ago, are effective in the therapy of breast and prostate cancer, respectively. Later, the discovery of steroid hormones and the steroid hormone receptors led to the concept that inhibition of steroid hormone receptor function by an antagonist prevents tumour growth. While the first anti-hormones, cyproterone acetate (CPA) and tamoxifen, were found accidentally, a deeper understanding of steroid hormone receptors as transcription factors enabled more rational, structure–activity relationship-based drug discovery programmes. This review will provide both a background on principles and an update on recent developments in the field of endocrine therapy of cancer.

Results from recent drug-finding programmes on new steroid hormone receptor antagonists will be reviewed. Among these, new steroidal and non-steroidal compounds

with increased potency and efficacy, improved tissue selectivity, and without any agonistic activity will be described. New pre-clinical and clinical data on development candidates will be reported. Advanced breast and prostate cancer is effectively managed by estrogen and androgen ablation, respectively. However, although steroid hormone receptors – estrogen receptor α (ERα) and androgen receptor (AR) – are still functionally expressed, this therapy fails in a majority of cases. As a novel strategy for the treatment of advanced breast and prostate cancer, the selective down-regulation of the receptor (ER or AR, respectively) has been proposed and this new therapeutic concept provides a significant inhibition of tumour growth in vivo.

In addition, it will be our intention to present a deeper insight into the biosynthesis of steroid hormones, which will allow definition of new targets and approaches for the treatment of endocrine-responsive cancer. Enzymes involved in mechanisms of steroid hormone biosynthesis might be novel targets for endocrine therapy. Moreover, further therapeutic indications for modulators of steroid hormone receptors will be discussed. In summary, many promising new opportunities for endocrine therapy of breast and prostate cancer are now arising.

Keywords Cancer · Steroid hormones · Estrogen · Androgen · Gestagen

Abbreviations

AACR	American Association for Cancer Research
AF-1/-2	Activation function 1 or 2
AI	Aromatase inhibitor
AR	Androgen receptor
ARE	Androgen response element
BPH	Benign prostate hyperplasia
CIS	*Carcinoma in situ*
CPA	Cyproterone acetate
CR	Complete response
DBD	DNA binding domain
DES	Diethylstilbestrol
DHEA	Dehydoepiandrostenedione
DHT	Dihydrotestosterone
E1	Estrone
E1S	Estrone sulfate
E2	17β-Estradiol
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EORTC	European Organisation for Research and Treatment of Cancer
ER	Estrogen receptor
ERE	Estrogen response element
ERKO	Estrogen receptor knock-out mouse
FSH	Follicle-stimulating hormone
GnRH	Gonadotropin-releasing hormone
GR	Glucocorticoid receptor
HRE	Hormone response element
HSD	17β-Hydroxysteroid dehydrogenase
HSP	Heat shock protein
IEN	Intraepithelial neoplasia
IGF-1	Insulin-like growth factor 1

IL-6	Interleukin 6
KGF	Keratinocyte growth factor
LBD	Ligand-binding domain
LH	Luteinising hormone
LHRH	Luteinising hormone releasing hormone
MAPK	Mitogen-activated protein kinase
MGA	Megestrol acetate
MPA	Medroxyprogesterone acetate
MMTV	Murine mammary tumour virus
MR	Mineralocorticoid receptor
NCI	National Cancer Institute
4-OHA	4-Hydroxyandrostendione
4-OHT	4-Hydroxytamoxifen
OR	Objective response
PIN	Prostate intraepithelial neoplasia
PR	Progesterone receptor
PR	Partial response
PRE	Progesterone response element
PRKO	Progesterone receptor knock-out mouse
PSA	Prostate-specific antigen
RAR/RXR	Retinoic acid receptors
USPSTF	US Preventive Services Task Force
SD	Stable disease
SERD	Selective estrogen receptor destabiliser
SERM	Selective estrogen receptor modulator
SRC	Steroid receptor co-activator
STS	Steroid sulfatase
TR	Thyroid hormone receptor

1 Introduction

1.1 Anti-hormones as Targeted Drugs

Endocrine therapy of cancer is based on at least one of the following principles:

- *Hormone deprivation:* Deprivation of endogenous hormones by inhibition of biosynthesis of hormones or due to removal or inactivation of the hormone producing tissue
- *Hormone antagonism:* Application of drugs that bind to and inhibit the steroid hormone receptors or different types of releasing hormone receptors
- *Hormone interference:* Application of high doses of hormones that either directly or via negative feedback mechanisms inhibit tumour growth

Most targets for the endocrine therapy are components of the hypothalamo–pituitary–gonadal/adrenal axis (Fig. 1). Interference with this finely tuned endocrine feedback loop can inhibit both, the hormone biosynthesis and the binding of endogenous hormones to steroid hormone receptors. The interference with the gonadotropin-releasing hormones (GnRH) inhibits the secretion of luteinising hormone (LH), follicle-stimulating hormone (FSH) or adrenocorticotropic hormone (ACTH) resulting in a decreased synthesis of the steroid hormones estrogen, progestin and androgen in the testes, ovaries or adrenal glands [1–4]. The estrogen, progesterone and androgen receptors (ER, PR, AR), which are activated by estrogens, progestins and androgens, respectively, are the downstream targets in endocrine-responsive tissues or in tumours. Historically, agonistic ligands are called hormones and antagonistic ligands are called anti-hormones.

Enzymes that are involved in steroid hormone biosynthesis or in steroid metabolism are also targets of anti-hormonal therapy. Recently, it was discovered that certain co-factors modulate the signalling of steroid hormone receptors in a tissue-selective fashion. By binding the receptor ligand complex, these co-activators and co-repressors are capable of either activating or repressing transcription, respectively [5].

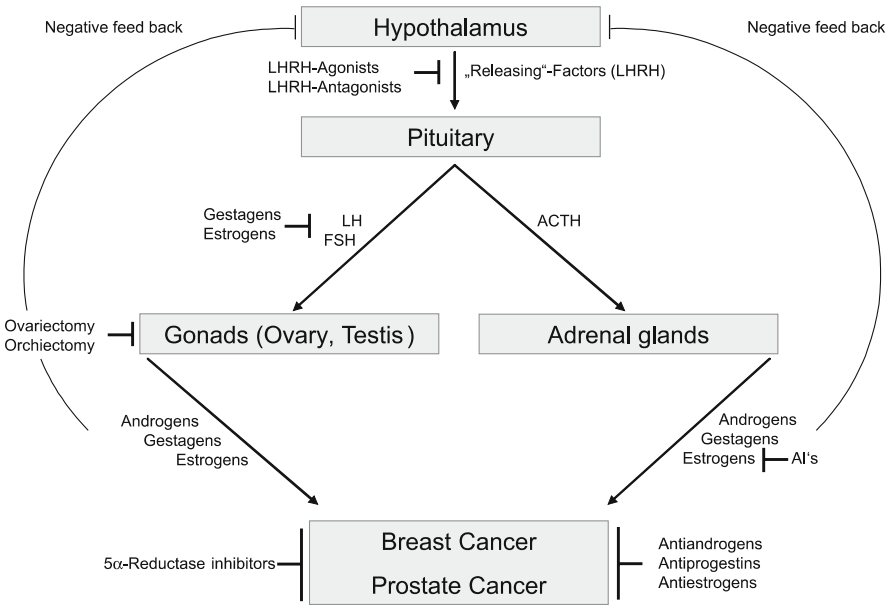


Fig. 1 Endocrine feedback cycle

1.2 Steroid Hormone Receptors

Nuclear hormone receptors are transcription factors that when bound by their cognate ligand affect biochemistry, cell biology, and physiology in nearly all human tissues. They are grouped into two subgroups, type I and II nuclear receptors. Type I nuclear receptors are also denominated as steroid hormone receptors because most of the natural ligands have a steroidal structure. Estrogen receptors (ERα, ERβ), progesterone receptors (PR-A, PR-B), the androgen receptor (AR), the glucocorticoid receptor (GR), and the mineralocorticoid receptor (MR) are important steroid hormone receptors.

The role of the steroid hormone receptors has extensively been defined with the help of natural and synthetic agonists and antagonists and with characterisation of transgenic and knock-out mice. In experimental studies, both approaches have been useful tools for validating that a physiological process is indeed mediated by the steroid hormone receptor under investigation.

The pharmacology of type II receptors is less well defined. Whereas for some receptors (TR, VDR, PPARα, γ) ligands or a physiological function has been elucidated, for others knowledge is still rudimentary (orphan nuclear receptors and the ER-related receptors, PPARs, RXR). However, even for orphan nuclear receptors, for which potential endogenous ligands have not been identified, an involvement in important physiological or metabolic processes has been observed and this provides evidence that they might also be potential therapeutic targets [6, 7].

Steroid hormone receptor antagonists are the best currently available options for the treatment of tumours associated with significant morbidity and mortality, even though their use is accompanied by undesirable side effects. Therefore, considerable efforts in pharmaceutical research have been directed towards identifying efficacious steroid hormone receptor ligands that are devoid of side effects. As it is not possible to cover all the steroid hormone receptors in this review, we will provide data on recent drug discovery efforts for a selected class of steroid hormone receptor antagonists, namely the anti-hormones of the ER, PR and AR and their pharmacology. We will focus on those projects where we envision novel drugs to emerge in the near future.

1.3 Steroid Hormone Receptors as Transcription Factors

The classical model, which described the pharmacology of steroid hormone receptors, hypothesised that antagonists function by competitively inhibiting agonist binding, blocking the receptor and inhibiting transcription. Due to the development of specific antagonists, and the identification of receptor isoforms, co-factors and the crystal structures, which are now available in complex with agonists and antagonists, it has become clear that for the

classic steroid hormone receptors ER, PR and AR this simple model does not adequately describe the pharmacology of steroid hormone receptors. In addition, interference with steroid hormone receptor signalling does not necessarily have to be directed against the receptors themselves. Our understanding of the molecular biology now offers new opportunities: For example, post-translational modifications leading to accelerated receptor inactivation or degradation might be exploited as new routes for interference with the stability of steroid hormone receptors. Steroid hormone receptor protein expression is most probably subject to a finely tuned regulation. The half-life of the steroid hormone receptors is rather short. Moreover, knowledge of epigenetic regulation of receptor expression [8], as well as about the function of proteins involved in receptor degradation, is becoming more and more comprehensive [9]. Interfering with the processes responsible for epigenetic regulation of steroid hormone receptor expression, protein stability, and the coordinated assembly of receptors, co-factors and the basal transcription machinery, and on steroid hormone receptor protein turnover are now moving more and more into the focus of drug discovery activities [10].

1.3.1
Estrogen Receptors ERα and ERβ

Although estrogens are mainly produced locally by the ovaries, they exert systemic effects on selected target tissues. The action of the estrogens is mediated by two different estrogen receptors (ERs), ERα and ERβ [11]. ERα and ERβ are products of different genes and exhibit a tissue- and cell type-specific expression. The ERα is expressed primarily in the uterus, liver, kidney and heart, and sometimes it is co-expressed with the ERβ in mammary, thyroid and adrenal glands, in bone, and in the brain, where they can form functional heterodimers and where ERβ in many instances opposes the actions of ERα [11]. ERβ in contrast, is expressed specifically in the ovaries, in prostate, lung, in the gastrointestinal tract, bladder, and in the hematopoietic and central nervous system [11].

Ligand binding induces conformational changes in the steroid hormone receptor leading to dimerisation, protein–DNA interaction at the cognate response elements, recruitment of co-factors, and the formation of the preinitiation complex (Fig. 2). It has long been recognised that estrogens promote proliferation of cancer cells. The current understanding is that downstream mediators of estrogen action stimulate cell cycle progression, particularly at the G1 to S transition, by inducing the expression of c-Myc and cyclin D1. In addition, estrogens also activate cyclin E-CDK2 [11]. These proteins are rapidly up-regulated in response to estrogen and initiate cell cycle progression and proliferation [12].

The functions of some domains of the ER have been defined using deletion mutants and site-directed mutagenesis as well as structural analyses [13].

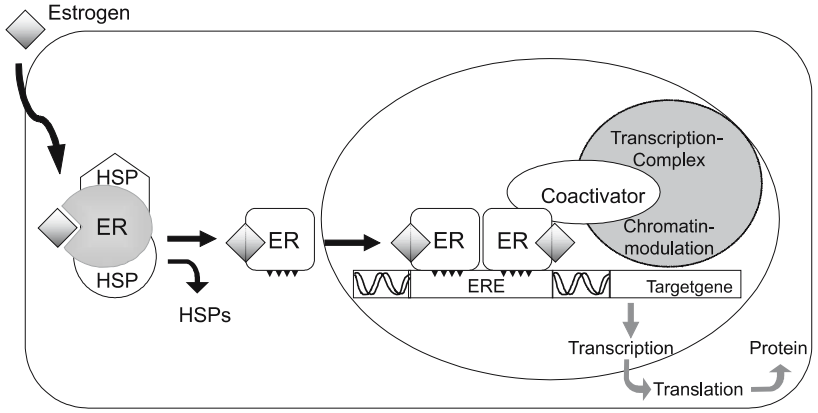


Fig. 2 Classic pathway of estrogen signal transduction. When an estrogen molecule binds to an estrogen receptor (*ER*), the receptor dissociates from the cytoplasmic chaperones, the receptor-associated heat shock proteins (*HSP*). The estradiol–ER complex then translocates into the nucleus, where it binds to estrogen response elements (*ERE*) on the DNA. Transcription requires the assembly of a transcription complex by associating various factors, such as steroid-receptor co-activators. The proposed mechanisms of stimulation includes the stabilisation of the pre-initiation complex, chromatin remodelling and interaction with other transcription factors

DNA-binding studies have indicated that the ER binds as a dimer, and both a motif within the DNA-binding domain (DBD) and a motif in the ligand-binding domain (LBD) of the ER are involved in dimerisation [14]. The ERα and ERβ share a high degree of sequence identity on the protein level, however, differential affinities to natural estrogen response elements (EREs) have been noted for the two receptors [15]. Even for the ERα and ERβ LBDs, which are highly conserved and exhibit similar affinities for the endogenous ligand estradiol, subtype-specific agonistic and antagonistic ligands with different affinities have been reported [16].

The physiological functions of the ER subtypes have been characterised in mice lacking the ERα, the ERβ, or both receptors [11]. Disruption of the ERα resulted in infertility of both male and female mice and inhibited the outgrowth of the mammary duct during puberty, whereas disruption of the ERβ had no effect on fertility and mammary gland development [17]. A number of ERα and ERβ isoforms have also been described, many of which alter estrogen-mediated gene expression [18].

The LBDs of the ERα and the ERβ share a similar overall architecture. Two separate transactivation domains (AF) mediate the transactivation of the ER: an N-terminal ligand-independent activation function (AF-1) and a C-terminal ligand-dependent activation function (AF-2), which is located within the LBD (Fig. 3). The surface of the AF-1 is composed of amino acids in helices 3, 4, 5 and 12, and the binding of ligands alters the position of helix

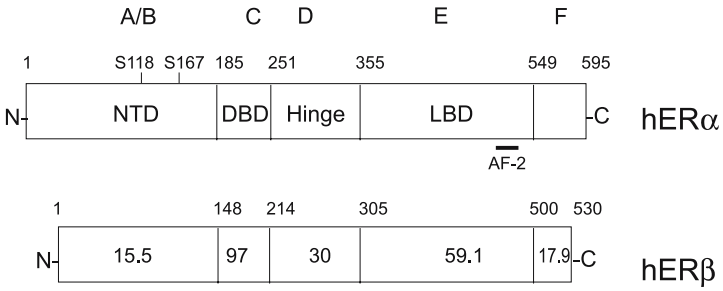


Fig. 3 Schematic representation of the human ERα and ERβ, together with their transactivation, DNA- and ligand-binding domains in the respective isoforms

12. The AF-1 domain in ERα is very active on a variety of estrogen responsive promoters, whereas the AF-1 is only minimally active in ERβ [19].

Crystallographic studies of the LBD of human ERα bound to either the agonist diethyl-stilbestrol or the selective antagonist 4-hydroxytamoxifen indicated that helix 12 is positioned over the ligand-binding pocket and forms an interaction surface for the recruitment of co-activators when the ERα LBD is complexed with agonists [20]. In contrast, when the LBD is complexed with an antagonist, helix 12 is displaced from its agonist position and occupies the hydrophobic groove formed by helices 3, 4 and 5. In consequence, helix 12 is dislocated and the interaction surface with the co-activator is disrupted [20]. However, Shiau et al. [21] identified compounds that are able to induce an agonistic conformation in the LBD of the ERα and an antagonistic conformation in the LBD of the ERβ.

The AF-2-dependent transcriptional activation of the two ERs is mediated via the recruitment of co-factors to estrogen-responsive promoters. Co-factors can be classified into co-activators, which promote ER activity, and into co-repressors, which attenuate ER activity. It has been suggested that co-repressors, many of which are histone deacetylases (HDACs), are recruited to ER target genes. In contrast, co-activor complexes often contain histone acetyltransferases (HATs), and thus an opposite transcriptional regulation is brought about. Indeed, the histone deacetylase 2 (HDAC2), which is recruited by co-repressor complexes such as N-CoR-SIN3, is required for the transcriptional repression of tamoxifen-bound ERα, and the loss of co-repressors might be one mechanism of tamoxifen resistance [22].

1.3.2 Progesterone Receptors

The physiological effects of progesterone are mediated by two progesterone receptor isoforms termed PR-A and PR-B. These arise from a single gene and act as ligand-activated transcription factors to regulate the expression of

target genes involved mainly in reproduction. The structure and functional properties of the PR isoforms and how functional differences between these proteins are likely to impact the overall role of these receptors in the reproductive systems have been discussed in detail by Conneely et al. [23]. The crystal structure of the LBD of the PR in complex with progesterone has been solved and it has a similar structure to the LBD of ERα in complex with estradiol [24].

In most cases, the expression of the PR is induced by estrogen, implying that many of the in vivo effects attributed to progesterone could also be the result of concomitantly administered estrogen. Therefore, to clearly define those physiological events that are specifically attributable to progesterone in vivo, a mouse model carrying a null mutation of the PR gene has been generated [25].

A null mutation of both PR isoforms, PR-A and PR-B, leads to pleiotropic reproductive abnormalities in mice [26, 27]. Male and female embryos with a homozygous deletion of the PR (PR-KO) developed normally to adulthood, but they displayed remarkable alterations in all reproductive organs. These alterations included an inability to ovulate, uterine hyperplasia and inflammation, severely limited mammary gland development, and an inability to exhibit sexual behaviour. As female mice with a homozygous deletion of the PR-A isoform have a lobuloalveolar developmental response, this indicates that the PR-B isoform is sufficient to mediate pregnancy-associated mammary development. The PR-KO model was also used to study the role of the PR in stroma and epithelium on ductal and lobuloalveolar development in the murine mammary gland [28]. Mammary gland transplantation experiments in PR-KO mice demonstrated that the luminal-epithelial compartment of the mammary gland is responsive to the progesterone-induced signalling. There is strong evidence that the PR may exert proliferative effects onto mammary epithelial cells that lack the PR through paracrine factors not yet identified [29, 30].

The PR-KO model was also used to define the controversial role of progesterone-initiated intracellular signalling in mammary gland tumourigenesis [31]. Performing tissue transplantation experiments in an established carcinogen-induced (7,12-dimethylbenz(a)anthracene, DMBA) mammary tumourigenesis model, it was shown that PR-KO mice have a marked reduction in the incidence of mammary gland tumours compared to isogenic wild-type mice. This observation indicates that in the absence of PR function, prolactin alone is not sufficient to induce the neoplastic transformation and that progesterone may activate mitogenic mediators of the prolactin pathway. Under these conditions, the epithelial cells might exhibit a low proliferative rate, and they might not be susceptible to malignant transformation upon administration of the carcinogen DMBA.

The luminal-epithelial compartment has not only been considered to be primarily responsive to the progesterone-induced proliferative signals and to be the primary site for the initial carcinogenic insult but, in addition, the

PR is highly expressed in this compartment [31]. One interpretation for the reduction of mammary gland tumourigenesis in PR-KO mice is that the progenitor cells for alveologenesis, the PR-expressing epithelial cells, are absent in the PR-KO mice. As the majority of mammary gland tumours are of alveolar origin, the absence of these progenitor cells might reduce the number of target cells susceptible to neoplastic transformation. These results strongly support the application of anti-progestins in the therapy of breast cancer because they might inhibit the prolactin-induced mitogenic activity on the luminal-epithelial compartment.

Depending on the tissue, progesterone has been classified as a hormone able to induce proliferation or differentiation. However, growth stimulation of the ERα- and PR-positive human breast cancer cell line T47D by progestins is restricted to one cell cycle, and is followed by growth arrest at the G1/S boundary of the second cell cycle [32, 33]. Afterwards, the application of additional progestins does not stimulate cell cycle progression but rather renders the cells resistant. During the progesterone-arrested state, the T47D cells upregulate expression of the epidermal growth factor receptor (EGFR) three- to fivefold and acquire sensitivity to the proliferative effects of EGF [34]. This led to the model put forward by Horwitz and co-workers [34], that progesterone is a competence factor that switches breast cancer growth from steroid hormone-dependence to growth factor-dependence. These effects include the attenuation of progestin responsiveness, decrease of the level of PR in cells treated with EGF [35], and progestin-dependent regulation of EGF and EGFR levels [36].

1.3.3 Androgen Receptor

The androgen receptor (AR), a transcription factor which is regulated via the binding of androgens, has two transactivation functions. In contrast to the ERα, ERβ and the PR, the C-terminal activation function AF-2 is only weakly transcriptionally active. The N-terminal region of the AR contains a stretch of variable length consisting of 6–30 glutamine and 3–18 glycine residues. The length of this polymorphism influences the transcriptional activity [37]. A nuclear localisation signal (NLS) spans the region between the DNA-binding domain and the hinge region. AR-regulated gene expression is responsible for male sexual differentiation and male pubertal changes. AR-specific ligands are widely used in a variety of clinical settings.

The first crystal structure of the LBD of the AR in complex with metribolone (R1881) was solved by Matias and colleagues in 2000 [38]. The LBD has a similar three-dimensional structure to the other agonist-bound steroid receptors, namely the ERα, ERβ and the PR [38]. The fact that all steroid hormone receptors bind similar hormone response elements (HREs) stands in sharp contrast to the specific activities elicited by application of the steroid

hormones. Specificity is achieved by enhancer elements on the DNA surrounding the HREs and the tissue- and cell type-specific expression of co-factors [37]. Intra-molecular interactions between the N-terminal activation function AF-1 and the C-terminal LBD may interfere with co-factor binding [37, 39]. The AR is also indirectly activated by cytokines and growth factors, namely IL-6, IGF-1, KGF and EGF. Furthermore, the AR is directly activated by phosphorylation and sumoylation [39–42].

In contrast to other steroid hormone receptors, a considerable number of mutations have been detected in the AR sequence, and have been characterised (a database is available at www.mcgill.ca/androgendb). As many mutations reside in the DNA-binding domain or in the LBD of the human AR, they result in different phenotypes. The mutation of amino acid residue 877 from threonine to alanine (T877A) is the most frequent [42]. The crystal structure has shown that the mutant AR-T877A has an enlarged ligand binding pocket, which may explain why the bulkier ligands progesterone and the glucocorticoids are able to activate this AR [38, 43, 44]. Whereas some AR mutations enhance the transcriptional activity, others completely inhibit all functions of the AR. The majority of these mutations are associated with human genetic diseases, in particular androgen insensitivity syndrome. The characteristic phenotype of men with complete androgen insensitivity syndrome (CAIS) are female external genitalia and an absence of internal and external male organs [45]. Considering the important role of steroid hormone receptors in reproduction and development, it is astonishing that mutations in the androgen receptor gene, which lead to a completely inactive receptor, elicit a rather mild phenotype.

1.4 Co-factors

Nuclear receptors exert their different transcriptional functions through interactions with and the recruitment of co-factors to responsive promoters. Co-factors are either positive or negative regulatory proteins and are classified as co-activators, which promote, or co-repressors, which attenuate the activity of nuclear hormone receptors [46]. The molecular mechanisms that regulate the mutually exclusive interactions of the nuclear receptor with either class of co-factors have been analysed by crystallographic studies. Functional and structural studies have shown that co-activators interact with the transactivation function (AF) of nuclear hormone receptors via short, leucine-rich motifs (LXXLL) termed “NR boxes”, thereby transducing hormonal signals to the basal transcription machinery [47].

Examples of co-activators are the steroid receptor co-activator (SRC) family [48] and the components of the mammalian mediator complex, which possesses chromatin remodelling ability and tethers activated steroid hormone receptors to the basal transcription machinery [49]. Additional co-

activators, such as histone acetyltransferases (HATs), CREB-binding protein (CBP) and the related p300 protein, are tethered to the nuclear hormone receptors through interactions with the SRC family of co-activators (Fig. 4) [50]. The recruitment of co-factors with histone modifying and chromatin remodelling activities by steroid hormone receptors overcomes the transcriptional repression mediated by histone deacetylases (HDACs), leading to active transcription via the general transcription machinery (Fig. 4). Important co-factors and their functions have been reviewed by Gao et al. [51].

In the absence of ligand, some nuclear hormone receptors associate with co-repressors, namely, SMRT (silencing mediator of retinoic acid and thyroid hormone receptors) and N-CoR (nuclear receptor co-repressor). Both, SMRT and N-CoR, recruit coregulatory protein SIN3 and histone deacetylases (HDACs) to form a large co-repressor complex that contains histone deacetylase activity, implicating histone deacetylation in transcriptional repression [52, 53].

The development of compounds that block the interaction of agonist-liganded steroid hormone receptors with co-activators might provide unique pharmacological agents for interrupting the signal transduction cascade. In first attempts, short peptides were generated by screening a phage display library against canonical LXXLL motifs [54]. Most of the peptides identified were able to discriminate between ERα and ERβ, and also between ERs complexed with ligands of different structure, including antagonists. Recently,

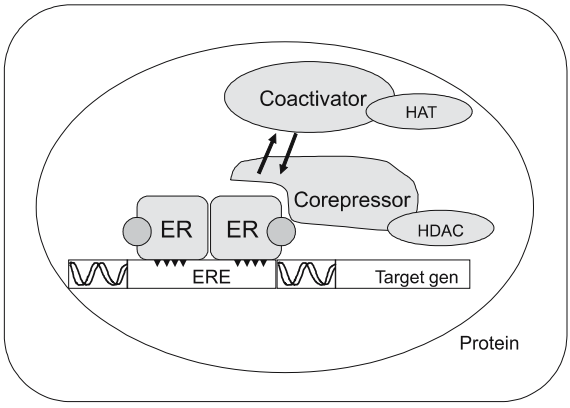


Fig. 4 Co-activator and co-repressor complexes are required for nuclear hormone receptor-mediated transcriptional regulation. The tissue-selective fine-tuning of gene transcription by nuclear hormone receptors is due to different co-regulatory complexes that have various functions and enzymatic activities. Co-activator complexes include factors that contain ATP-dependent chromatin remodelling activity often associated with histone acetyltransferase (HAT) activity. Co-repressors include ATP-dependent chromatin remodelling complexes, which function as platforms for the recruitment of several subcomplexes that often contain histone deacetylase (HDAC) activity

approaches to identify small molecular weight inhibitors of co-activator binding (CBI) based on three-dimensional data of steroid hormone receptors and co-activator complexes have been reported [55]. Analyses were made to see whether CBIs are able to block the interaction of a LXXLL sequence-containing peptide (NR box of co-activators) with the ERα. The best CBIs, which effectively blocked the interaction of the peptide with the ERα, were found in a pyrimidine series [55]. Although blocking protein–protein interaction is usually very difficult to accomplish, the results provide a proof-of-principle that effective small molecule CBIs can be generated.

In cells of the mammary gland, either in normal epithelial or in cancerous cells, the packaging of chromosomal DNA into chromatin restricts the access of the transcription machinery, thereby causing transcriptional repression. The basic N-termini of histones are subject to post-translational modifications, including lysine acetylation, lysine and arginine methylation, serine phosphorylation and ubiquitinylation [56]. It has been proposed in the "histone code hypothesis" that the intricate pattern of modifications of the N-terminal histone tail influences gene regulation [57].

The potential impact of the chromatin structure on ERα- and ERβ-mediated transcriptional activities was investigated using an in vitro chromatin assembly assay. These experiments have shown that the AF-1 domain of ERα, but not of ERβ, contains a transferable activation domain, which permits the ERα to efficiently activate transcription on chromatin templates [58]. Furthermore, the co-activators CBP/p300 and SRC have to be recruited to the ERα in order to maximally enhance transcription on ERα-susceptible chromatin templates. The p300/CBP–SRC complex, when interacting with the AF-1 of the ERα, is primarily involved in the stable formation of the pre-initiation complex of transcription [59].

1.5 Ligand-Independent Activation of Steroid Hormone Receptors and Non-genomic Effects of Steroids

Estradiol is capable of eliciting fast biological effects in several tissues (e.g. in bone, breast, brain and vasculature). Estradiol for example induces vasodilatation within minutes of application. This observation led to the hypothesis that estrogens may induce "non-genomic" effects, i.e. effects that are independent of de novo RNA transcription and protein biosynthesis. In order to distinguish the classic action of estradiol from the non-genomic action, it has to be shown that the steroidal effect is rapid, i.e. it occurs within seconds or minutes, and cannot be accounted for by the induction of gene expression or protein biosynthesis mediated by the steroid hormone receptors. It has been proposed that the non-genomic effects are mediated via steroid hormone binding sites in the cell membrane, which are linked with intracellular signal transduction pathways [60]. It has been recognised that different pools

of ERα protein exist in the cell: a fraction of the ERα is localised to the endoplasmic reticulum and the plasma membrane [61]. One model involves events that are initiated by the ERα, which includes activation of G proteins, caveolins and receptor tyrosine kinases.

Experimental evidence indicates that translocation of the ERα to the membrane in the absence of estrogen is dependent on the caveolin-1 protein. Dependent on the cellular context, the membrane-localised ERα seems to be capable of activating c-Src [62]. In breast cancer cells it has been demonstrated that this can activate kinases, in particular the EGFR tyrosine kinase, imparting cellular growth and survival signals [63]. This demonstrates that membrane-associated ERα might utilise a classic growth factor signalling cascade in breast cancer cells [63]. The anti-estrogen ICI 182,780 inhibits translocation of the ERα to the membrane and the association of the signal transduction complex. Additional non-genomic effects for classic steroid hormone receptors have been reported [64] (Fig. 5). Moreover, the interaction of ligand-bound PR with c-Src activates Ras, Raf and the MAPK-cascade.

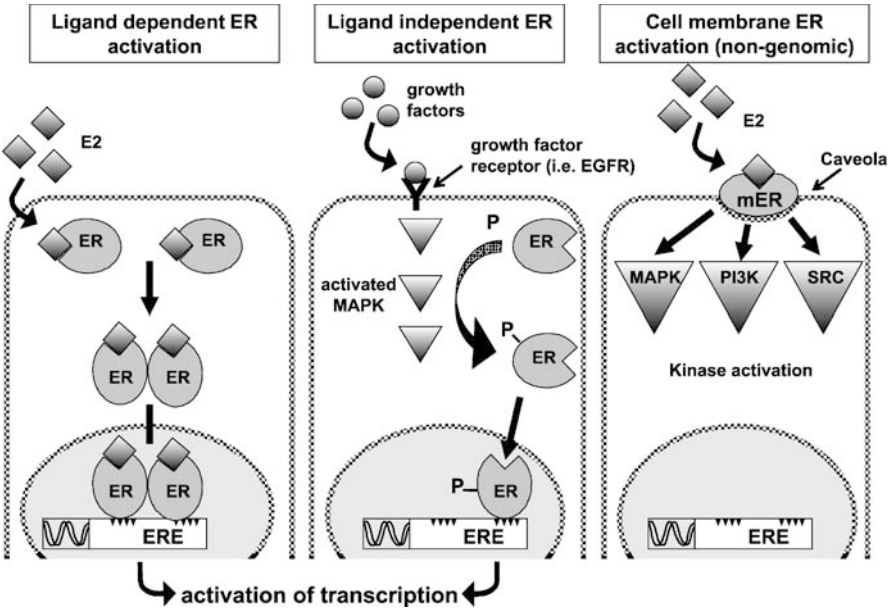


Fig. 5 Ligand-dependent versus ligand-independent ER activation. The estrogen receptor can be activated by estrogen (*left-hand panel*) or independently of estrogen, for example by growth factors that increase the activity of protein kinases that phosphorylate different sites on the ER. In this model (*centre panel*), the unbound but activated receptor will then exert transcriptional effects. In the case of the non-genomic estrogen-signalling pathway (*right-hand panel*), cell-membrane estrogen receptors are located in cell-membrane invaginations called caveolae. Their activity is linked to the mitogen-activated protein kinase pathway, resulting in a rapid, non-genomic effect

Recently, a membrane steroid hormone receptor for estradiol has been identified. The GPR30 is a G protein-coupled receptor. G protein-coupled receptors (GPCRs) are 7-transmembrane spanning proteins that interact with heterotrimeric G proteins. Upon ligand binding and exchange of GDP for GTP, the Gα-GTP dissociates from the G$\beta\gamma$ subunits. Sometimes the G$\beta\gamma$ subunits also activate different signal transduction cascades. GPR30 is activated by estradiol, tamoxifen and ICI 182,780 in the absence of ERα or ERβ protein [65–67]. With its long N-terminus, it has sequence homology to the GPCRs angiotensin II1A, interleukin-8A, and chemokine type 1 receptor. Therefore, it has been proposed that the endogenous ligand of GPR30 might be a peptide or a chemokine. However, attempts to deorphanise, i.e. to identify the potential endogenous peptidergic ligand of the GPR30 have not been successful [68].

The GPR30 is expressed ubiquitously and expression in placenta, breast, ovaries, prostate, neural tissue, heart, endothelial, hepatic and lymphoid tissue has been shown [69]. GPR30 is co-expressed with the ERα in breast cancer tissue and breast cancer cell lines [70]. An exception is the human breast cancer cell line SK-BR-3, which is negative for the classic steroid hormone receptors ERα and ERβ but which does express the GPR30 endogenously. E2, but also the ERα antagonists 4-OH-tamoxifen and ICI 182,780 bind to cell membrane preparations of these cells with high affinity [67]. HEK-293 cells, which neither express the ERα, ERβ nor the GPR30, were transfected with a cDNA coding for GPR30. In membrane preparations of HEK-293 cells, GPR30-bound radio-labelled E2 was displaced by E2, 4-OH-tamoxifen, and ICI 182,780 [67]. In contrast, knock-down of endogenous GPR30 in SK-BR-3 cells with specific small interfering RNAs (siRNAs) directed against the GPR30, decreased the binding of E2. Binding of E2 to GPR30 activates the stimulatory G protein Gαs and, via activation of adenylate cyclase, cAMP levels are increased [67].

There is currently some debate about the subcellular localisation of GPR30. While Revankar and colleagues [66] were able to show that GPR30 is predominantly localised to the endoplasmic reticulum in COS-7 cells over-expressing GPR30 using a fluorescent marker coupled to E2, Thomas and colleagues [67] in contrast proposed in their supplementary material that GPR30 is localised to the plasma membrane but only a diffuse staining is visible [67]. A localisation of GPCRs to the endoplasmic reticulum is surprising and it disagrees with previous results by others that have shown that non-genomic, fast signalling is initiated at the plasma membrane. Currently, it is not clear whether or not the GPR30 is subject to disturbed post-translational processing and trafficking to the plasma membrane in COS-7 cells.

Interestingly, both the SERM 4-OH-tamoxifen and the "pure" anti-estrogen ICI 182,780 are antagonists of the genomic response of the nuclear ERα. However, they are agonists of the non-genomic, fast response at the GPR30 [66, 67]. So far, there is no proof that GPR30 plays a role in breast cancer. Nevertheless, it is tempting to speculate that resistance to anti-estrogens

in breast cancer might be brought about by an activation of GPR30-dependent pathways by tamoxifen or ICI 182,780, which stimulate proliferation or inhibit apoptosis.

Furthermore, post-translational modifications activate steroid hormone receptors in a ligand-independent fashion (Fig. 5), as shown for the ERα which is phosphorylated on serine residue 118 in the AF-1 domain by the Erk1/2 kinase [71]. In vitro, the serine-118 phosphorylated ERα is transcriptionally active in a ligand-independent fashion.

2
Chemistry and Pharmacology of Endocrine Therapy

2.1
Hormone Deprivation – Inhibition of Steroid Hormone Biosynthesis

2.1.1
Aromatase Inhibitors

2.1.1.1
Rationale for the Use of Aromatase Inhibitors in Cancer Treatment

One approach to interfere with ER signalling is to reduce the circulating level of its ligand estradiol by inhibiting the enzyme aromatase. Aromatisation is the last step in the synthesis of estradiol. This reaction is catalysed by the P450 aromatase mono-oxygenase complex that is present in the smooth endoplasmic

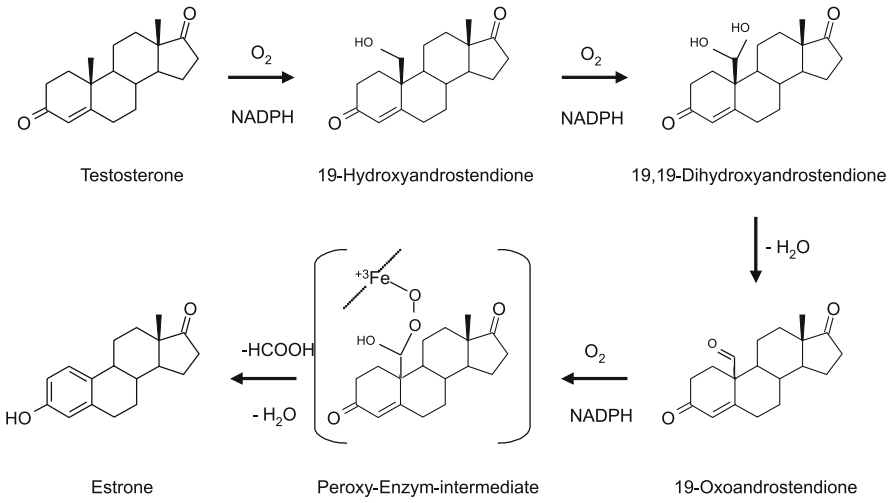


Fig. 6 Enzymatic reactions leading to aromatisation, according to Brueggemeier [73]

reticulum of placenta and granulosa cells of ovarian follicles. In three consecutive hydroxylating reactions, estrone and estradiol are synthesised from their precursors androstenedione and testosterone, respectively (Fig. 6). The final hydroxylating step in aromatisation does not require enzymatic action and is not product-sensitive. Aromatase is also present in peripheral tissues, including adipose tissue, liver, muscle, brain and breast cancer tissue. In the peri-menopausal period, the ovaries, as a result of the complete loss of primordial follicles, stop producing estrogens. This leads to a steady decline in ovarian estradiol production although serum estradiol concentrations can vary considerably. In post-menopausal women, approximate plasma estradiol levels are 20 pmol/L, and most of the estradiol is formed by peripheral, extra-gonadal conversion of testosterone. As peripheral aromatase activity increases with age, peripheral estrogen production approximately doubles. Estrone is the predominant estrogen in these women [72]. There is substantial information that breast cancer tissue contains all the enzymes responsible for the local biosynthesis of estradiol from circulating precursors. Aromatase inhibitors may therefore also inhibit enhanced in situ estrogen synthesis in both breast cancer tissue and non-malignant adjacent tissue, i.e. adipose tissues [73, 74].

2.1.1.2 Chemistry of Aromatase Inhibitors

Development of aromatase inhibitors (AIs) began as early as the 1970s and has expanded greatly in the past three decades. Consequently, numerous comprehensive reviews on aromatase inhibitors have been published, see for example [73].

Competitive Aromatase Inhibitors

Competitive AIs are chemical compounds that compete with the substrate androstenedione for non-covalent binding to the active site of the enzyme to decrease the amount of product formed. Initially, research and development of AIs started with the synthesis and biochemical characterisation of competitive inhibitors.

As a common chemical feature, non-steroidal AIs contain a hetero-atom, which binds to the heme iron of cytochrome P450 and is thus involved in the hydroxylation reaction by which estrone and estradiol are synthesised from their obligatory precursors androstenedione and testosterone. Aminoglutethimide was the first AI to be studied in breast cancer patients and is therefore referred to as a first-generation AI. Due to the unspecific inhibition of other heme iron-containing enzymes, aminogluthetimide had severe side effects. Therefore, research and development was directed towards more specific second-generation (imidazole type: fadrozole) and third-generation (triazole type: vorozole, anastrozole, letrozole) AIs (Fig. 7). The competitive inhibitor fadrozole is more potent and selective than aminoglutethimide, but

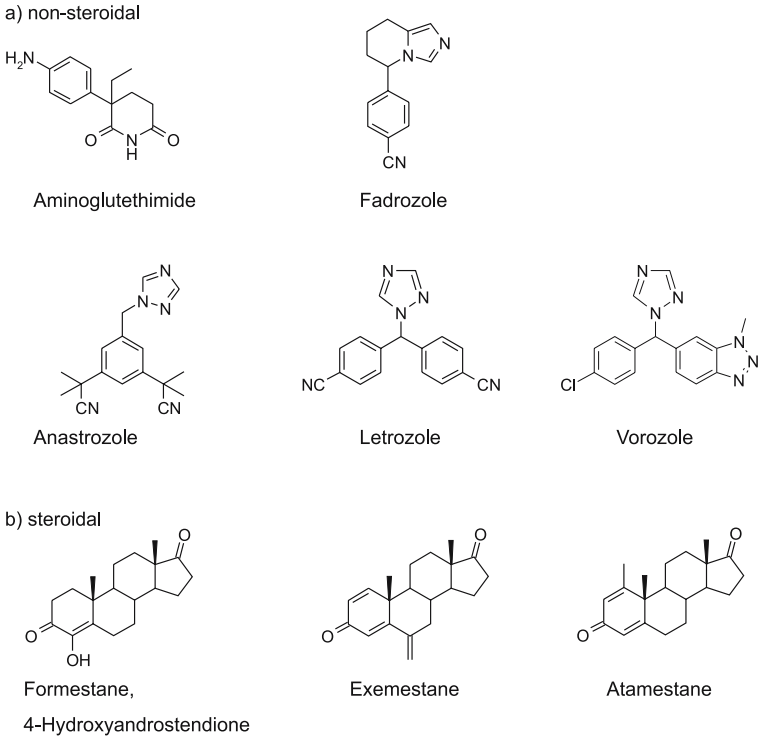


Fig. 7 Chemical structures of aromatase inhibitors

still shows residual non-selective inhibition. A higher degree of specificity has been obtained with the third-generation AIs, which are derivatives of triazoles. These new agents are 100–3000 times more potent than aminoglutethimide, and all inhibit whole-body aromatisation by more than 96%. The first triazole analogue was vorozole, which potently inhibits the aromatase with an apparent K_i of 1.3 nM in human placental microsomes [75]. Anastrozole inhibits the aromatase with an IC_{50} of 15 nM in human placental microsomes and is selective over several other cytochrome P450 enzymes [76]. The triazole derivative letrozole is a potent inhibitor of aromatase with an IC_{50} of 11.5 nM in human placental microsomes. It exerts no effect on the biosynthesis of other steroid hormones such as aldosterone, progesterone or corticosterone [77].

Steroidal Aromatase Inhibitors

Steroidal AIs have been synthesised using the structure of androstenedione as starting point for the chemical optimisation programme (Fig. 6). These inhibitors bind to the aromatase in the same manner as the substrate androstenedione. The structure–activity relationship (SAR) of the androstenedione derivatives has extensively been investigated. Although several of these

compounds exhibited apparent K_i values in the low nanomolar range and were competitive inhibitors, none of these compounds has been further developed [73].

A different type of steroidal inhibitor is termed an irreversible, suicide, or mechanism-based aromatase inhibitor and examples are 4-hydroxyandrostenedione, exemestane, and atamestane (Fig. 7). Irreversible AIs are initially recognised by the aromatase as alternative substrates and are then transformed via an NADPH-dependent mechanism into reactive intermediates, which inactivate the aromatase enzyme. Irreversible inhibitors can have distinct advantages because these inhibitors are highly enzyme-specific, they produce prolonged inhibition, and often they exhibit a low toxicity. The most effective irreversible inhibitors exhibit a short half-time of inactivation ($t_{1/2}$) and a rapid inactivation rate. Atamestane is a competitive and irreversible inhibitor of estrogen biosynthesis [78]. Exemestane (FCE 24304; 6-methylenandrosta-1,4-diene-3,17-dione) was the second irreversible AI to be described. Exemestane has similar properties to 4-hydroxyandrostendione, with a similar affinity (K_i of 26 nM versus 29 nM) and a slow inactivation rate ($t_{1/2}$ of 13.9 versus 2.1 min). Exemestane does not interfere with 5α-reductase. Besides weak binding to the AR, the compound did not bind to other steroid hormone receptors. Due to the high selectivity and potency of the available third-generation AIs, the chance to identify compounds with improved characteristics appears rather low. This scepticism is reflected in the very limited number of new drug finding programmes reported in the last few years that have focussed on the aromatase as target.

Flavonoids

Several flavonoids inhibit the aromatase, thus decreasing estrogen biosynthesis and circulating estrogen levels [79]. Flavonoids encompass flavones, isoflavones, flavanones and flavonols, with a benzopyranone ring system as the common chemical scaffold. Approaches to develop synthetic flavonoids, chromone or xanthone analogues with enhanced aromatase inhibitory activity have identified several selective and potent compounds [73]. Generally, flavones and flavanones (chrysin has an IC_{50} value of 0.5 μM) are more potent inhibitors of the aromatase than isoflavones (biochanin A with an IC_{50} value of 113 μM). By introducing functional groups onto the isoflavone core, the potency was enhanced approximately 160-fold. A synthetic pyridyl isoflavone analogue exhibited an IC_{50} value of approximately 210 nM and an apparent K_i value of 220 nM. Considering the rather weak potency of flavonoid-based AIs in comparison to the triazole derivatives, this is very likely the reason why there has been little effort to develop AIs on a flavonoid scaffold.

Recently, dual aromatase-sulfatase inhibiton (DASI) was introduced as a new targeting approach [80]. It is based on the hypothesis that additional inhibition of the steroid sulfatase (STS) should reduce estrone levels signifi-

cantly [81] and should thus provide a novel approach for the treatment of breast cancer. A series of DASIs based on the AI YM511 was developed [80]. By introducing the pharmacophore for STS inhibition (i.e. a phenol sulfamate ester) into the AI letrozole, a new structural class of dual inhibitors was generated. Although these dual inhibitors exhibited a lower activity in vitro, with IC_{50} values of 30 μM for aromatase and > 10 μM for STS in human choriocarcinoma JEG-3 cells, some in vivo activity on STS was observed [80]. Clearly, there is still plenty of room for improvement and chemical optimisation.

2.1.1.3 Pharmacology of Aromatase Inhibitors

Due to the limited specificity of the first- and second-generation AIs, this class of compounds has been neglected therapeutically for a long time. However, recent results from the clinical development of the selective and potent third-generation AIs have provided accumulating evidence that AIs are an alternative endocrine therapy for treating patients with advanced breast cancer. Several randomised clinical trials demonstrated that AIs are superior to tamoxifen in the neo-adjuvant and first-line treatment of advanced breast cancer [73]. Among these, three relevant clinical studies showed that the third-generation AIs, anastrozole, letrozole and exemestane, are superior to tamoxifen in patients with advanced disease. These results led to a series of clinical trials comparing AIs with tamoxifen in the adjuvant setting [82].

Primary objectives of the "Arimidex, Tamoxifen, Alone or in Combination" (ATAC) trial, were to discover whether anastrozole is at least as effective as tamoxifen in post-menopausal women with localised breast cancer, and/or offers benefits in safety or tolerability over tamoxifen in this group of patients. In total, 9366 patients were recruited and randomised to either 5 years of the AI anastrozole alone, tamoxifen alone or a combination of both. Three efficacy analyses have been completed with median follow-ups of 33 months, 47 months and 68 months. The completed treatment analysis at a median follow-up of 68 months showed that disease-free survival (DFS) and time to recurrence remained consistently in favour of anastrozole compared with tamoxifen. The benefit of anastrozole over tamoxifen was most striking in women with steroid hormone receptor-positive tumours and the absolute difference in benefit continued to increase over time for anastrozole versus tamoxifen. Overall survival (OS), however, was similar in both treatment groups [83]. Unexpectedly, the combination treatment arm had to be closed because of low efficacy. Patients that were treated with the third-generation AIs had significantly lower incidences of hot flushes, vaginal bleeding, vaginal discharge, endometrial cancer, ischaemic cerebrovascular events, venous thromboembolic events and deep venous thromboembolic events compared with those treated with tamoxifen. However, anastrozole induced a signifi-

cantly higher number of incidences of arthralgia and fractures than the bone-sparing tamoxifen.

Exemestane is currently under investigation in several clinical studies: in the TEAM study, which originally was designed to compare exemestane with tamoxifen for 5 years, and in the study EXEM027, comparing exemestane to placebo for 2 years in low-risk ER positive patients [84]. Due to the results of the Intergroup Exemestane Study (IES) the protocol of the TEAM study was amended such that sequential tamoxifen followed by exemestane was compared against exemestane alone. In the IES, 4742 patients were enrolled to investigate whether exemestane, when given to post-menopausal women after receiving adjuvant tamoxifen therapy for 2–3 years for primary breast cancer, could prolong disease-free survival as compared with continued tamoxifen therapy. At the second interim analysis, it was shown that switching to exemestane significantly improved DFS compared with continued tamoxifen. There was no significant difference in OS. The results of the updated analysis, at a median follow-up of 37.4 months, demonstrated that the benefit of switching to exemestane compared with tamoxifen was maintained. The updated safety data confirmed that patients switching to exemestane experienced fewer gynaecological symptoms, vaginal bleeding, muscle cramps, myocardial infarction and thromboembolic events compared with continued tamoxifen. Switching to exemestane continued to be associated with a significantly higher incidence of diarrhoea, arthralgia and adverse musculoskeletal events compared with continued tamoxifen. In summary, these results show that switching to exemestane after 2–3 years of tamoxifen therapy is associated with a reduced risk of breast cancer recurrence. In general, these data strengthen the evidence for the superiority of third-generation AIs over tamoxifen as adjuvant treatment [84].

A large ongoing randomised trial investigating primary adjuvant endocrine treatment with either letrozole or tamoxifen, demonstrated a 19% improvement in DFS and a significantly reduced risk of distant recurrences for patients treated with letrozole [85]. As there appears to be no additional benefit from continuing tamoxifen therapy beyond 5 years, there is a risk of breast cancer recurrence beyond 5 years. Therefore, a trial was initiated to investigate the benefits of continued adjuvant treatment with an AI beyond 5 years with tamoxifen. The MA 17 trial demonstrated that 5 years of adjuvant letrozole therapy after 5 years of adjuvant tamoxifen therapy in postmenopausal women with early stage breast cancer significantly improved DFS compared with placebo in the total population but there was no significant difference in OS [86].

Adverse events observed during letrozole treatment were similar to those seen during anastrazole treatment but with a significantly higher incidence of hot flushes, arthritis, arthralgia and myalgia, and a significantly lower incidence of vaginal bleeding compared with placebo. In addition, newly diagnosed osteoporosis was more frequently seen in the letrozole group com-

pared with placebo. These results, published in the last few years [87], led to the recommendation that switching to an AI might offer advantages over continued tamoxifen treatment for women who have already received adjuvant tamoxifen therapy.

The superior results of AIs compared to tamoxifen in the adjuvant and first-line advanced settings have started to change the endocrine treatment sequence for breast cancer patients. As switching to an AI allows patients to receive a drug that might prove more effective, it might avoid the development of tamoxifen resistance and it might also offer a better tolerability. The use of an AI should now be considered as the preferred initial adjuvant endocrine treatment for post-menopausal women with hormone receptor-positive localised breast cancer [84]. These changes, however, require new options for the second-line treatment of advanced disease.

There are now two main groups of patients to consider when sequencing endocrine treatment in breast cancer: those who progressed on or after adjuvant or first-line advanced tamoxifen treatment, and those who progressed on or after adjuvant or first-line advanced AI treatment. There is a need for treatments that are effective and that are not cross-resistent with tamoxifen or AIs.

Atamestane, a steroidal AI, which was developed by Schering for treatment of benign prostate hyperplasia (BPH), is now in phase III clinical trials, being evaluated in combination therapy with toremifen against letrozole. Results reported at the ASCO meeting 2006 in Atlanta, revealed a identical time to progression (11.2 months) in the two arms [88]. The pharmacology of atamestane was investigated in mice, rats, rabbits, dogs, monkeys and humans. In all species tested, atamestane has no other intrinsic hormonal or anti-hormonal activities, and does not inhibit other cytochrome P450-dependent enzymes involved in the adrenal steroidogenesis besides aromatase [78, 89]. However, it does inhibit the estrogen-induced negative feedback loop.

Currently, anastrozole and letrozole are efficacious in early-stage, locally advanced, and metastatic disease and thus they present with the most complete data set for the different stages of breast cancer. Although it seems rather unlikely that one will be able to detect differences with respect to clinical effects at the tumour level, the indirect comparison of different AIs suggests a stronger evidence for the use of exemestane compared with other AIs for breast cancer therapy [90].

Importantly, extended biological knowledge and large-scale profiling of gene expression of breast cancer specimens [91] will most probably help to define which patients might benefit most from treatment with an AI. This technology also offers the possibility to explore mechanisms of therapy resistance, including estrogen hypersensitisation or the switch from hormone to growth factor pathways. Based on the hypothesis that estrogen deprivation induced by AIs could sensitise tumours to the treatment with estrogens at pharmacological doses, those tumours that developed resistance to AIs have

been treated with estrogens at high doses. During this regimen objective responses were observed in ten out of 29 patients, and some responses lasted for more than 2 years [92].

Another observation focusses on cyclooxygenase 2 (COX2) expression in breast cancer and the regulation of aromatase expression [93]. Although, thus far, no study reported intra-tumoural estrogen levels in patients treated with a COX2 inhibitor, the potential influence of COX2-derived signals on estrogen synthesis [94] provided the background for implementing celecoxib in adjuvant and breast cancer prevention trials in concert with AIs. Whether combining a COX2 inhibitor with an aromatase inhibitor may improve therapeutic outcome is still awaiting data.

2.1.2 17β-Hydroxysteroid-dehydrogenase Inhibitors

2.1.2.1 Rationale for the Use of 17β-Hydroxysteroid-dehydrogenase Inhibitors in Cancer Treatment

Besides AIs, inhibitors of other steroidogenic enzymes have the potential to reduce circulating or tissue levels of active estrogens by blocking their biosynthetic pathway. The 17β-hydroxysteroid dehydrogenases (17β-HSDs) play an important role in the modification of steroid hormones such as estrogen and androgen (Fig. 8). In the last decade, several isoforms of 17β-HSD have been discovered [95]. The enzymatic activities of the different members of the 17β-HSD family are ubiquitous in human tissues. The type 1 or human placenta estradiol dehydrogenase (17β-HSD1) catalyses the final step

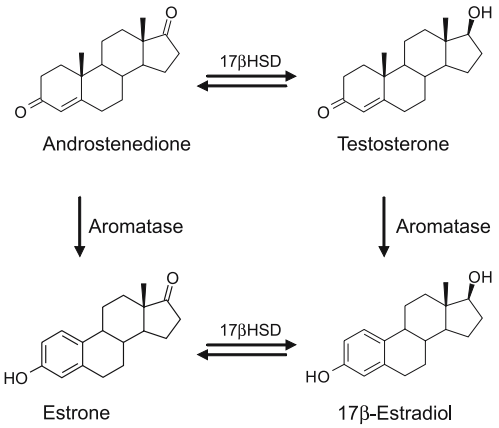


Fig. 8 Enzymatic mechanism involved in the formation of estrogens: role of the 17β-HSD pathway

in the biosynthesis of 17β-estradiol (E2) via the reduction of estrone (E1) using NADPH or NADH as co-factor [96]. 17β-HSD1 is expressed in many steroidogenic tissues, including breast tissue, and has been found to be active in breast cancer cells and could therefore be a target for breast cancer therapy. The potential therapeutic effects of inhibiting this enzyme have been the rationale of the search for selective inhibitors. Although several inhibitors have been reported in the literature [96], none of them is available in the clinic because an in vivo efficacy of selective 17β-HSD inhibitors has not been demonstrated [96].

2.1.2.2 Chemistry of 17β-HSD Inhibitors

The synthesis of inhibitors from several structural classes has been reported. A review article by Poirier [96] focussing on 17β-HSD inhibitors summarised all known activities on 17β-HSD inhibitors. In addition to giving an up-to-date description of inhibitors of 17β-HSD isoforms 1–8, this review provides information on the isoform selectivity and residual estrogenic or androgenic activity. Both, derivatives of steroidal and non-steroidal structure, have been described as 17β-HSD inhibitors. Among them are substituted agonists like estrogens, progestins and phytoestrogens, which either irreversibly or reversibly inhibit the 17β-HSD1 (e.g. bromoacetoxy or alkylamide derivatives) [97].

Several potent anti-estrogens also have a potential to be used as 17β-HSD inhibitors because some of them have a dual site of inhibitory action: they block both the estrogen receptor (anti-estrogen effect) and estrogen formation (inhibitory effect on 17β-HSD). Despite the complexity of a dually active agent, such inhibitors have interesting properties suitable for their potential use in the treatment of estrogen-sensitive diseases, but their potency and selectivity for 17β-HSD1 have to be improved [98].

As more insight into the three-dimensional structure of 17β-HSD has been gained in recent years [99], information on the three-dimensional structure of the catalytic site can now be derived. Based on these data, novel inhibitors have been discovered. Amides containing an aromatic pyridyl moiety have been found to give the best inhibition, indicating that the pyridyl group interacts with the active site. The chemical optimisation and pharmacological evaluation of these novel inhibitors is currently ongoing [99].

2.1.2.3 Pharmacology of 17β HSD Inhibitors

Historically, the 17β-HSDs have been classified as “reversible” enzymes, i.e. being able to catalyse both reductive and oxidative conversions. However, Luu-The and colleagues [95, 100] observed that, although the activity of 17β-

HSDs in homogenised cells is reversible, their activity in intact cells is mainly uni-directional. Based on these findings, each member of the 17β-HSDs family has now been classified as either reductive or oxidative. The effects of 17β-HSD inhibition on tumor cell proliferation has been evaluated only in the human breast cancer cell lines MCF-7 and T47D. Compounds from the class of pure steroidal anti-estrogens (ICI 164,384) inhibit the conversion from E1 to E2 by 53%. However, the high IC_{50} values which range between 0.5–5 μM preclude the use of these compounds in vivo [101]. Clinical and pre-clinical observations demonstrated that progestins can interfere with 17β-HSD expression and activity. Inhibiton of both, oxidative and reductive activities have been reported [102]. Whether 17β-HSD inhibition alone is sufficient to suppress the growth of breast cancer or whether a combination with an aromatase inhibitor might provide additional clinical benefit still remain open questions.

2.1.3
Steroid Sulfatase Inhibitors

2.1.3.1
Rationale for the Use of Steroid Sulfatase Inhibitors in Cancer Treatment

The biologically inactive estrone sulfate (E1S) and dehydro-epiandrosterone-sulfate (DHEAS) are the most abundant circulating estrogenic precursors in the plasma of post-menopausal women [103]. Desulfation of inactive steroid-3-*O*-sulfates by estrone-sulfatase (STS) plays a key role in the regulation of levels of receptor-active estrogenic steroids (estradiol and androstenediol) in breast cancer cells (Fig. 9). There is strong evidence suggesting that estrone sulfatase (STS) and DHEA-sulfatase are the same enzyme [103].

Although the affinity of androstenediol for the ER is much lower than that of estradiol, the plasma concentration of androstenediol is 100-fold higher than that of estradiol and it is presumed that the amount of circulating androstenediol is sufficient to stimulate hormone-dependent breast cancer cells. In addition, the activity of the STS and the tissue concentration of estrone sulfate were found to be higher in tumour than in normal breast tissue [104]. This led to the hypothesis that the sulfatase pathway, where the STS converts E1S into E1, provides most of the E1 for the last step of E2 formation. Circulating estrone sulfate is seen as a reservoir for the local formation of free, biologically active estrogens by an intracrine mechanism, particularly after the menopause. STS is located in the endoplasmic reticulum and accepts a range of substrates. The structure of the STS has been determined at 2.6 Å resolution by X-ray crystallography and is used for molecular modelling of inhibitors [105]. The presence of two different isozymes was shown by Zhu et al. [106]. In summary, STSes have been implicated in the growth of hormone-dependent breast cancer, and they might be an important target for

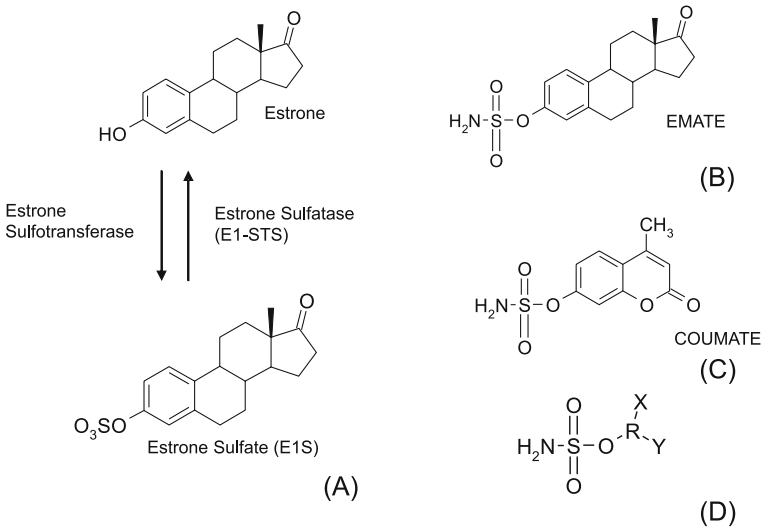


Fig. 9 Enzymatic mechanism involved in the formation of estrogens. *A* The sulfatase pathway. *B,C* Structure of the potent STS inhibitors EMATE and COUMATE. *D* New pharmacophore for the inhibition of estrone sulfatase: *R* general carbon backbone (aromatic or aliphatic), *X* electron withdrawing groups (e.g. nitro), *Y* additional functionality including fused or adjacent/remote ring structures so as to meet the log *P* requirement

endocrine therapy. There is now considerable interest in discovering how to control and block this enzyme.

2.1.3.2 Chemistry of Steroid Sulfatase Inhibitors

Research on STS inhibition is still at its early stage, but several steroidal and non-steroidal compounds are under investigation. From the large series of steroidal compounds estrone-3-*O*-sulfamate (EMATE) (Fig. 9) was found to be the most potent inhibitor of STS. EMATE is an irreversible STS inhibitor with an IC_{50} for STS inhibition of 65 pM (measured in MCF-7 cells with estrone sulfate as the substrate at a concentration of 2 nM) [107]. All further structural modifications, either on the sulfamate group, on alternative groups, or by varying the steroidal backbone (androgens, progestins, cholestane) resulted in compounds that potently inhibit the STS; however, in general they were less potent than EMATE [108]. The major drawback of most of the steroidal STS inhibitors is their intrinsic estrogenicity. Therefore, non-steroidal compounds devoid of estrogenic activity and based on different backbones (phenols, indoles, flavones, stilbenes, coumarin, tetrahydronaphthol, tyramines and even ethyl alcohol) were synthesised [109]. One prominent example for a non-steroidal inhibitor is the

coumarine 4-methylcoumarin-7-*O*-sulfamate derivative, COUMATE, with an IC_{50} of 380 nM. SAR analyses led to a simplified pharmacophore (Fig. 9) with the sulfamate group attached to a carbon backbone as the most important group within potential non-steroidal inhibitors of STS [110]. Potent sulfamoyloxy-substituted stilbenes with anti-estrogenic activity were synthesised and evaluated as inhibitors of STS by Walter et al. [111]. These compounds inhibited the STS with IC_{50} values in the submicromolar range. The data disclosed in the recent publications have confirmed STS as an attractive target for a range of potential indications, primarily for the therapy of estrogen-dependent breast cancer. Now, the new STS inhibitors await validation of their therapeutic potential in clinical trials.

2.1.3.3 Pharmacology of Steroid Sulfatase Inhibitors

The available information on the pharmacology of STS inhibitors is restricted to a few in vitro and in vivo models. From all steroidal compounds, the irreversible inhibitor EMATE was found to be the most potent STS inhibitor with an IC_{50} of 65 pM [107]. However, it has been proposed that the sulfamate moiety of EMATE irreversibly binds to the active site and releases the steroidal backbone estrone. This means that EMATE, although a potent inhibitor of STS, counteracts its own effect by releasing estrone. The most potent derivative from a series of sulfamoyloxy-substituted stilbenes inhibited the growth of MCF-7 breast cancer cells with an IC_{50} value of 13 nM [111].

Recently Shields-Botella et al. [112] reported novel, orally active STS inhibitors. Several compounds were synthesised and explored for the treatment of estrogen-dependent breast cancer. The compounds TX 1299, TX 1492 and TX 1506 proved to be inhibitors of STS in the choriocarcinoma cell line JEG-3 at an IC_{50} of 5–70 nM and in the breast cancer cell line MCF-7 at an IC_{50} of 0.07–0.7 nM. They were shown to be negative in estrogenicity assay in human endometrial adenocarcinoma Ishikawa cells. In vivo potency of TX 1299, was evaluated in comparison with COUMATE. TX 1299 showed anti-uterotrophic activity in adult ovariectomised rats supplemented with estrone sulfate (E1S) without residual estrogenic activity. In addition, the measurement of uterine sulfatase levels confirmed the complete inhibition of the enzyme STS within the target organ [112]. These preliminary studies indicate that non-steroidal compounds are potent and rather selective in vitro, efficacious in vivo, and results from further studies are awaited eagerly. However, it turns out that most of the non-steroidal compounds may hit additional targets, i.e. the estrogen receptor, or the aromatase, or even inhibit tubulin polymerisation [81]. As it is difficult or nearly impossible to attribute the general anti-proliferative effects of such inhibitors to one mechanism, the therapeutic relevance of STS inhibition still has to be shown. Compounds which are able to inhibit two enzymes could, however, have the potential to totally block

the synthesis of estrogens. As a consequence, a number of research groups have directed their research into compounds that are dual inhibitors of aromatase and STS [113]. The compound YM 511, as an example of a dual aromatase and STS inhibitor (DASI), was already mentioned in the section on AIs [80].

2.1.4
5α-Reductase Inhibitors

2.1.4.1
Rationale for the Use of 5α-Reductase Inhibitors in Cancer Treatment

Steroid 5α-reductase is a membrane bound, NADPH-dependent enzyme that is responsible for the selective, irreversible conversion (reduction) of 4-ene-3-oxosteroids into the corresponding 5α-3-oxosteroids (Fig. 10). Two genes code for 5α-reductase activity, the 5α-reductase type 1 and type 2 (5αR-1 and 5αR-2), and they are only 50% homologous on the protein level [114]. 5αR-1 is mainly expressed in the sebaceous glands of the skin and in the liver, whereas 5αR-2 is expressed in androgen-sensitive tissues, i.e. prostate, epididymis and other reproductive tissues [115]. The 5α-reductases are important regulators of endocrine action in androgen-sensitive cells. The 5αR-2 isoenzyme has a high affinity for the most important substrate testosterone (K_m 4–50 nM) while the affinity of the 5αR-1 for testosterone is considerably lower (K_m 1–5 μM). The physiological roles of testosterone and dihydrotestosterone (DHT) are quite different. In males, testosterone determines the modification of external genitalia, increases the muscle mass, deepens the voice, and affects spermatogenesis, sexual potency and male sexual behaviour. DHT is responsible for the increase of body hair and facial hair and the enlargement of the prostate. The abnormal production of DHT has been associated with diseases of the prostate and the skin, and high interest has been paid to the synthesis of 5α-reductase inhibitors for the treatment of DHT-related pathologies. Further evidence for a role of the 5αR-2 in the pathogenesis of DHT-related disorders comes from the clinical phenotype of 5αR-2 deficiency. In individuals with a total deficiency of 5αR-2, the prostate remains undeveloped, facial and body hair growth patterns are more feminine in character, and the temporal regression of the hair line is significantly reduced, indicating that 5αR-2 is involved in prostatic diseases and to some extent in androgenic alopecia [116]. For this reason, the development of 5αR-2-specific inhibitors for the treatment of DHT-dependent pathologies has been a major focus of pharmaceutical research.

Whether the selective inhibition of 5αR-2 may provide an advantage over inhibition of both 5αR-2 and 5αR-1, will have to be shown by comparing finasteride (rather type 2-selective) and dutasteride (a type 1 and type 2 inhibitor) (Fig. 10). At present, no selective 5αR-1 inhibitor is available for the

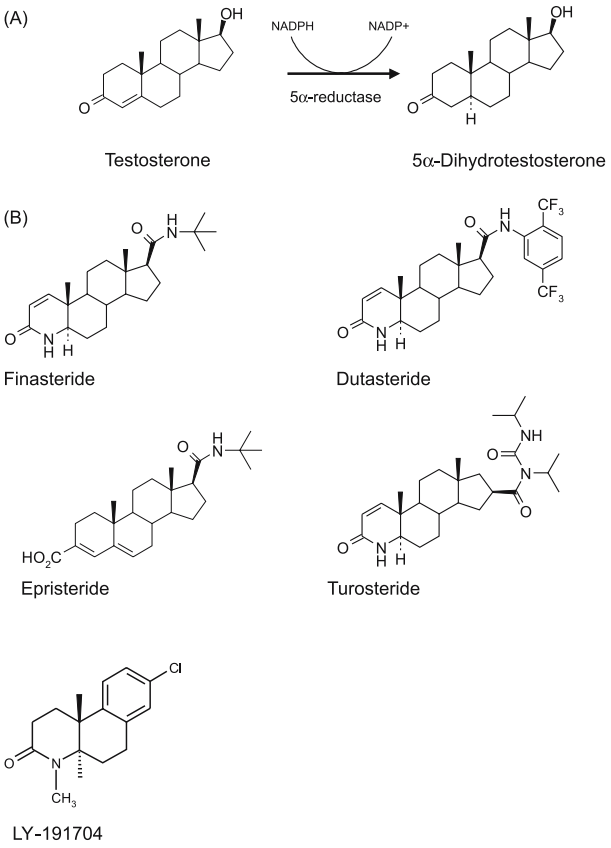


Fig. 10 *A* Enzymatic mechanism of 5α-reductase and *B* structures of 5α-reductase inhibitors

treatment of 5αR-1-related pathologies but pharmaceutical research is active in this field [117].

2.1.4.2 Chemistry of 5α-Reductase Inhibitors

The first 5αR-2 inhibitors were synthesised by modifying the structure of the natural substrate testosterone. Substitution of one carbon atom at the A- or B-ring by an hetero-atom, led to the discovery of potent inhibitors of 5αR-2 such as the 4-azasteroids, 6-azasteroids, 10-azasteroids, as well as steroidal carboxylic acid inhibitors [118]. Among all these compounds, only the 4-azasteroides finasteride and dutasteride are approved for the treatment of benigne prostate hyperplasia (BPH). Dutasteride (GI198745) emerged from a family of 6-azasteroids through modification of the steroidal struc-

ture [119]. Epristeride, a steroid with an carboxylic acid in the A-ring, is a potent non-competitive steroidal inhibitor of 5αR-2 with weaker type 1 activity [120].

Following a paradigm shift in the field, the recognition of "potential" undesired endocrine actions of steroidal compounds directed activity towards research on non-steroidal inhibitors. As three-dimensional structures for the two 5α-reductase enzymes are not available, SAR-driven compound design was not possible. Nevertheless, in the last few years, some groups in academia and in the pharmaceutical industry have pursued research on non-steroidal compounds that inhibit human 5α-reductase type 1 and type 2 [121]. Several classes of non-steroidal inhibitors have been reported so far. They were designed by removing one or more rings from the (aza)steroidal structures. The most potent inhibitor of the benzoquinolinones series is LY191704 [122], with an IC_{50} of 8 nM. Among compounds with benzoquinolizinone structure, some potent dual inhibitors with IC_{50} values ranging between 93 and 166 nM for both isozymes, were identified [123]. Benzoquinolizinone-based inhibitors are very potent type 1-selective inhibitors with an IC_{50} of 7.6 nM [124].

Novel substituted benzoylbenzoic acids and phenylacetic acids inhibitors have been synthesised that exhibit IC_{50} values in the nanomolar range. The compounds turned out to be potent and selective human 5αR-2 inhibitors. The phenylacetic acid derivatives, equipotent to finasteride and more potent than the analogous benzoic acids (IC_{50} values: 5 versus 23 nM) were the strongest inhibitors in this class [125]. Analogues of ONO3805 are selective inhibitors of human 5αR-1, although not very potent, with IC_{50} of 310 nM towards type 1 isozyme and > 100 000 towards type 2 [126]. Alpha-1 adrenergic antagonists relax the smooth muscle of the prostate, thereby decreasing the resistance to urine flow and improving BPH symptoms. Research on non-steroidal compounds with dual inhibitory action on the α1-adreno receptor and 5αR-2 in rat models is currently ongoing and these compounds could represent a true innovation for the treatment of BPH [127, 128].

Numberless phytotherapeutic preparations for the treatment of BPH are on the market. However, the active ingredients and the mode of action remain unknown for most of them. *Serenoa repens* (also known as saw palmetto from the American dwarf palm) has been investigated in a number of scientific experiments and in clinical trials. It has been proposed that it inhibits the 5αR-2. Since *Serenoa repens* has no effect on serum prostate-specific antigen (PSA) levels, the mode of action might certainly differ from the mode of action of finasteride or dutasteride [129].

2.1.4.3 Pharmacology of 5α-Reductase Inhibitors

Finasteride, the first 5α-reductase inhibitor, was introduced more than a decade ago. It competitively inhibits 5αR-2 but is only weakly active against

type 1. It reduces human serum DHT levels by 65–70% and prostatic DHT levels by 85–90% [130]. The efficacy, safety and ability to reverse the natural progression of benign prostatic hyperplasia have convincingly been demonstrated. Since serum testosterone levels are unaffected, side effects such as decreased libido, fertility and sexual function are rare. Treatment with finasteride results in shrinkage of the prostate gland by inducing apoptosis and atrophy of the epithelial cells with little effect on the stroma [130]. As finasterides selectively inhibit the 5αR-2, the prostate still receives androgenic stimuli from the residual 30% of serum DHT and 10% of intraprostatic DHT, which has been generated by the 5αR-1.

More recently, dutasteride, has emerged as alternative treatment option. Dutasteride is a dual type 1 and type 2 5α-reductase inhibitor with 60-fold stronger inhibition of type 2 than finasteride and it is also potent antagonist of the 5αR-1. A phase II study in 399 men compared DHT suppression by various doses of dutasteride, 5 mg finasteride and placebo at the end of a 24-week treatment period. Dutasteride decreased serum DHT levels by $98.4\% \pm 1.2\%$ at a dose of 5 mg. In comparison, finasteride caused a decrease of $70.8\% \pm 18.3\%$. Dutasteride thus appears to achieve stronger, less variable, and almost maximal serum DHT suppression compared to finasteride [131]. In men with symptomatic BPH, long-term treatment with dutasteride resulted in sustained and continued improvements in symptoms and flow rate [132]. The other dual 5α-reductase inhibitor, epristeride, irreversibly binds to the enzyme and this results in the formation of an unproductive complex of testosterone, enzyme and NADPH. As testosterone is caught in a trap, the reciprocal intraprostatic testosterone increase seen with finasteride should not occur. Despite this, phase II clinical trials with epristeride showed only a 74% reduction in intraprostatic DHT, compared to the 85–90% reduction seen with finasteride. So far, no results regarding phase III clinical trials have been published [130].

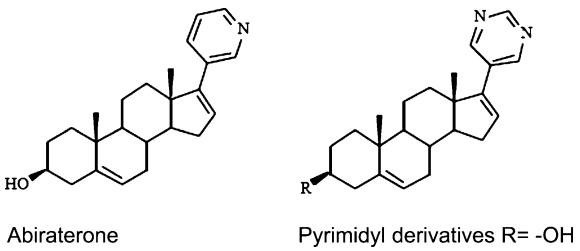
The Prostate Cancer Prevention Trial (PCPT) tested, in a prospective randomised trial, whether finasteride treatment prevents prostate cancer growth. The trial demonstrated a nearly 25% reduction in the prevalence of prostate cancer compared with placebo and provided proof of principle that a chemoprevention strategy using an endocrine agent such as a 5α-reductase inhibitor can be effective. Recent evidence suggests that an increased expression of the 5α-reductase type 1 in prostate cancer compared to benign prostate tissue also makes dutasteride an attractive compound to be studied in this malignant disease [133, 134]. The REDUCE (Reduction by Dutasteride of Prostate Cancer Events) trial will use the dual 5α-reductase inhibitor dutasteride in a group of men identified at increased risk of developing prostate cancer to determine whether treatment with dutasteride will provide an effective chemoprevention strategy [135]. The lack of appropriate animal models for BPH and the observed differences in sensitivitiy of rat versus human 5α-reductase enzymes are major hurdles for the pharmacological character-

isation of all recently synthesised non-steroidal inhibitors. This means that finally only clinical trials in humans might give useful information for the development of drugs for the treatment of BPH.

2.1.5 CYP-17 Inhibitors

The bioconversion of cholesterol to testosterone and DHT in the testes and adrenal glands proceeds via two routes: one involves pregnenolone and dehydro-epiandrosterone and the other involves progesterone and androstenedione. The crucial steps of this process are mediated by a single cytochrome P450 monooxygenase called CYP-17, which displays two enzymatic activities. On the one hand it is a 17α-hydroxylase, which stereospecifically hydroxylates pregnenolone and progesterone at C17, and on the other hand it is a 17,20-lyase, which catalyses the side-chain cleavage of the 17-hydroxylated derivatives of pregnenolone and progesterone resulting in the biosynthesis of the 17-keto-androgens: dehydro-epiandrosterone and androstenedione. A recent review by Leroux summarises the development of new steroidal and non-steroidal inhibitors of CYP-17 [136]. As androgens are implicated in the development and progression of prostatic diseases, this enzyme has become a promising therapeutic target. In order to avoid undesired side effects, CYP-17 targeted androgen biosynthesis inhibitors have to be very specific so that they will not influence corticoid biosynthesis. Attempts were made to obtain selective steroidal as well as non-steroidal inhibitors of CYP-17 [137].

In the last few years, several inhibitors based on a steroidal backbone (pregnenolone and progesterone) were developed by attaching a functional group, which complexes the heme iron into the 17-position. This modification prevented CYP-17 from catalysing the hydroxylation step. Compounds with a 3-pyridyl group in the 17-position, like abiraterone, were the most active (Fig. 11) [138]. Abiraterone was the only steroidal compound used in a clinical trial. Recently, the group of Hartmann et al. [139] reported that pyrimidyl derivatives are potent inhibitors, two to three times more active



Abiraterone Pyrimidyl derivatives R= -OH

Fig. 11 Inhibitors of P450 CYP-17

than abiraterone (IC_{50} 24 nM versus 73 nM) [139]. These compounds could be promising candidates for clinical evaluation. Steroidal compounds often bind to the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) and thus elicit side effects. However, non-steroidal inhibitors based on the triazole backbone of ketoconazole or bifonazole were less potent in vitro (IC_{50} 130 nM) and inactive in vivo due to rapid metabolic degradation [140].

2.2 Hormone Antagonists – Blockade of Steroid Hormone Receptors

2.2.1 Anti-estrogens

2.2.1.1 Rationale for the Use of Anti-estrogens in Cancer Treatment

The effects observed after surgical oophorectomy and the discovery of steroid hormones and the steroid hormone receptors led to the concept that inhibition of steroid hormone receptor function by antagonists should prevent tumour growth. While the first anti-estrogen, tamoxifen, was found accidentally, a deeper understanding of the estrogen receptor as a transcription factor enabled more rational, SAR-based drug discovery. The introduction of the anti-estrogen tamoxifen has changed the treatment of all stages of breast cancer. It is still the method of choice not only for the treatment of advanced disease in pre- and post-menopausal women but also for prevention in women at high risk for developing breast cancer [141]. Tamoxifen has some interesting side effects that render this compound so unique that the term selective estrogen receptor modulator (SERM) was coined for this class of compounds. Although tamoxifen is an anti-estrogen, the drug is a partial estrogen receptor agonist with regard to estrogen-like effects, which are mainly beneficial but in some cases can be harmful. The reduced estrogenicity is not reflected in a reduction in the incidence of blood clots but in the consistent ability to decrease the serum levels of low density lipoprotein (LDL). Unlike estrogen, tamoxifen does not increase high-density lipoprotein (HDL). Proliferative effects on the endometrium have been reported. The partial estrogenicity of tamoxifen maintains bone density in post-menopausal women and a decrease in hip, wrist and spinal fractures has been noted [142].

Resistance to tamoxifen is a complex phenomenon and there is evidence that relapse under tamoxifen therapy is linked to the estrogenicity of the drug. Both, the great success of tamoxifen and its liabilities have boosted the search for new analogues in the past 25 years with the goal of identifying a compound with increased anti-tumour activity and with reduced side effects. A second generation of structurally related triphenyl-ethylenes like

droloxifene, toremifene and idoxifene has been developed but these compounds were not superior to tamoxifen [143]. Even raloxifene was not further evaluated as a breast cancer treatment when early clinical trials showed less activity than tamoxifen in therapy of advanced breast cancer. Nevertheless, raloxifene was more effective in prevention of osteoporosis and was successfully developed as the first SERM for this indication. Only later was a reduction of the incidence of breast cancer observed as a beneficial side effect in the placebo-controlled raloxifene trials [144].

Current interest in new SERMs has built on the experiences with the two prototypical anti-estrogens of the SERM type, namely tamoxifen and raloxifene, with tamoxifen having strong anti-tumour activity and with raloxifene having an improved safety profile and offering bone protection. With the goal of combining only the positive effects and thus decreasing the potential for drug resistance in breast cancer, a third generation of SERM compounds is currently under development.

The observation that as long as the ERα is present, transcription of estrogen-responsive genes and tumour growth may still be stimulated by small amounts of estrogens or partially agonistic anti-estrogens, or even (in a ligand-independent fashion) by growth factor-mediated ERα phosphorylation [145], resulted in the development of a new generation of pure anti-estrogens, the selective estrogen receptor destabilisers or SERDs. In 1991, the first prototype of a so-called pure anti-estrogen (fulvestrant, or ICI 182,780) was introduced [146]. This compound lacks estrogen agonistic activity and it induces a rapid reduction of protein levels of ERα. As those compounds have no agonistic activity but rather destabilise the ERα protein and completely disrupt ERα-mediated growth stimulation, SERDs represent an important therapeutic option for breast cancer treatment and appear to be an effective approach even for tamoxifen-resistant breast cancer.

Results from drug-finding programmes on new anti-estrogens will be reviewed in this section. Drug discovery programmes have focussed on the identification of pure and orally available anti-estrogens. A number of pure anti-estrogens were synthesised and their SAR characterised [147]. These new steroidal anti-estrogens are highly active, pure ER-antagonists that lead to an efficient degradation of the ERα protein without any agonistic activity. Data obtained in pre-clinical tumour models in mice and rats showed a high potency with regard to growth inhibition of ERα-positive breast cancer [148].

2.2.1.2 Chemistry of Anti-estrogens

Approaches from medicinal chemistry that resulted in the discovery of first- and second-generation SERMS have been reviewed extensively [149] and will not be discussed in this section. The more recently published chemical structures of anti-estrogen were derived from different classes, e.g. stilbestriol,

hexestriol, phenylindoles, napthalenes, and benzothiophenes [149]. Arzoxifene (Fig. 12) is a third-generation SERM of the benzothiophene class that is currently under development for cancer treatment [150]. Replacement of the carbonyl function of raloxifene with an ether oxygen and methylation of the phenolic hydroxyl group resulted in a tenfold increase in anti-estrogenic potency in vivo and in vitro.

Lasofoxifene, a diaryltetrahydro-naphthalene derivative is a further third-generation SERM that is structurally distinct from raloxifene [151] (Fig. 12). The compound was discovered during a programme aiming at the synthesis of novel SERMs with good oral bioavailability and higher potency in vivo. In order to circumvent the poor oral bioavailability and limited in vivo potency associated with phenolic groups of benzothiophene derivatives such as raloxifene, which are extensively glucuronidated in the intestinal wall, non-planar phenols such as dihydro- and tetrahydro-naphthalene derivatives, which are poorer substrates for glucuronidation, were introduced. This led to the concept of modulating phenolic glucuronidation to obtain pharmacokinetically superior SERMs. Lasofoxifene is more resistent to glucuronidation and this resulted in an improved oral bioavailability.

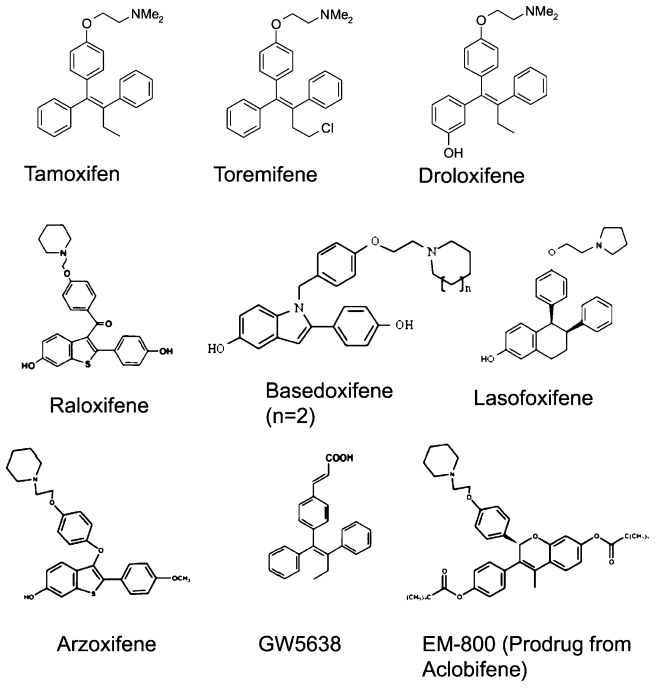


Fig. 12 Chemical structures of first and second generation non-steroidal anti-estrogens (tamoxifen and derivatives), and novel third generation SERMs

Non-steroidal analogues of the potent 7α-substituted steroidal anti-estrogens were synthesised with the goal of identifying orally active, pure anti-estrogens [152]. The benzopyrene derivatives aclobifene (EM-652) and its prodrug EM-800 are active anti-estrogens in human breast cancer cells in vitro as well as in nude mice in vivo [153]. Although EM-652 and EM-800 have frequently been proposed as pure anti-estrogens, partial estrogenicity was observed in some experiments (Hoffmann, unpublished results). GW5638 is a SERM with a rather conventional triphenylethylene structure that was identified in a screen for compounds that are mechanistically distinct from tamoxifen

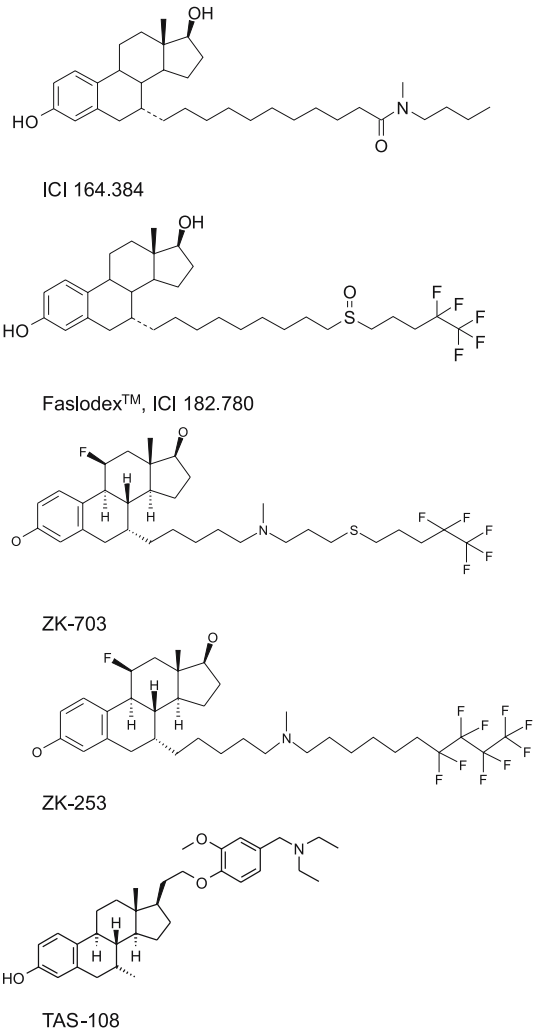


Fig. 13 Chemical structures of steroidal anti-estrogens

and raloxifene. In contrast to tamoxifen, the dimethylamino-ethyoxy group is replaced by an acrylate side chain. This structural modification should result in beneficial estrogenic properties, but unlike tamoxifen, it is a more potent antagonist in breast cancer cells and has no uterotrophic behaviour [154, 155]. Bazedoxifene represents an example of a SERM that has a phenylindole structure. Bazedoxifene acetate represents a further promising new structural class of SERMs; however, it is currently developed for osteoporosis treatment only because it might cause less uterine and vasomotor side effects than the SERMs that are currently used for osteoporosis treatment [156].

ICI 164,384 and fulvestrant (ICI 182,780) (Fig. 13) represent the first generation of pure anti-estrogens with ER destabilising activity [146]. These compounds are 7-α-substituted analogues of 17β-estradiol. SAR studies identified fulvestrant (ICI 182,780), a compound with significantly increased anti-estrogenic potency. As fulvestrant has a very low bioavailability when administered orally it has to be applied by intramuscular injection [146].

Further drug-finding activities focussed on the identification of an orally available pure anti-estrogen with strong anti-tumour activity in endocrine-responsive breast cancer. Rational drug design led to the synthesis of the steroidal compound ZK-703 (Fig. 13). The distinguishing features of ZK-703 are an amino-function in the 7α-side chain and a fluorine atom in the 11β-position of the molecule. Although the activity of ZK-703 was encouraging, this compound still was less effective when administered orally than when administered subcutaneously. Therefore, the metabolically labile thioether moiety of ZK-703 was replaced by two methylene groups which resulted in the compound ZK-253. Preliminary in vitro studies in liver microsomes indicated an enhanced metabolic stability for ZK-253, which eventually may lead to an increased bioavailability [148].

TAS-108 (SR16234) is a novel and orally active steroidal compound with a proposed additional molecular mode-of-action that is different from that of SERMs such as tamoxifen and raloxifene [157]. TAS-108 is a full estrogen receptor-α antagonist, and it should also recruit co-activator transcriptional intermediary factor 2 to ER-β, which may have a preventive effect on bone loss [157].

2.2.1.3 Pharmacology of Anti-estrogens

From a clinical perspective, tamoxifen is still the first and only SERM worth mentioning from the first- and second-generation compounds. The pharmacology of tamoxifen has been reviewed extensively [142]. The NSABP-11 adjuvant trial and the Breast Cancer Prevention Trial (BCPT-1) are some of the milestones in the history of tamoxifen [141].

Palliative and adjuvant phase III trials with toremifene demonstrated that this compound is as effective as tamoxifen [158, 159]. Toremifene is less estro-

genic, which results in a different side-effect profile. While lipid levels were similar, the proliferative effects on the endometrium were reduced and the osteoporosis protection was less effective under toremifene treatment [160]. Although droloxifene was more potent in pre-clinical assays, it was significantly less active than tamoxifen in a randomised phase III trial in advanced breast cancer [161]. Levormeloxifene and idoxifene were noted to increase uterine prolapse and incontinence during phase III trials and therefore the trials were terminated prematurely [162].

Raloxifene was successfully approved for osteoporosis prevention after initial failure in breast cancer studies [144]. In the randomised, double-blind "MORE" study (Multiple Outcomes of Raloxifene) a 72% decrease in the incidence of invasive breast cancer was found after 4 years of raloxifene therapy, besides the prevention of osteoporosis [144]. A "CORE" (Continuing Outcomes Relevant to Evista) trial was conducted to examine the effect of an additional 4 years of raloxifene therapy on the incidence of invasive breast cancer in women who participated in the MORE trial and who agreed to continue in the CORE trial. Women who had randomly been assigned to receive raloxifene (either 60 or 120 mg/day) in MORE were now assigned to receive raloxifene (60 mg/day) in CORE ($n = 3510$) and women who had been assigned to receive placebo in MORE continued on placebo in CORE ($n = 1703$). Over the period of the 8 years of both trials, the incidence of invasive breast cancer and of ER-positive invasive breast cancer was reduced by 66% and 76%, respectively, in the raloxifene group compared with the placebo group. Further advantages of raloxifene are the abundant stimulation of the uterus and the lowering of serum lipid concentrations. During the CORE trial, the relative risk of thromboembolism in the raloxifene group compared with that in the placebo group was 2.17 (95% CI 0.83–5.70). This increased risk, also observed in the MORE trial, persisted over the 8 years of both trials [163].

These findings led the National Surgical Adjuvant Breast and Bowel Project (NSABP) to design and launch the STAR trial (P-2, the Study of Tamoxifen And Raloxifene). The trial was designed to recruit a total of 22 000 postmenopausal women that are randomly assigned to receive either tamoxifen (20 mg/day) or raloxifene (60 mg/day) in a double-blind design and the results were reported recently [164]. Raloxifene is as effective as tamoxifen in reducing the risk of invasive breast cancer and has a lower risk of thromboembolic events and cataracts but a non-statistically significant higher risk of non-invasive breast cancer. The risk of other cancers, fractures, ischemic heart disease and stroke is similar for both drugs.

The novel SERMs, which include bazedoxifene and ospemifene (also known as deaminohydroxy-toremifene), are being investigated for the prevention and treatment of osteoporosis in post-menopausal women in phase III clinical trials [151, 165]. The non-steroidal SERM lasofoxifene (CP-336156) [151], currently under consideration by the Food and Drug Administration for both prevention of osteoporosis and urogenital atrophy, may have potential for

breast cancer therapy. Lasofoxifene has been reported to be in phase III clinical studies in breast cancer, but so far no data have been published.

Currently, very large and cost-intensive clinical trials are necessary to prove efficacy of SERMS in breast cancer. This might be one of the reasons why no further follow-up compounds have been introduced into the clinic. Development and implementation of biomarkers and surrogate endpoints in clinical trials of breast cancer treatment and prevention might help to improve this unsatisfying situation [166]. First attempts were recently reported with arzoxifene, a new SERM with strong anti-estrogenic activity in breast cancer which lacks agonist activity in the uterus [167]. Arzoxifene was explored as a potential chemoprevention agent in a multi-centre study in women with newly diagnosed ductal carcinoma in situ or T1/T2 invasive breast cancer. In a phase IB trial, 76 postmenopausal women were randomised to 20 mg of arzoxifene versus matched placebo. Serum specimens collected at entry and at re-excision and were assayed for various hormones and growth factors. In parallel, tissues from biopsies (ERα positive and/or PR positive) were evaluated immunohistochemically for proliferation markers Ki-67 and proliferating cell nuclear antigen (PCNA). In this trial, an increase in serum sex hormone binding globulin (SHBG) was noted, as well as a decrease in insulin-like growth factor 1 (IGF1) and IGF binding protein-3 (IGFBP3) ratio ($P < 0.007$ versus control/placebo). For 58 evaluable women, a decrease in ERα expression for arzoxifene was observed compared with no change with placebo ($P = 0.0068$). However, the decrease of proliferation markers after treatment with arzoxifene was not statistically significant when compared to the placebo group. This might be due to the confounding effect of stopping hormone replacement therapy before entry into the study. The effects on biomarkers reported in this study reveal that arzoxifene remains a reasonable candidate for an additional study as a breast cancer chemoprevention agent [168].

The prodrug EM-800 and its active metabolite EM-652 are orally active anti-estrogens. EM-652, however, was misclassified as an orally active pure anti-estrogen. SAR of EM-652 predicted that EM-652 would be a SERM with potential cross-resistance to tamoxifen and analogues. In-house experiments demonstrated that EM-800 retains some estrogenic activity (9–18% of estradiol) in the rat uterus assay (Hoffmann, data not shown). EM-800 is a potent, orally active anti-tumour agent in experimental tumour models. Beneficial effects on bones and lipids, observed in pre-clinical and first clinical studies, also support the classification of EM-652 as a SERM. Results from one phase II study in tamoxifen-resistant patients have been reported for EM-652. The clinical benefit rate (CRs + PRs + SDs) was 35% with a median duration of response of 8 months. Similar results were observed in large studies performed in a comparable population of patients who had failed tamoxifen therapy and who received the pure steroidal anti-estrogen fulvestrant. Here, 44.6% and 42.2% of the women, respectively, had clinical benefit rates [169].

The pure steroidal anti-estrogen fulvestrant (ICI 182,780) is the only compound from the class of anti-estrogens that induces the degradation of the ERα protein, culminating partially in an abrogation of estrogen-induced gene transcription. The different mechanism of action suggested a lack of cross-resistance with other SERMS such as tamoxifen. Indeed, first pre-clinical studies have confirmed that fulvestrant has the potential to inhibit the growth of tamoxifen-resistant human breast cancer cell lines [170]. The rate of degradation of estradiol-occupied ERα appeared to be directly correlated with the transcriptional activity. The analysis of the ubiquitination pathways revealed that the ERα is hypo-ubiquitinated in presence of tamoxifen. In the presence of fulvestrant, the ERα is hyper-ubiquitinated. It is likely that the ligand-induced conformational changes in ERα influence its degradation by modulating its interaction with components of the 26S proteasome [171].

Fulvestrant has been evaluated in two randomised phase III trials in post-menopausal women with advanced disease after progression on prior anti-estrogen therapy. In both trials, fulvestrant was at least as effective as anastrozole. In a prospectively designed combined analysis of the results from both trials, median time to progression (TTP) was 5.5 months for fulvestrant versus 4.1 months for anastrozole [172]. Fulvestrant and tamoxifen have been compared as first-line treatments in a trial including post-menopausal women with advanced breast cancer. In this study, the between-treatment difference was non-significant (median TTP 6.8 versus 8.3 months) [173].

Pre-clinical studies demonstrated that the destabilisation of the ERα occurs dose-dependently. Although fulvestrant at 250 mg was shown to be effective, the level of ERα down-regulation achieved in a clinical setting has not yet matched the degree of down-regulation seen in pre-clinical studies. Indeed, it was shown recently that a more frequent application of fulvestrant leads to a stronger down-regulation of the ERα [173]. In women receiving an i.m. injection of either 6 or 18 mg of a short-acting fulvestrant formulation daily for 7 days prior to surgery, a significant dose-dependent reduction in the median ERα levels was evident. Whether strong initial ERα down-regulation may impact the long-term efficacy of the drug still has to be shown, but it may allow earlier identification of patients who respond to treatment. As the down-regulation of the ERα is a dose-dependent process that seems to correlate with response, it is assumed that more potent selective estrogen receptor destabilisers (SERDs), which achieve a further reduction of the levels of ERα, may have an impact on the long-term efficacy. Drug discovery programmes, therefore, focussed on the identification of pure and orally available anti-estrogens with improved potency. A number of pure anti-estrogens were synthesised and their SAR characterised [147]. The novel compound ZK-703 is a pure estrogen antagonist that efficiently destabilises the ERα protein in T47D breast cancer cells. The concentration required for destabilisation (0.129 nM) is lower than the concentration required for anti-proliferative activity (IC_{50} 2.1 nM), suggesting that receptor

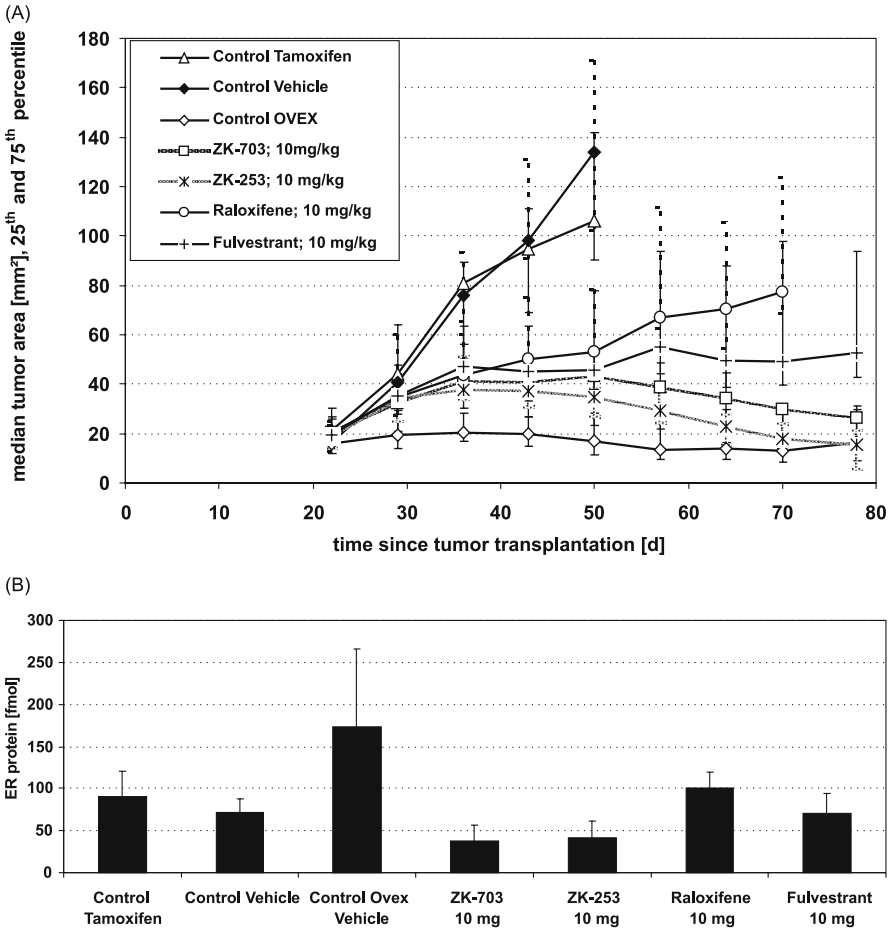


Fig. 14 ZK-253 effects on tamoxifen-resistant breast cancer xenograft tumours. Estrogen-dependent MCF-7/TAM tumours were implanted on day 0 into one flank of 70 estrogen- and tamoxifen-supplemented nude mice. After tumours had reached approximately 25 mm^2 in size (after about 22 days), mice were randomised into seven groups (10 mice each): three control groups (control tamoxifen, control vehicle or control ovariectomy without estradiol), and the four treatment groups (ZK-703, ZK-253, raloxifene or fulvestrant) each at 10 mg/kg subcutaneously daily. Treatment was continued either until the end of the experiment or until tumours reached a median of approximately 100 mm^2 (larger tumours were observed in some mice). The tumours were then removed, snap frozen, and used for analysis of ER levels. **a** Xenograft tumour growth curves. Data are expressed as medians with interquartile ranges. **b** ERα levels. Data are expressed as mean with upper 95% CI

destabilisation strongly contributes to the inhibition of tumour cell growth. The promising pharmacological profile of ZK-703 appears to provide an advantage for the treatment of breast cancer. Although the potency and effi-

cacy of ZK-703 is very encouraging, this compound was less effective after oral than after subcutaneous administration. In contrast, ZK-253, a structurally optimised derivative of ZK-703, retained the anti-proliferative activity of ZK-703 even when given orally. Treatment with ZK-703 and ZK-253 resulted in prolonged growth control of the tamoxifen-resistant MCF-7/TAM xenograft tumour mouse model, and occurrence of resistance was not observed (Fig. 14a). From these experiments it was concluded that tamoxifen-resistant tumours were not cross-resistant to ZK-703 and ZK-253; and consequently these drugs should be active against tumours that developed a resistance to the triphenylethylene class of anti-estrogens. Treatment with either of the two novel anti-estrogens, but not with tamoxifen, raloxifene or fulvestrant resulted in measurable ERα destabilisation in the MCF-7/TAM xenograft tumour model (Fig. 14b). These data suggest that decreased ERα levels in MCF-7 tumours, as caused by treatment with the novel pure anti-estrogens ZK-253 and ZK-703, contribute to the sustained growth inhibition of estrogen-dependent tumour cells (Fig. 14b). If, however, the anti-estrogen no longer destabilises the ERα protein, as observed with fulvestrant in the MCF-7/TAM xenograft tumour model (Fig. 14a), this might result in an acquired cross-resistance to tamoxifen. Broad and direct comparisons with other anti-estrogens such as tamoxifen, fulvestrant and EM-800 in hormone-sensitive breast cancer models completed the pharmacological characterisation of ZK-253. The novel pure anti-estrogen ZK-253 was remarkably superior to all other anti-estrogens in all hormone-sensitive breast cancer models that were investigated [148].

2.2.2
Progesterone Receptor Antagonists

2.2.2.1
Rationale for the Use of Progesterone Receptor Antagonists in Cancer Treatment

It is well known that progesterone in physiological concentrations – beside estradiol – may be required for the proliferation of mammary carcinomas [174]. Therefore, it is expected that progesterone receptor (PR) antagonists (PRAs) will be able to block the growth of those mammary carcinomas that express a functional PR, and that PR antagonists might be promising new tools for breast cancer therapy [175]. Although these compounds require a functional PR in order to block tumour growth, there is strong experimental evidence that PR antagonist-mediated tumour growth inhibition is not solely based on progesterone antagonism. The ability of these compounds to induce tumour cell differentiation that leads to apoptosis is a unique ability compared to all other endocrine therapies [176].

In the last few years, considerable progress has been made in elucidating the mechanism of action of PR antagonists. According to the classical

mechanism, PR antagonists bind to the PR and modulate PR-dependent gene transcription. In addition, it has been demonstrated that the biological response to a PR antagonist involves more factors and that this response is not only the result of competition for progesterone [176]. In order to clearly define those physiological effects that are specifically attributable to progesterone in vivo, a mouse knock-out model carrying a null mutation of the PR gene (PRKO) was generated. Male and female embryos homozygous for the PRKO developed normally to adulthood but they displayed significant defects in the development of the reproductive organs [177]. The PRKO model was also used to define the controversial role of progesterone-initiated signalling in mammary gland tumourigenesis [178]. Combining tissue transplantation with an carcinogen (DMBA)-induced mammary tumourigenesis model, a marked reduction in mammary tumour incidence in PRKO mice as compared with isogenic wild-type mice was observed. This section of the review will focus on the pre-clinical and clinical development of new PR antagonists with high specificity for the PR and high selectivity for treatment of breast cancer. PR antagonists and selective progesterone receptor modulators (SPRMs) display direct anti-proliferative effects in the endometrium, justifying their use in the treatment of myomas and endometriosis [179]. Interestingly, clinical data show that treatment with these compounds is not associated with hypo-estrogenism and bone loss [179]. The notion that an anti-progestin such as mifepristone has been linked to drug-induced abortion has unfortunately restricted the involvement of the major pharmaceutical companies in the development of PR antagonists and SPRMs.

2.2.2.2 Chemistry of Progesterone Receptor Antagonists

Since the discovery of the anti-progestin mifepristone, hundreds of similar compounds have been synthesised. This family includes pure progesterone receptor antagonists (PR antagonists) and SPRMs, which have mixed agonist–antagonist properties. The discovery of RU38486 (RU486; mifepristone), the first compound with pronounced anti-glucocorticoid and anti-gestagenic activities, was a starting point for drug discovery and medicinal optimisation [180]. The steroid nucleus was modified at various positions. Among them, 11β-aryl-substituted PR antagonists with reduced anti-glucocorticoid activity were synthesised and characterised [181]. One of the members of this class is onapristone (ZK98299). Onapristone has a similar structure to mifepristone, which is a 19-norprogesterone derivative, also with 11β-aryl substitutions as Org33628 and Org31710. Asoprisnil (J867) is a hydrophobic oxime with substitutions at the 11 position (Fig. 15) [182].

SAR analyses revealed that unwanted endocrine side effects such as anti-glucocorticoid activities were reduced by modifying position C-17. Among a variety of modifications, the 17α-pentafluoroethyl side chain (Fig. 15) led

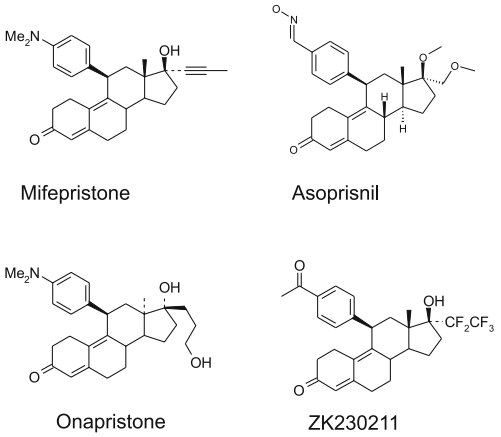


Fig. 15 Chemical structures of progesterone receptor antagonists

to a new anti-progestin (ZK230211) with a pronounced anti-progestagenic activity and low or no other endocrine activities [183]. Non-steroidal PR antagonists and SPRMs have also been developed [184], but are currently not clinically evaluated. Eremmophilane-type sesquiterpenes, isolated as bacterial metabolites from *Penicillium obtalum* about 7 years ago, have received some attention as progesterone receptor modulators [185]. Tetrahydrobenzindolone analogues such as CP8661, CP8668 and CP8863, and analogues of aryl-substituted benzimidazolones, benzoxazinones and oxindoles are also potent PR antagonists [186].

2.2.2.3 Pharmacology of Progesterone Receptor Antagonists

PR antagonists such as onapristone, ZK112993, ZK136798 and ZK230211, which are highly selective for the progesterone receptor and possess a reduced anti-glucocorticoid activity compared to mifepristone, exerted a strong anti-tumour activity in a panel of hormone-dependent mammary tumour models [187–189]. Observations from these pre-clinical experiments in different model systems led to the conclusion that the strong anti-tumour activity of these "pure" PR antagonists in breast cancer does not only depend on the classic anti-hormonal mechanism. For the first time Michna et al. (1992) described that the morphological pattern in experimental breast tumours after treatment with PR antagonists differs totally from that after treatment with tamoxifen, high doses of estrogen, or ovariectomy [190]. By using light and electron microscopy they found that the anti-tumour action of PR antagonists is accompanied by the initiation of differentiation by induction of active secretory glandular formations while undifferentiated epithelial tumour cells disappeared. Mammary glands and breast tumours from

onapristone-treated rats displayed morphological features of terminal differentiation with the appearance of apoptotic cell death. In addition, flow cytometry studies revealed an accumulation of the tumour cells in the G0–G1 phase of the cell cycle, together with a significant and biologically relevant reduction in the number of cells in the G2M- and S-phases, which may result from induction of differentiation [176]. The ability of PR antagonists to reduce the number of cells in S-phase may offer a clinical advantage, since it has been established that the S-phase fraction is a highly significant predictor of disease-free survival among axillary node-negative patients with diploid mammary tumours. In contrast, conventional endocrine therapies for breast cancer such as tamoxifen as well as ovariectomy did not alter in the distribution of cells in the cell cycle phases [190]. It can be concluded from these data that PR antagonists clearly differ in their mode of action from compounds used in established endocrine treatment strategies for breast cancer.

To date, the results of five phase II clinical trials with PR antagonists in patients with metastatic breast cancer have been reported [191]. In postmenopausal women, two studies with mifepristone as second- or third-line treatment for metastatic breast cancer showed an objective response rate (complete response plus partial response) of 10 and 13% and stable disease in 54 and 40% of patients, respectively. A third study was conducted using mifepristone as first-line treatment. An objective response rate of 11% and a stable disease rate of 39% was reported [191].

Onapristone was the first PR antagonist investigated as an alternative endocrine agent for the treatment of advanced breast cancer. In a phase II study, onapristone was given at a dose of 100 mg/day to 118 patients with metastatic breast cancer resistant to tamoxifen. The objective response rate was 10%, and in 39% of the patients there was stable disease for at least 3 months. The overall time to progression was 4 months [192]. In an explorative phase II clinical trial [193], 19 patients with either locally advanced breast cancer ($n = 12$) or who were elderly, unfit patients with primary breast cancer ($n = 7$) received onapristone at 100 mg/day. Seventeen of the 19 tumours expressed the ER while 12 of the 18 tumours tested expressed the PR. Tumour remission was categorised according to the criteria of the International Union Against Cancer. One patient was withdrawn after 4.5 months. Of the remaining 18 patients, ten (56%) showed a partial response and two (11%) a durable static disease ($\geq$ 6 months), giving an overall tumour remission rate of 67%. This confirmed that onapristone does induce tumour responses in human breast cancer. The median duration of remission was 70 weeks. Studies are ongoing to investigate whether onapristone induces a differentiation of these human breast cancer cells similar to the changes seen in the in vivo models described above. Due to the fact that some patients developed abnormalities in liver function tests, the development programme for onapristone was terminated.

Nevertheless, these clinical results suggest a potential benefit of adding PR antagonists to the panel of options for the treatment of endocrine-responsive breast cancer, especially in order to extend the therapeutic options in anti-estrogen refractory diseases. The extension of endocrine treatments to other tumour entities is also a promising approach for further developments. El Etreby et al. demonstrated that applying the PR antagonist mifepristone in combination with 4-hydroxytamoxifen increases the induction of apoptosis additively and down-regulates the apoptosis-inhibitory protein Bcl-2 in the human breast cancer cell line MCF7 [194]. These results suggest a potential clinical benefit of adding a PR antagonist to anti-estrogen therapy of breast cancer patients. The effects of PR antagonists (onapristone) has also been investigated in other tumour types, both in classic endocrine-sensitive tumours such as prostate cancer and in non-classic endocrine-sensitive ones as gastrointestinal tumours [188].

By definition, SPRMs have mixed agonist–antagonist properties and occupy an intermediate position in the spectrum of compounds active on the progesterone receptor. Asoprisnil differs from the previously described PR antagonists because it is a partial PR agonist. Asoprisnil treatment in cynomolgus monkeys resulted in endometrial atrophy and reversibly suppressed menstruation [195]. Two randomised, placebo-controlled, dose-finding phase II studies of asoprisnil (5, 10 and 25 mg) have been conducted in patients suffering from endometriosis-induced pain. All three asoprisnil doses significantly reduced the average daily combined non-menstrual pelvic pain/dysmenorrhea scores at all treatment months compared with placebo. All effective doses of asoprisnil showed similar effects on pain; however, the effect on bleeding pattern was dose-dependent. A separate study with an identical design using lower asoprisnil doses (0.5, 1.5 and 5 mg) showed that 5 mg is the minimum effective dose for pain relief in subjects with endometriosis [196]. Both studies also confirmed the favourable safety and tolerability profiles of asoprisnil during short-term treatment. Adverse events were evenly distributed among treatment and placebo groups and were generally mild and self-limiting. No serious drug-related adverse events were reported during treatment or follow-up period.

2.2.3 Anti-androgens

2.2.3.1 Rationale for the Use of Anti-androgens in Cancer Treatment

Prostate cancer is the most frequently diagnosed malignancy in males and ranks second only to lung cancer in terms of annual mortality. Great efforts have been made in the past to develop novel approaches to the treatment of prostate cancer. The two main options for the treatment of prostate cancer are

limited to surgical removal by radical prostatectomy (if the tumour is organ-confined) or endocrine therapy (if the tumour has crossed the capsule). One major problem in the treatment of non-organ confined prostate cancer is that the currently available therapies are only palliative. For locally advanced or metastatic prostate cancer the only effective therapies are those targeting the androgen receptor (AR). As most prostate cancer cells initially grow androgen-dependently, androgen withdrawal results in the apoptosis and inhibition of tumour proliferation. Although the majority of patients (80%) responds to endocrine therapy, almost all prostate cancer patients undergo a relapse after a median duration of 12–18 months [197]. As no effective therapies are available for such patients, there is a high medical need for better therapies. The common therapies targeting the AR can be divided into ligand depletion by reduction of serum testosterone levels of testicular origin via orchiectomy, application of LHRH analogues, high dose estrogens, and the blockade of the AR.

Failure of androgen ablation therapy is likely to result from progression of prostate tumours to androgen independency that may have different reasons such as: (1) AR activation by residual androgens in androgen hypersensitive tumour cells, (2) mutant ARs activated by other endogenous steroid hormones, (3) ligand-independent AR activation by growth factor-mediated signal tranduction pathways and (4) insufficient efficacy of available anti-androgens.

It was shown that expression of the AR is not decreased or lost in advanced prostate tumours but instead is frequently increased and in addition the AR gene is amplified [198]. Furthermore, mutations that convert the AR into a promiscuous receptor that can be activated not only by androgens but also by various other hormones and growth factors have been associated with tumour progression [199]. It was also shown that the AR can be activated in a ligand-independent fashion in the absence of hormones by various growth factors such as insulin-like growth factor (IGF-1), keratinocyte growth factor (KGF), epidermal growth factor (EGF) and cAMP-activating compounds [200]. All these findings point out that prostate tumours may use several pathways to circumvent their androgen requirements to adapt to an androgen-deprived environment. Nevertheless, the key regulatory protein involved both in the androgen-dependent and the androgen-independent growth of prostate tumours seems to be the AR.

The currently available anti-androgens have a relatively low AR affinity when compared to anti-hormones that are specific for the ER and the PR. This is reflected by IC_{50} values in the higher nanomolar range in in vitro transactivation assays. Based on the evidence outlined above it becomes obvious that there is room for improved prostate cancer therapies targeting the AR. The AR might be a potential target for antisense or RNA interference (RNAi) therapies to inhibit a key regulatory step in the androgen signal cascade. A further approach is based on the positive experience with SERDs such as fulvestrant

in the therapy of breast cancer. These compounds are pure anti-estrogens and in addition they destabilise the receptor protein and lead to a complete disruption of steroid receptor-mediated growth stimulation. Complete blockade of all AR functions by AR destabilising anti-androgens could represent an important therapeutic option for prostate cancer treatment.

Attempts have been made to generate highly potent anti-androgens that completely block all AR-mediated effects (ligand-dependent or -independent, mediated by wild-type or mutated AR). Such compounds should be of superior efficacy when used in first-line therapy, they should delay the tumour relapse, and they should be effective after relapse under classic androgen ablation therapy. The successful development of selective estrogen receptor modulators (SERMs) has triggered a strong interest in developing selective androgen receptor modulators (SARMs). A SARM should be an antagonist or weak agonist in the prostate, but an agonist in the pituitary and muscle. For an ideal SARM, the antagonist activity in the prostate should inhibit the growth of prostate cancer cells or it may even prevent the growth of nascent or undetected prostate cancer, while the selective agonist activity in the muscle and bone should prevent muscle-wasting conditions, hypogonadism or age-related fragility.

2.2.3.2 Chemistry of Anti-androgens

The biology and structure–activity relationships for the emerging class of AR antagonists have been discussed in many reviews [201]. Based on their structure, the known AR antagonists can be classified as steroidal or non-steroidal. A few steroidal compounds have been used as anti-androgens, including cyproterone acetate (CPA), which was originally developed for other therapeutic purposes. When CPA was evaluated for the originally intended use, the anti-androgenic activity manifested as an undesired side effect. CPA is a progestin that suppresses gonadotropin release and binds to the AR with relatively high affinity and inhibits the growth of prostate cancer cells [202].

It was proposed that non-steroidal ligands will have a high specificity for the AR, improve oral bioavailability, and achieve tissue selectivity. In addition, they might allow more flexible chemical modifications if deemed necessary. First, substituted toluidides such as bicalutamide, flutamide and nilutamide were developed as non-steroidal anti-androgens (Fig. 16). Unlike the steroidal CPA, these toluidides are considered to be pure anti-androgens because they possess little if any intrinsic androgenic activity when bound to wild-type AR and they do not cross-react with any of the other steroid receptors [203]. As such, the non-steroidal anti-androgens are mainly used to treat androgen-sensitive prostate cancer or BPH.

A variety of investigational anti-androgens are in development. These compounds have not yet been evaluated clinically but demonstrate potent

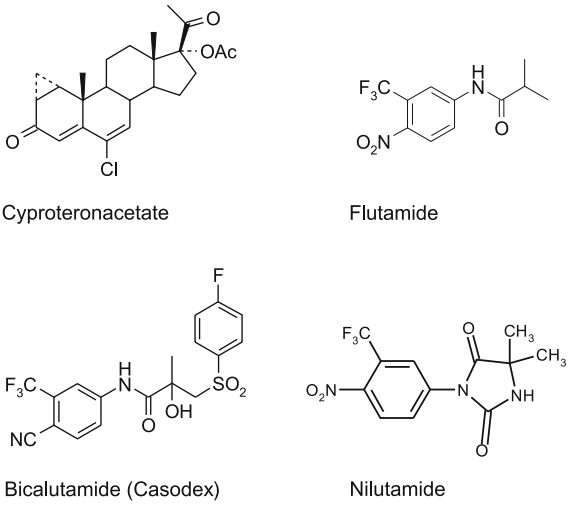


Fig. 16 Chemical structures of anti-androgens

anti-androgenic activity in in vitro and pre-clinical models. Structural modification led to the development of a series of hydantoin derivatives that act as AR antagonists [204]. The lead compound BMS-564929 binds to the AR with high affinity and specificity and acts with high selectivity in the target tissues [203]. A series of quinolone derivatives bind to the AR in the nanomolar range and work as AR antagonists. In intact male rats, the lead compound LG120907 showed antagonist activity in the prostate and seminal vesicle without raising the plasma levels of luteinising hormone and testosterone. However, the tissue selectivity observed in rats has not yet been demonstrated in humans [205].

Structural modifications of bicalutamide led to the discovery of the first generation of selective androgen receptor modulators (SARMs). These compounds not only bind to the AR with an affinity in the nanomolar range, but they also show tissue selectivity in animal models [206].

The mechanism by which the AR distinguishes between agonists and antagonists was studied for non-steroidal AR ligands using site-directed mutagenesis of the AR and structural evidence of the mechanism by which non-steroidal ligands interact with the wild-type AR. Furthermore, mutations at the amino acid residues Trp-741, Thr-877, Met-895, W741L, T877A and M895T allow for accommodation of larger ligands such as corticosteroids and non-steroidal antagonists within the AR binding pocket. Additionally, it was demonstrated that R(-)bicalutamide stimulates transcriptional activation of AR harbouring the T877A or M895T point mutation [207]. These studies provide a strong rationale for further AR-based drug design activities with the aim of identifying novel non-steroidal AR antagonists that overcome this type of resistance. Furthermore, the concept of AR destabilising compounds has

been developed in the last few years and the first results will be published in the near future.

2.2.3.3 Pharmacology of Anti-androgens

Anti-androgens competitively bind to the AR-LBD. In clinical use are CPA, flutamide, nilutamide and bicalutamide. The first steroidal anti-androgen CPA has been reviewed extensively [202]. CPA suppresses gonadotropin release and leads to a decrease of testosterone levels. Flutamide and bicalutamide are non-steroidal compounds widely used in prostate cancer treatment. Bicalutamide [208] has replaced flutamide and nilutamide as the anti-androgen of choice for prostate cancer treatment since it has less side effects and a longer half-life. It is therefore administered at a relatively lower dose of 50 mg/day. Response rates of bicalutamide in phase II clinical trials were comparable to those of CPA and flutamide [209]. In ongoing phase III studies, bicalutamide was compared with androgen ablation or maximal androgen blockade. Interim analyses confirmed the improved tolerability of bicalutamide; however, the compound failed to improve survival [210, 211].

Combined androgen blockade, first proposed by Labrie, is under investigation in a number of randomised studies. For example, in a recent study the combination of flutamide or nilutamide with a GnRH agonist has been evaluated. Although the combination seems to improve time to progression (TTP) and overall survival (OS), final data have not yet been published [212]. Whereas the EORTC trial reported significant advantages of a combination of goserelin plus flutamide [213], this combination failed in other studies [214]. A meta-analysis showed a 5% increase of the 5-year survival under combination therapy [215]. A final consensus for endocrine treatment options for prostate cancer, comparable to the St. Gallen consensus for breast cancer, still has to be established. In some clinical trials, finasteride, an inhibitor of the 5α-reductase type 2, has been added to the combined androgen blockade (goserelin and flutamide) [216].

Flutamide was the first drug used in prostate cancer therapy for which the withdrawal syndrome was reported. In that study, 40% of patients showed a decline in prostate specific antigen (PSA) levels after cessation of flutamide from the therapeutic protocol. The decline in PSA levels was associated with an improvement of the clinical symptoms. Based on these paradoxical observations, the concept of sequenced androgen ablation was proposed [217]. Several phase II clinical studies were performed, demonstrating safety and tolerability, however, a direct comparison in randomised phase III trials is necessary [218].

Although administration of all currently available anti-androgens in most cases leads to stabilisation of the disease, survival of prostate cancer patients has still not been significantly improved. There are several mechanisms by

which AR antagonists acquire agonistic properties. A possible approach to control prostate cancer growth is to develop therapies that down-regulate AR expression. However, the AR is also implicated in regulating prostate differentiation and a better understanding of the physiological role of the AR might be achieved when critical co-activators associated with prostate cancer progression have been identified. These co-activators, if drugable, may be regarded as novel targets for the therapy of prostate cancer.

In the bicalutamide prostate cancer programme, the adjuvant treatment of patients with advanced prostate carcinoma (T1–T4; N0/NX, M0) with bicalutamide (150 mg, once a day) was evaluated. 4052 patients were randomised to bicalutamide with best standard care (either radiation, prostatectomy or "watchful waiting"), whereas 4061 patients received a placebo with best standard care. An initial reduction of prostate cancer recurrence, which was observed in an interim analysis, later was not confirmed [219]. As the survival time in the bicalutamide treatment group was decreased, the study was terminated ahead of time.

A recent study shows that inhibition of AR expression results in prostate tumour growth inhibition in vivo. These promising results further underline the key role of the AR in prostate tumour growth and warrant further testing down-regulation of the AR as a treatment for prostate cancer. As the function of the AR seems to be mediated exclusively through genomic mechanisms, one can also envision drugs that prevent AR nuclear translocation or impair assembly of AR transcription complexes on target genes. Finally, it will be important to determine in more detail which mechanisms are responsible for anti-androgen resistance implicated in therapy failure [220].

2.3 Hormone Interference – Estrogens and Progestins

Prior to the introduction of tamoxifen, high-dose estrogens such as diethylstilbestrol (DES) or ethinyl estradiol were generally considered the endocrine treatment of choice for post-menopausal women with breast cancer and for men with prostate cancer [221]. Subsequently, the use of estrogens declined, but data from recent clinical trials underline that these drugs have a similar efficacy as tamoxifen and are able to produce responses, even in patients who have received extensive prior endocrine therapy. However, the use of these agents is limited by their toxicity profile. The mode of action of high dose estrogens is still under discussion. Besides a negative feedback regulation of the hormonal cycle, cytotoxic effects and induction of apoptosis have been proposed [222]. A modification of the treatment schedule and new formulations like sulfamates, phosphates or esters, which avoid the primary liver passage of the estrogen, may lead to a renaissance of this very effective treatment option for breast and prostate cancer patients who are resistant to anti-hormones [221].

High-dose progestins are used as last-line endocrine therapy [223]. They inhibit the adrenal steroid biosynthesis. The decrease of estrogen levels is comparable to that caused by the administration of aromatase inhibitors. In post-menopausal women, the progestin megestrol acetate (MGA) decreases serum plasma level of DEAH, androstenedione and cortisol to less than 10% [223, 224].

2.3.1 Inhibitors of Releasing Hormones

2.3.1.1 Rationale for the Use of Inhibitors of Releasing Hormones in Cancer Treatment

Prostate cancer and breast cancer are both stimulated by steroid hormones. The synthesis of estrogens and androgens is initiated by gonadotropins. In pre-menopausal women, gonadotropin-releasing hormone (GnRH) is released from the hypothalamus in a pulsatile fashion and is carried by the portal veins directly to the anterior pituitary gland where it binds to GnRH receptors, stimulating the release of luteinising hormone (LH) and follicle stimulating hormone (FSH) (Fig. 17). The ligand-bound receptors cluster and are taken up into the pituitary cells. These inactivated G protein-coupled GnRH receptors are replaced by newly synthesised receptors on the cell surface, ready for the next pulse of GnRH. LH stimulates the ovaries to produce

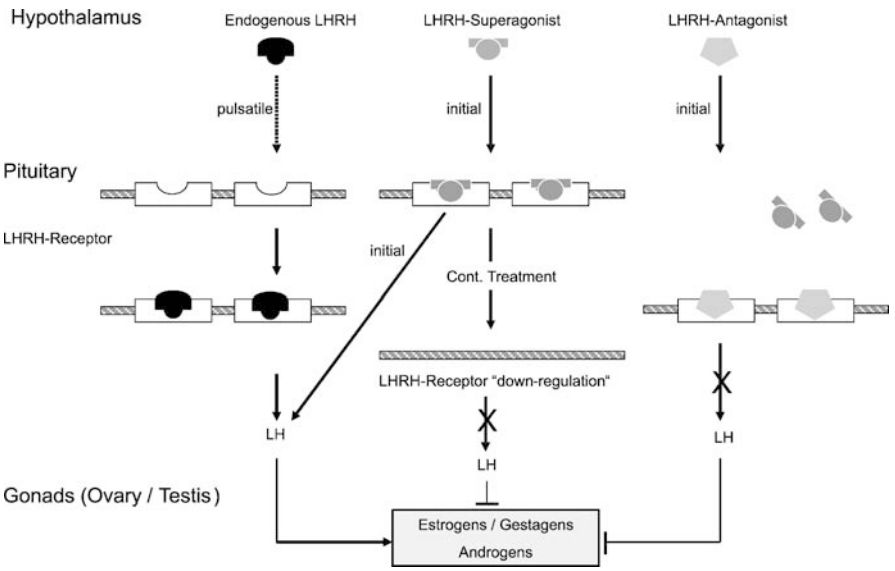


Fig. 17 Mode of action of GnRH analogues

estrogens, including estradiol. This process is responsible for producing up to 90% of circulating estradiol, depending on the phase of the menstrual cycle. The aromatisation of androgens by the adrenal glands is responsible for the synthesis of the remaining 10% estradiol in pre-menopausal women and for all estradiol in post-menopausal women (Fig. 17).

Long-term administration of GnRH superagonists effectively down-regulates the GnRH receptors in the pituitary gland, whereas GnRH antagonist can directly block the release of gonadotropins [225, 226].

2.3.1.2
Chemistry of Inhibitors of Releasing Hormones

The luteinising hormone-releasing hormone (LHRH; one of the GnRH peptides) is an endogenous decapeptidic hormone with a short plasma half-life. Based on the sequence of LHRH, amino acids in positions 2, 3 and 7 were replaced leading to potent analogues with long half-lives. Further improvements were achieved by systematic SAR approaches, and the use of modified amino acids. In the past few years, more than 3000 analogues of LHRH have been synthesised, with substitutions in up to seven positions [227, 228]. Agonistic analogues, such as decapeptyl, zoladex, leuprolide and buserelin, more potent than LHRH itself and available in depot formulations, have important clinical application in gynecology and oncology. Potent antagonists of LHRH such as cetrolelix, ganerelix and abarelix, suitable for clinical use, have been synthesised likewise. All relevant LHRH superagonists and LHRH antagonists are listed in Table 1 (modified from Kiesel et al. [229]).

2.3.1.3
Pharmacology of Inhibitors of Releasing Hormones

The action of GnRH analogues are mediated by high-affinity receptors for GnRH found on the cell membrane of the pituitary gland. An acute administration of GnRH agonists induces a marked release of LH and FSH. However, continuous stimulation of the pituitary by chronic administration of GnRH agonists inhibits the hypophyseal-gonadal axis via "down-regulation" of GnRH receptors in the pituitary, desensitisation of the pituitary gland, and a suppression of circulating levels of LH, estrogens or androgens. This down-regulation of GnRH receptors, produced by the sustained administration of GnRH agonists, provides the basis for the clinical applications of GnRH superagonists in gynecology and oncology [230].

Antagonists of GnRH exhibit no intrinsic activity, but compete with GnRH for the same receptor. GnRH antagonists produce a competitive blockade of GnRH receptors and cause an immediate inhibition of the release of gonadotropins and steroid hormones. The principal mechanism of action of GnRH antagonists was thought to be based only on a competitive occupancy

Table 1 LHRH agonists and antagonists

Name		Relative potency	Aminoacid sequence 1	2	3	4	5	6	7	8	9	10
LHRH-agonists												
LHRH		1	pyroGlu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly-NH_2
		2										NH-Et
		3						D-Ala				
		14						D-Ala				NH-Et
Triptorelin	Decapeptyl							D-Trp				Gly-NH_2
Leuprorelin	Leuprolid, Lupron	15						D-Leu				NH-Et
Buserelin	Suprefact	20						D-Ser (*t*Bu)				NH-Et
Goserelin	Zoladex							D-Ser (*t*Bu)				Aza-Gly-NH_2
Nafarelin								D-Nal(2)				Gly-NH_2
Deslorelin		144						D-Trp				NH-Et
Histrelin		210						D-His (ImBzl)				NH-Et
LHRH-agonists												
Antide			Ac-D-Nal	D-Phe(4Cl)	D-Pal(3)		Nic-Lys	D-Nic-Lys		*i*Pr-Lys		D-Ala
Ramorelix			Ac-D-Nal	D-Phe(4Cl)	D-Trp			D-Ser (Rha)		Aza-Gly-NH_2		
Cetrorelix	Cetrotid		Ac-D-Nal(2)	D-Phe(4Cl)	D-Pal(3)			D-hArg(Et_2) [D-Cit]		D-Ala		
Antarelix			Ac-D-Nal	D-pCl-Phe	D-Pal(3)			D-HCl		*i*Pr-Lys		D-Ala
ORG30850			Ac-D-Phe-(4Cl)	D-Phe(4Cl)	D-Pal(3)			D-Lys				D-Ala
Abarelix	PPI-149		*N*-Ac-D-Nal(2)	D-pCl-Phe	D-Pal(3)			Asn		*i*Lys		Gly-NH_2
Ganirelix	Orgalutran		*N*-Ac-D-Nal(2)	D-pCl-Phe	D-Pal(3)			D-hArg(Et_2)		I-hArg (Et_2)		D-Ala

of GnRH receptors, but it was shown that administration of the GnRH antagonist cetrorelix induces a down-regulation of pituitary GnRH receptors and a decrease in the levels of mRNA for GnRH receptors [231].

GnRH analogues have had a great impact on the endocrine therapy of prostate cancer. Administration of GnRH agonist alone or in combination with anti-androgens is currently the preferred treatment for men with advanced prostate cancer. In about 70% of cases GnRH agonists are selected for primary treatment [232]. Administration of anti-androgens prior to and during early therapy with agonist can prevent the disease flare [233]. The most important therapeutic advantage of "chemical castration" using GnRH analogues is the reversible inhibition of steroid hormone biosynthesis.

Clinical trials in patients with advanced prostate cancer show that a GnRH antagonist could be beneficial as a monotherapy for patients with prostate cancer and metastases in the brain, spine, liver and bone marrow in whom the GnRH agonist cannot be used as single drug, due to the possibility of a flare-up [234]. GnRH antagonists immediately decrease the levels of gonadotropin and testosterone and greatly reduce the time of onset of therapeutic effects. In addition, treatment with GnRH antagonists can produce long-term improvement in patients with symptomatic BPH [234] and they are a treatment option for patients with BPH who are considered under surgical risk.

Experimental studies have clearly demonstrated that GnRH agonists are effective agents for the treatment of estrogen-dependent breast cancer. This suggested that GnRH agonists should be considered as endocrine therapy for breast cancer. Various clinical trials carried out since the early 1980s demonstrated a regression of tumour mass and the disappearance of metastases in pre- and post-menopausal women with breast cancer. These studies showed that GnRH agonists are efficacious for the treatment of pre-menopausal women with ER-positive breast cancer achieving response rates of 53% [235]. GnRH superagonists are now the treatment of choice for pre-menopausal women with ER-positive breast cancer. In the adjuvant treatment, GnRH superagonists and chemotherapy have comparable effects on survival, however, the endocrine therapy is better tolerated [236]. Side effects in pre-menopausal women are typical menopausal symptoms, which are reversible [229]. Additional studies evaluating GnRH superagonists in endometrial and ovarian cancer an ongoing [229, 237, 238].

3 Conclusion and Outlook

Pharmacological knowledge, gained with the discovery of steroid hormones, their nuclear receptors and their function in normal and malignant tissues, has been successfully translated into the first targeted drugs in oncology.

Blocking steroid receptor function, by antagonists or inhibitors of steroid synthesis, inhibits or even prevents breast or prostate tumour growth. While the first anti-hormones were found accidentally, a deeper understanding of the steroid receptors as transcription factors and of the pathways of steroid hormone synthesis enabled more rational, structure–activity relationship-based drug discovery. Steroid hormone antagonists still have unspecific side effects and improvement of receptor and even tissue selectivity is the challenge for future research in this field. The development of compounds that block the interaction of agonist-liganded nuclear hormone receptors with co-factors might provide unique pharmacological agents for interrupting the signal transduction cascade [239].

In summary, research on steroid receptor action will provide many opportunities to further improve treatment of hormone-dependent cancers.

References

1. Florio S, Pagnini U, Crispino A, Pacilio C, Crispino L, Giordano A (2002) Front Biosci 7:1590
2. Ortmann O, Diedrich K (1999) Hum Reprod 14:194
3. Burger HG, Dudley E, Mamers P, Robertson D, Groome N, Dennerstein L (2002) Novartis Found Symp 242:161
4. Piltonen T, Koivunen R, Morin-Papunen L, Ruokonen A, Huhtaniemi IT, Tapanainen JS (2002) Hum Reprod 17:620
5. Smith CL, O'Malley BW (2004) Endocr Rev 25:45
6. Ariazi EA, Jordan VC (2006) Curr Top Med Chem 6:181
7. Tirona RG, Kim RB (2005) J Pharm Sci 94:1169
8. Fu M, Wang C, Zhang X, Pestell RG (2004) Biochem Pharmacol 68:1199
9. Reid G, Denger S, Kos M, Gannon F (2002) Cell Mol Life Sci 59:821
10. Metivier R, Reid G, Gannon F (2006) EMBO Rep 7:161
11. Hewitt SC, Harrell JC, Korach KS (2005) Annu Rev Physiol 67:285
12. Butt AJ, McNeil CM, Musgrove EA, Sutherland RL (2005) Endocr Relat Cancer S1:S47
13. Nettles KW, Greene GL (2005) Ann Rev Physiol 67:309
14. Glass CK (1994) Endocr Rev 15:391
15. Klinge CM (2001) Nucleic Acids Res 29:2905
16. Harrington WR, Sheng S, Barnett DH, Petz LN (2003) Mol Cell Endocrinol 206:13
17. Couse JF, Korach KS (1999) Estrogen Endocrine Rev 20:358
18. Ramsey TL, Risinger KE, Jernigan SC, Mattingly KA, Klinge CM (2004) Endocrinology 145:149
19. Pike AC, Brzozowski AM, Hubbard RE, Bonn T, Thorsell AG, Engstrom O, Ljungren J, Gustafsson J-Å, Carlquist M (1999) EMBO J 18:4608
20. Shiau AK, Barstad D, Loria PM, Cheng L, Kushner PJ, Agard DA, Greene GL (1998) Cell 95:927
21. Shiau AK, Barstad D, Radek JT, Meyers MJ, Nettles KW, Katzenellenbogen BS, Katzenellenbogen JA, Agard DA, Greene GL (2002) Nat Struct Biol 9:359
22. Lavinsky RM, Jepsen K, Heinzel T, Torchia J, Mullen TM, Schiff R, Del-Rio AL, Ricote M, Ngo S, Gemsch J, Hilsenbeck SG, Osborne CK, Glass CK, Rosenfeld MG, Rose DW (1998) Proc Natl Acad Sci USA 95:2920

23. Conneely OM, Lydon JP, De Mayo F, O'Malley BW (2000) J Soc Gynecol Investig 7:S25
24. Williams SP, Sigler PB (1998) Nature 393:392
25. Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr, Shyamala G, Conneely OM, O'Malley BW (1995) Genes Dev 15:2266
26. Chappell PE, Lydon JP, Conneely OM, O'Malley BW, Levine JE (1997) Endocrinology 138:4147
27. Conneely OM, Jericevic BM, Lydon JP (2003) J Mammary Gland Biol Neoplasia 8:205
28. Humphreys RC, Lydon J, O'Malley BW, Rosen JM (1997) Mol Endocrinol 11:801
29. Brisken C, Park S, Vass T, Lydon JP, O'Malley BW, Weinberg RA (1998) Proc Natl Acad Sci USA 95:5076
30. Kurita T, Young P, Brody JR, Lydon JP, O'Malley BW, Cunha GR (1998) Endocrinology 139:4708
31. Lydon JP, Ge G, Kittrell FS, Medina D, O'Malley BW (1999) Cancer Res 59:4276
32. Musgrove EA, Lee CS, Cornish AL, Swarbrick A, Sutherland RL (1997) Mol Endocrinol 11:54
33. Lange CA, Richer JK, Shen T, Horwitz KB (1998) J Biol Chem 273:31308
34. Groshong SD, Owen GI, Grimison B, Schauer IE, Todd MC, Langan TA, Sclafani RA, Lange CA, Horwitz KB (1997) Mol Endocrinology 11:1593
35. Sarup JC, Rao KVS, Fox CF (1988) Cancer Res 48:5071
36. Lange CA, Richer JK, Horwitz KB (1999) Mol Endocrinol 6:829
37. Gelmann EP (2002) J Clin Oncol 20:3001
38. Matias PM, Donner P, Coelho R, Thomaz M, Peixoto C, Macedo S, Otto N, Joschko S, Scholz P, Wegg A, Basler S, Schaefer M, Egner U, Carrodono MA (2000) J Biol Chem 275:26164
39. Sommer A, Haendler B (2003) Curr Opin Drug Disc Dev 6:702
40. Brinkmann AO, Blok KL J, De Ruiter PE, Doesburg GP, Stekette K, Berrevoets CA, Trapman J (1999) Steroid Biochem Mol Biol 69:307
41. Poukka H, Karvonen U, Jaenne OA, Palvimo JJ (2000) Proc Natl Acad Sci USA 97:14145
42. Feldman BJ, Feldman D (2001) Nat Rev Cancer 1:34
43. Matias PM, Carrondo MA, Coelho R, Thomaz M, Zhao XY, Wegg A, Crusius K, Egner U, Donner P (2002) J Med Chem 45:1439
44. Sack JS, Kish KF, Wang C, Attar RM, Kiefer SE, An Y, Wu GY, Scheffler JE, Salvati ME, Krystek SR Jr, Weinmann R, Einspahr HM (2001) Proc Natl Acad Sci USA 98:4904
45. Brinkmann AO (2001) Mol Cell Endocrinol 179:105
46. Perissi V, Rosenfeld MG (2005) Nat Rev Mol Cell Biol 6:542
47. Gronemeyer H, Gustafsson JA, Laudet V (2004) Nat Rev Drug Discov 3:950
48. Liao L, Kuang SQ, Yuan Y, Gonzalez SM, O'Malley BW, Xu J (2002) Steroid Biochem Mol Biol 83:3
49. Kumar R, Wang RA, Barnes CJ (2004) Mol Carcinog 41:221
50. Kumar V, Carlson JE, Ohgi KA, Edwards TA, Rose DW, Escalante CR, Rosenfeld MG, Aggarwal AK (2002) Mol Cell 10:857
51. Gao X, Loggie BW, Nawaz Z (2002) Mol Cancer 1:7
52. Lavinsky RM, Jepsen K, Heinzel T, Torchia J, Mullen TM, Schiff R, Del-Rio AL, Ricote M, Ngo S, Gemsch J, Hilsenbeck SG, Osborne CK, Glass CK, Rosenfeld MG, Rose DW (1998) Proc Natl Acad Sci USA 95:2920
53. Yoon HG, Chan DW, Huang ZQ, Li J, Fondell JD, Qin J, Wong J (2003) EMBO J 22:1336
54. Hall JM, Chang CY, McDonnell DP (2000) Mol Endocrinol 14:2010

55. Rodriguez AL, Tamrazi A, Collins ML, Katzenellenbogen JA (2004) J Med Chem 47:600
56. Berger SL (2001) Oncogene 20:3007
57. Strahl BD, Allis CD (2000) Nature 403:41
58. Cheung E, Schwabish MA, Kraus WL (2003) EMBO J 22:600
59. Acevedo ML, Kraus WL (2003) Mol Cell Biol 23:335
60. Lösel R, Wehling M (2003) Nat Rev Mol Cell Biol 4:46
61. Govind AP, Thampan RV (2003) Mol Cell Biochem 253:233
62. Evinger AJ 3rd, Levin ER (2005) Steroids 70:361
63. Santen RJ, Song RX, Zhang Z, Kumar R, Jeng MH, Masamura S, Lawrence J Jr, MacMahon LP, Yue W, Berstein L (2005) J Steroid Biochem Mol Biol 95:155–165
64. Boonyaratanakornkit V, Edwards DP (2004) Essays Biochem 40:105
65. Filardo EJ, Quinn JA, Frackelton AR Jr, Bland KI (2002) Mol Endocrinol 16:70
66. Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER (2005) Science 307:1625
67. Thomas P, Pang Y, Filardo EJ, Dong J (2005) Endocrinology 146:624
68. Feng Y, Gregor P (1997) Biochem Biophys Res Commun 231:65
69. Owman C, Blay P, Nilsson C, Lolait SJ (1996) Biochem Biophys Res Commun 228: 285
70. Carmeci C, Thompson DA, Ring HZ, Francke U, Weigel RJ (1997) Genomics 45: 607
71. Martin LA, Farmer I, Johnston SR, Ali S, Dowsett M (2005) Endocr Relat Cancer 12:75
72. Dowsett M, Folkerd E, Doody D, Haynes B (2005) Breast 14:452
73. Brueggemeier RW, Hackett JC, Diaz-Cruz ES (2005) Endocr Rev 26:331
74. Smith IE, Dowsett M (2003) N Engl J Med 348:2431
75. Wouters W, Snoeck E, De Coster R (1994) Breast Cancer Res Treat 30:89
76. Plourde PV, Dyroff M, Dukes M (1994) Breast Cancer Res Treat 30:103
77. Demers LM (1994) Breast Cancer Res Treat 30:95
78. Nishino Y, Schneider MR, Michna H, el Etreby MF (1989) J Steroid Biochem 34:435
79. Pouget C, Fagnere C, Basly JP, Besson AE, Champavier Y, Habrioux G, Chulia AJ (2002) Pharm Res 19:286
80. Wood PM, Woo LW, Humphreys A, Chander SK, Purohit A, Reed MJ, Potter BV (2005) J Steroid Biochem Mol Biol 94:123
81. Reed MJ, Purohit A, Woo LW, Newman SP, Potter BV (2005) Endocr Rev 26:171
82. Goss PE (2003) Am J Clin Oncol 26:27
83. Howell A, Cuzick J, Baum M, Buzdar A, Dowsett M, Forbes JF, Hoctin-Boes G, Houghton J, Locker GY, Tobias JS, ATAC Trialists' Group (2005) Lancet 365:60
84. Jones SE (2006) Clin Breast Cancer 6(Suppl 2):41
85. Thurlimann B, Keshaviah A, Coates AS, Mouridsen H, Mauriac L, Forbes JF, Paridaens R, Castiglione-Gertsch M, Gelber RD, Rabaglio M, Smith I, Wardly A, Price KN, Goldhirsch A, Breast International Group (BIG) 1-98 Collaborative Group (2005) N Engl J Med 353:2747
86. Ingle JN, Tu D, Pater JL, Martino S, Robert NJ, Muss HB, Piccart MJ, Castiglione M, Shepherd LE, Pritchard KI, Livingston RB, Davidson NE, Norton L, Perez EA, Abrams JS, Cameron DA, Palmer MJ, Goss PE (2006) Breast Cancer Res Treat 99(3):295 (Epub ahead of print)
87. Goss PE, Ingle JN, Martino S, Robert NJ, Muss HB, Piccart MJ, Castiglione M, Tu D, Shepherd LE, Pritchard KI, Livingston RB, Davidson NE, Norton L, Perez EA, Abrams JS, Cameron DA, Palmer MJ, Pater JL (2005) J Natl Cancer Inst 97:1262

88. Goss PE, Bondarenko IN, Manikhas GN, Pendergrass KB, Miller WH (2006) J Clin Oncol 24:525
89. Henderson D, Norbisrath G, Kerb U (1986) J Steroid Biochem 24:303
90. Berry J (2005) Clin Ther 27:1671
91. Miller WR, Anderson TJ, White S, Larionov A, Murray J, Evans D, Krause A, Dixon JM (2005) J Steroid Biochem Mol Biol 95:83
92. Lönning PE, Taylor PD, Anker G (2001) Breast Cancer Res Treat 67:111
93. Brodie AM, Lu Q, Long BJ, Fulton A, Chen T, Macpherson N, DeJong PC, Blankenstein MA, Nortier JW, Slee PH, van de Ven J, van Gorp JM, Elbers JR, Schipper ME, Blijham GH, Thijssen JH (2001) J Steroid Biochem Mol Biol 79:41
94. Osborne CK, Arteaga CL (2003) J Clin Oncol 21:285
95. Luu-The V (2001) Steroid Biochem Mol Biol 76:143
96. Poirier D (2003) Curr Med Chem 10:453
97. Tremblay MR, Poirier D (1998) J Steroid Biochem Molec Biol 66:179
98. Pasqualini JR, Chetrite GS (2005) J Steroid Biochem Mol Biol 93:221
99. Allan GM, Lawrence HR, Cornet J, Bubert C, Fischer DS, Vicker N, Smith A, Tutill HJ, Purohit A, Day JM, Mahon MF, Reed MJ, Potter BV (2006) J Med Chem 49:1325
100. Luu-The V, Zhang Y, Poirier D, Labrie F (1995) J Steroid Biochem Mol Biol 55:581
101. Santner SJ, Santen RJ (1993) J Steroid Biochem Mol Biol 45:383
102. Poutanen M, Moncharmont B, Vihko R (1992) Cancer Res 52:290
103. Reed MJ, Purohit A, Woo LW, Newman SP, Potter BV (2005) Endocr Rev 26:171
104. Suzuki T, Miki Y, Nakamura Y, Moriya T, Ito K, Ohuchi N, Sasano H (2005) Endocr Relat Cancer 12:701
105. Ahmed S, James K, Owen CP (2002) J Steroid Biochem Mol Biol 82:425
106. Zhu BT, Fu JH, Xu S, Kauffman FC, Conney AH (1998) Biochem Biophys Res Commun 246:45
107. Woo LW, Howarth NM, Purohit A, Hejaz HA, Reed MJ, Potter BV (1998) J Med Chem 41:1068
108. Winum JY, Scozzafava A, Montero JL, Supuran CT (2005) Med Res Rev 25:186
109. Ahmed S, James K, Owen CP, Patel CK, Patel MB (2001) Bioorg Med Chem Lett 11:3001
110. Ahmed S, Owen CP, James K, Sampson L, Patel CK (2002) Curr Med Chem 9:263
111. Walter G, Liebl R, von Angerer E (2004) Arch Pharm (Weinheim) 337:634
112. Shields-Botella J, Bonnet P, Duc I, Duranti E, Meschi S, Cardinali S, Prouheze P, Chaigneau AM, Duranti V, Gribaudo S, Riviere A, Mengual L, Carniato D, Cecchet L, Lafay J, Rondot B, Sandri J, Pascal JC, Delansorne R (2003) J Steroid Biochem Mol Biol 84:327
113. Woo LW, Sutcliffe OB, Bubert C, Grasso A, Chander SK, Purohit A, Reed MJ, Potter BV (2003) J Med Chem 46:3193
114. Russell DW, Wilson JD (1994) Annu Rev Biochem 63:25
115. Steers WD (2001) Urology 58:17
116. Katz MD, Cai LQ, Zhu YS, Herrera C, DeFillo-Ricart M, Shackleton CH, Imperato-McGinley J (1995) J Clin Endocrinol Metab 80:3160
117. Gerst C, Dalko M, Pichaud P, Galey JB, Buan B, Bernard BA (2002) Exp Dermatol 11:52
118. Bratoeff E, Cabeza M, Ramirez E, Heuze Y, Flores E (2005) Curr Med Chem 12:927
119. Frye SV (2006) Curr Top Med Chem 6:405
120. Ranjan M, Diffley P, Stephen G, Price D, Walton TJ, Newton RP (2002) Life Sci 71: 115
121. Occhiato EG, Guarna A, Danza G, Serio M (2004) J Steroid Biochem Mol Biol 88:1

122. Hirsch KS, Jones CD, Audia JE, Andersson S, McQuaid L, Stamm NB, Neubauer BL, Pennington P, Toomey RE, Russell DW (1993) Proc Natl Acad Sci USA 90:5277
123. Ferrali A, Menchi G, Occhiato EG, Danza G, Mancina R, Serio M, Guarna A (2005) Bioorg Med Chem Lett 15:145
124. Occhiato EG, Ferrali A, Menchi G, Guarna A, Danza G, Comerci A, Mancina R, Serio M, Garotta G, Cavalli A, De Vivo M, Recanatini M (2004) J Med Chem 47:3546
125. Salem OI, Frotscher M, Scherer C, Neugebauer A, Biemel K, Streiber M, Maas R, Hartmann RW (2006) J Med Chem 49:748
126. Ishibashi K, Nakajima K, Sugioka Y, Sugiyama M, Hamada T, Horikoshi H, Nishi T (1999) Chem Pharm Bull 47:226
127. Fukuta Y, Fukuda Y, Higashino R, Yoshida K, Ogishima M, Tamaki H, Takei M (1999) J Pharmacol Exp Ther 290:1013
128. Tarter TH, Vaughan ED Jr (2006) Curr Pharm Des 12:775
129. Marks LS, Partin AW, Epstein JI, Tyler VE, Simon I, Macairan ML, Chan TL, Dorey FJ, Garris JB, Veltri RW, Santos PB, Stonebrook KA, deKernion JB (2000) J Urol 163: 1451
130. Foley CL, Kirby RS (2003) Curr Opin Urol 13:31
131. Djavan B, Milani S, Fong YK (2005) Expert Opin Pharmacother 6:311
132. Roehrborn CG, Lukkarinen O, Mark S, Siami P, Ramsdell J, Zinner N (2005) BJU Int 96:572
133. Thomas LN, Lazier CB, Gupta R, Norman RW, Troyer DA, O'Brien SP, Rittmaster RS (2005) Prostate 63:231
134. Iczkowski KA, Qiu J, Qian J, Somerville MC, Rittmaster RS, Andriole GL, Bostwick DG (2005) Urology 65:76
135. Gomella LG (2005) Curr Opin Urol 15:29
136. Leroux F (2005) Curr Med Chem 12:1623
137. Barrie SE, Haynes BP, Potter GA, Chan FC, Goddard PM, Dowsett M, Jarman M (1997) J Steroid Biochem Mol Biol 60:347
138. Njar VC, Brodie AM (1999) Curr Pharm Des 3:163
139. Haidar S, Ehmer PB, Barassin S, Batzl-Hartmann C, Hartmann RW (2003) J Steroid Biochem Mol Biol 84:555
140. Hartmann RW, Palusczak A, Lacan F, Ricci G, Ruzziconi R (2004) J Enzyme Inhib Med Chem 19:145
141. Fisher B (1999) Eur J Cancer 35:1963
142. Levenson AS, Jordan VC (1999) Eur J Cancer 35:1974
143. Robertson JF (2004) Cancer Treat Rev 30:695
144. Cauley JA, Norton L, Lippman ME, Eckert S, Krueger KA, Purdie DW, Farrerons J, Karasik A, Mellstrom D, Ng KW, Stepan JJ, Powles TJ, Morrow M, Costa A, Silfen SL, Walls EL, Schmitt H, Muchmore DB, Jordan VC, Ste-Marie LG (2001) Breast Cancer Res Treat 65:125
145. Sommer S, Fuqua SA (2001) Semin Cancer Biol 11:339
146. Wakeling AE, Dukes M, Bowler J (1991) Cancer Res 51:3867
147. Bohlmann R, Bittler D, Fritzemeier KH, Heinrich N, Hoffmann J, Hofmeister H, Künzer H. Lessl, Lichtner R, Nishino Y, Parczyk K, Sauer G, Schneider MR (2001) Cancer Res Clin Oncol 127:42
148. Hoffmann J, Bohlmann R, Heinrich N, Hofmeister H, Kroll J, Kunzer H, Lichtner RB, Nishino Y, Parczyk K, Sauer G, Gieschen H, Ulbrich HF, Schneider MR (2004) J Natl Cancer Inst 96:210
149. Jordan VC (2003) J Med Chem 46:883
150. Munster PN (2006) Expert Opin Investig Drugs 15:317

151. Gennari L (2005) Curr Opin Investig Drugs 6:1067
152. Gauthier S, Caron B, Cloutier J, Dory YL, Favre A, Larouche D, Mailhot J, Ouellet C, Schwerdtfeger A, Leblanc G, Martel C, Simard J, Merand Y, Belanger A, Labrie C, Labrie F (1997) J Med Chem 40:2117
153. Labrie F, Labrie C, Belanger A, Simard J, Giguere V, Tremblay A, Tremblay G (2001) J Steroid Biochem Mol Biol 79:213
154. Willson TM, Norris JD, Wagner BL, Asplin I, Baer P, Brown HR, Jones SA, Henke B, Sauls H, Wolfe S, Morris DC, McDonnell DP (1997) Endocrinology 138:3901
155. Wu YL, Yang X, Ren Z, McDonnell DP, Norris JD, Willson TM, Greene GL (2005) Mol Cell 18:413
156. Komm BS, Kharode YP, Bodine PV, Harris HA, Miller CP, Lyttle CR (2005) Endocrinology 146:3999
157. Buzdar AU (2005) Clin Cancer Res 11:906
158. Vogel CL (1998) Oncology (Williston Park) 12:9
159. Holli K (2002) Eur J Cancer 38:37
160. Marttunen MB, Hietanen P, Tiitinen A, Ylikorkala O (1998) J Clin Endocrinol Metab 83:1158
161. Buzdar A, Hayes D, El-Khoudary A, Yan S, Lonning P, Lichinitser M, Gopal R, Falkson G, Pritchard K, Lipton A, Wolter K, Lee A, Fly K, Chew R, Alderdice M, Burke K, Eisenber P (2002) Breast Cancer Res Treat 73:161
162. Albertazzi P, Sharma S (2005) Climacteric 8:214
163. Martino S, Cauley JA, Barrett-Connor E, Powles TJ, Mershon J, Disch D, Secrest RJ, Cummings SR (2004) J Natl Cancer Inst 96:1751
164. Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN, Bevers TB, Fehrenbacher L, Pajon ER Jr, Wade JL III, Robidoux A, Margolese RG, James J, Lippman SM, Runowicz CD, Ganz PA, Reis SE, McCaskill-Stevens W, Ford LG, Jordan VC, Wolmark N (2006) JAMA 295:2727
165. Gruber C, Gruber D (2004) Curr Opin Investig Drugs 5:1086
166. Kelloff GJ, Lippman SM, Dannenberg AJ, Sigman CC, Pearce HL, Reid BJ, Szabo E, Jordan VC, Spitz MR, Mills GB, Papadimitrakopoulou VA, Lotan R, Aggarwal BB, Bresalier RS, Kim J, Arun B, Lu KH, Thomas ME, Rhodes HE, Brewer MA, Follen M, Shin DM, Parnes HL, Siegfried JM, Evans AA, Blot WJ, Chow WH, Blount PL, Maley CC, Wang KK, Lam S, Lee JJ, Dubinett SM, Engstrom PF, Meyskens FL Jr, O'Shaughnessy J, Hawk ET, Levin B, Nelson WG, Hong WK (2006) Clin Cancer Res 12:3661
167. Sato M, Turner CH, Wang T, Adrian MD, Rowley E, Bryant HU (1998) J Pharmacol Exp Ther 287:1
168. Fabian CJ, Kimler BF, Anderson J, Tawfik OW, Mayo MS, Burak WE Jr, O'Shaughnessy JA, Albain KS, Hyams DM, Budd GT, Ganz PA, Sauter ER, Beenken SW, Grizzle WE, Fruehauf JP, Arneson DW, Bacus JW, Lagios MD, Johnson KA, Browne D (2004) Clin Cancer Res 10:5403
169. Labrie F, Champagne P, Labrie C, Roy J, Laverdiere J, Provencher L, Potvin M, Drolet Y, Pollak M, Panasci L, L'Esperance B, Dufresne J, Latreille J, Robert J, Samson B, Jolivet J, Yelle L, Cusan L, Diamond P, Candas B (2004) J Clin Oncol 22:864
170. Wakeling AE (1993) J Steroid Biochem Mol Biol 47:107
171. Wijayaratne AL, McDonnell DP (2001) J Biol Chem 276:35684
172. Howell A, Pippen J, Elledge RM, Mauriac L, Vergote I, Jones SE, Come SE, Osborne CK, Robertson JF (2005) Cancer 104:236
173. Robertson JF, Come SE, Jones SE, Beex L, Kaufmann M, Makris A, Nortier JW, Possinger K, Rutqvist LE (2005) Eur J Cancer 41:346

174. Ismail PM, Amato P, Soyal SM, DeMayo FJ, Conneely OM, O'Malley BW, Lydon JP (2003) Steroids 68:779
175. Parczyk K, Schneider MR (1996) J Cancer Res Clin Oncol 122:383
176. Michna H, Nishino Y, Neef G, McGuire WL, Schneider MR (1992) J Steroid Biochem Mol Biol 41:339
177. Brisken C, Park S, Vass T, Lydon JP, O'Malley BW, Weinberg RA (1998) Proc Natl Acad Sci USA 95:5076
178. Lydon JP, Ge G, Kittrell FS, Medina D, O'Malley BW (1999) Cancer Res 59:4276
179. Chwalisz K, Perez MC, Demanno D, Winkel C, Schubert G, Elger W (2005) Endocr Rev 26:423
180. Belanger A, Philibert D, Teutsch G (1981) Steroids 37:361
181. Klijn JGM, Setyono-Han B, Foekens JA (2000) Steroids 65:825
182. Elger W, Bartley J, Schneider B, Kaufmann G, Schubert G, Chwalisz K (2000) Steroids 65:713
183. Fuhrmann U, Hess-Stumpp H, Cleve A, Neef G, Schwede W, Hoffmann J, Fritzemeier KH, Chwalisz K (2000) J Med Chem 43:5010
184. Negro-Vilar A (2000) J Soc Gynecol Investig 7:53
185. Allan GF, Sui Z (2005) Mini Rev Med Chem 5:701
186. Terefenko EA, Kern J, Fensome A, Wrobel J, Zhu Y, Cohen J, Winneker R, Zhang Z, Zhang P (2005) Bioorg Med Chem Lett 15:3600
187. Schneider MR, Michna H, Nishino Y, el Etreby MF (1989) Eur J Cancer Clin Oncol 25:691
188. Hoffmann J, Lichtner RB, Fuhrmann U, Michna H, Parczyk K, Neef G, Chwalisz K, Schneider MR (2002) Effects of Progesterone Receptor Antagonists on Breast Cancer. In: Robertson JFR, Nicholson RI, Hayes DF (eds) Endocrine Management of Breast Cancer. Dunitz
189. Hoffmann J, Sommer A (2005) J Steroid Biochem Mol Biol 93:191
190. Michna H, Gehring S, Kuhnel W, Nishino Y, Schneider MR (1992) J Steroid Biochem Mol Biol 43:203
191. Klijn JG, Setyono-Han B, Sander HJ, Lamberts SW, de Jong FH, Deckers GH, Foekens JA (1994) Hum Reprod 9:181
192. Jonat W, Giurescu M, Robertson JFR (2002) The Clinical Efficacy of Progesterone Antagonists in Breast Cancer. In: Robertson JFR, Nicholson RI, Hayes DF (eds) Endocrine Management of Breast Cancer. Martin Dunitz Ltd, London
193. Robertson JF, Willsher PC, Winterbottom L, Blamey RW, Thorpe S (1999) Eur J Cancer 35:214
194. El Etreby MF, Liang Y (1998) Breast Cancer Res Treat 49:109
195. Chwalisz K, Perez MC, Demanno D, Winkel C, Schubert G, Elger W (2005) Endocr Rev 26:423
196. Chabbert-Buffet N, Meduri G, Bouchard P, Spitz IM (2005) Hum Reprod Update 11:293
197. Anderson J (2003) BJU Int 91:455
198. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanen R, Palmberg C, Palotie A, Tammela T, Isola J, Kallioniemi OP (1995) Nat Genet 9:401
199. Zhao XY, Malloy PJ, Krishnan AV, Swami S, Navone NM, Peehl DM, Feldman D (2000) Nat Med 6:703
200. Culig Z, Hobisch A, Cronauer MV, Radmayr C, Trapman J, Hittmair A, Bartsch G, Klocker H (1994) Cancer Res 54:5474
201. Culig Z, Bartsch G, Hobisch A (2004) Curr Cancer Drug Target 4:455
202. Neumann F (1994) Exp Clin Endocrinol 102:1

203. Gao W, Bohl CE, Dalton JT (2005) Chem Rev 105:3352
204. Salvati ME, Balog A, Wei DD, Pickering D, Attar RM, Geng J, Rizzo CA, Hunt JT, Gottardis MM, Weinmann R, Martinez R (2005) Bioorg Med Chem Lett 15:389
205. Bohl CE, Chang C, Mohler ML, Chen J, Miller DD, Swaan PW, Dalton JT (2004) J Med Chem 47:3765
206. Gao W, Reiser PJ, Coss CC, Phelps MA, Kearbey JD, Miller DD, Dalton JT (2005) Endocrinology 146:4887
207. Bohl CE, Miller DD, Chen J, Bell CE, Dalton JT (2005) J Biol Chem 280:37747
208. Furr BJ, Tucker H (1996) Urology 47:13
209. Tyrrell CJ, Denis L, Newling D, Soloway M, Channer K, Cockshott ID (1998) Eur Urol 33:39
210. Tyrrell CJ, Kaisary AV, Iversen P, Anderson JB, Baert L, Tammela T, Chamberlain M, Webster A, Blackledge G (1998) Eur Urol 33:447
211. Iversen P, Tyrrell CJ, Kaisary AV, Anderson JB, Van Poppel H, Tammela TL, Chamberlain M, Carroll K, Melezinek I (2000) J Urol 164:1579
212. Denis L (1994) Prostate 5:17
213. Denis LJ, Keuppens F, Smith PH, Whelan P, de Moura JL, Newling D, Bono A, Sylvester R (1998) Eur Urol 33:144
214. Eisenberger MA, Blumenstein BA, Crawford ED, Miller G, McLeod DG, Loehrer PJ, Wilding G, Sears K, Culkin DJ, Thompson IM Jr, Bueschen AJ, Lowe BA (1998) N Engl J Med 339:1036
215. Schmitt B, Wilt TJ, Schellhammer PF, DeMasi V, Sartor O, Crawford ED, Bennett CL (2001) Urology 57:727
216. Leibowitz RL, Tucker SJ (2001) Oncologist 6:177
217. Dawson NA (2000) Curr Oncol Rep 2:409
218. Grossfeld GD, Chaudhary UB, Reese DM, Carroll PR, Small EJ (2001) Urology 58:240
219. Mcleod DG (2002) Urology 60:13
220. Eder IE, Hoffmann J, Rogatsch H, Schafer G, Zopf D, Bartsch G, Klocker H (2002) Cancer Gene Ther 9:117
221. Ingle JN (2002) Breast Cancer Res 4:133
222. Mabjeesh NJ, Escuin D, LaVallee TM, Pribluda VS, Swartz GM, Johnson MS, Willard MT, Zhong H, Simons JW, Giannakakou P (2003) Cancer Cell 3:363
223. Pasqualini JR, Ebert C, Chetrite GS (2001) Gynecol Endocrinol 6:44
224. Lundgren S, Helle SI, Lonning PE (1996) Clin Cancer Res 2:1515
225. Schally AV (1999) Peptides 20:1247
226. Florio S, Pagnini U, Crispino A, Pacilio C, Crispino L, Giordano A (2002) Front Biosci 7:1590
227. Beckers T, Reilander H, Hilgard P (1997) Anal Biochem 251:17
228. Hovelmann S, Hoffmann SH, Kuhne R, ter Laak T, Reilander H, Beckers T (2002) Biochemistry 41:1129
229. Kiesel LA, Rody A, Greb RR, Szilagyi A (2002) Clin Endocrinol (Oxf) 56:677
230. Schally AV, Halmos G, Rekasi Z, Arencibia JM (2001) The actions of LH-RH agonists, antagonists, and cytotoxic analogs on the LH-RH receptors on the pituitary and tumors. In: Devroey P (ed) Infertility and reproductive medicine clinics of North America. Saunders, Philadelphia, pp 12, 17–44
231. Pinsky J, Lamharzi N, Halmos G, Groot K, Jungwirth A, Vadillo-Buenfil M, Kakar SS, Schally AV (1996) Endocrinology 137:3430
232. Schally AV, Comaru-Schally AM, Plonowski A, Nagy A, Halmos G, Rekasi Z (2000) The Prostate 45:158
233. Denmeade SR, Isaacs JT (2002) Nat Rev Cancer 2:389

234. Gonzalez-Barcena D, Vadillo Buenfil M, Garcia Procel E, Guerra-Arguero L, Cardenas Cornejo I, Comaru-Schally AM, Schally AV (1994) Eur J Endocrinol 131:286
235. Jonat W (2002) Eur J Cancer 38:39
236. Robertson JF, Blamey RW (2003) Eur J Cancer 39:861
237. Emons G, Schulz KD (2000) Rec Res Cancer Res 153:83
238. Asbury RF, Brunetto VL, Lee RB, Reid G, Rocereto TF (2002) Am J Clin Oncol 25(6):557
239. Hall JM, McDonnell DP (2005) Mol Interv 5:343

Top Med Chem (2007) 1: 83–132
DOI 10.1007/7355_2006_004

Published online: 13 December 2006

Inhibition of Growth Factor Signaling by Small-Molecule Inhibitors of ErbB, Raf, and MEK

Eli M. Wallace (✉) · Tammie C. Yeh · Ellen R. Laird · James F. Blake · Joseph Lyssikatos

Array BioPharma, Inc., 3200 Walnut Street, Boulder, CO 80301, USA
ewallace@arraybiopharma.com

1 Introduction . . . 85

2 Biological Background and Rationale . . . 85

3 Structural Biology . . . 88
3.1 EGFR . . . 89
3.2 B-Raf . . . 92
3.3 MEK1 and MEK2 . . . 93

4 Inhibitors of the ErbB Family . . . 95
4.1 Selective Inhibitors Targeting Individual ErbB Family Members . . . 96
4.1.1 Erlotinib . . . 96
4.1.2 Gefitinib . . . 99
4.1.3 Erlotinib and Gefitinib: Comparison of Clinical Outcomes . . . 101
4.1.4 CP-724714 . . . 101
4.2 EGFR/ErbB2 Dual Inhibitors . . . 102
4.2.1 Lapatinib . . . 102
4.2.2 BMS 599626 . . . 105
4.2.3 ARRY-334543 . . . 106
4.2.4 Canertinib . . . 106
4.2.5 BIBW 2992 . . . 107
4.2.6 HKI-272 . . . 107
4.3 Direct Comparison of Select EGFR/ErbB2 Inhibitors . . . 108
4.4 Multi-Kinase Inhibitors . . . 109
4.4.1 AEE788 . . . 109
4.4.2 XL647 . . . 111

5 Inhibitors of Raf . . . 113

6 Inhibitors of MEK . . . 117
6.1 ARRY-142886 (AZD6244) . . . 117
6.2 PD0325901 . . . 120

7 Biomarkers . . . 121

8 Conclusions and Outlook . . . 124

References . . . 126

Abstract Approval of Gleevec and its demonstrated therapeutic value marked the full recognition of kinase inhibitors as a relevant means of cancer treatment. A significant number of kinase inhibitors have recently entered clinical practice for the treatment of a variety of cancers and many others are in advanced stages of clinical research. Among these are several promising agents that act in the pathway that initiates with the growth factor receptors EGFR/ErbB2 and signals through Raf and MEK to the MAP kinase ERK. This chapter focuses on the biological rationale, enzymatic and pharmacological properties, and clinical status of several inhibitors of EGFR/ErbB2, Raf, and MEK1/2. Coverage is given of those compounds that have gained approval or are being evaluated in human clinical trials.

Keywords EGFR inhibitor · ErbB2 inhibitor · Raf inhibitor · MEK inhibitor · Kinase inhibitor · Cancer · ERK

Abbreviations

Abl	Abelson leukemia virus tyrosine kinase
Akt	Protein kinase B (PKB)
AUC	Area under the curve
Bcr-Abl	Breakpoint cluster region–Abelson leukemia virus tyrosine kinase
bid	Twice a day
CDK2	Cyclin-dependent kinase 2
CML	Chronic myelogenous leukemia
DLT	Dose-limiting toxicity
EGF	Epidermal growth factor
EGFR (ErbB1, HER-1)	Epidermal growth factor receptor
ErbB2	HER-2
ErbB3	HER-3
ErbB4	HER-4
ERK1/2	Extracellular signal-regulated kinase 1/2
ERK5	Extracellular-signal regulated kinase 5
FAK	Focal adhesion kinase
FGF	Fibroblast growth factor
FGFR	Fibroblast growth factor receptor
FISH	Fluorescence in situ hybridization
Flt1	Fms-like tyrosine kinase 1
Flt3	Fms-like tyrosine kinase 3
HER	Human epidermal growth factor receptor
HGFR	Hepatocyte growth factor receptor
Hsp27	Heat shock protein 27
Hsp90	Heat shock protein 90
IGF-1R	Insulin-like growth factor-1 receptor
IR	Insulin receptor
IRK	Insulin receptor kinase
JAK	Janus kinase
KDR (VEGFR2)	Kinase domain receptor (vascular endothelial growth factor receptor 2)
MEK1/2	Mitogen-activated protein kinase kinase 1/2
MTD	Maximum tolerated dose
NSCLC	Non-small cell lung cancer

P38	p38 Mitogen-activated protein kinase
PAK	p21 Activated kinase
PBMC	Peripheral blood mononuclear cells
PDGFR	Platelet-derived growth factor receptor
PFS	Progression-free survival
PI-3 kinase	Phosphatidylinositol 3-kinase
PKA	Protein kinase A or cAMP-dependent protein kinase
PMA	Phorbol 12-myristate 13-acetate
qd	Once a day
RCC	Renal cell carcinoma
RMSD	Root mean square deviation
SCID	Severe combined immunodeficiency
SOS	Son of sevenless
STAT	Signal transducer and activator of transcription
TGFα	Transforming growth factor α
TGI	Tumor growth inhibition
tid	three times a day
VEGF	Vascular endothelial growth factor

1 Introduction

Cancer can result from dysregulated growth factor signaling. Given that many kinases play pivotal roles in this process, it is not surprising that several kinase inhibitors are now at the forefront of drug discovery for cancer treatment. This chapter will focus on several of the major kinase targets in growth factor signaling (e.g., EGFR/ErbB2, Raf, and MEK1/2) and the development of principal compounds in each class. Covering this important area in some depth requires limiting this review to small-molecule agents that have been either approved or evaluated in human clinical studies. The characterization of these compounds in enzymatic, cellular, animal, and human clinical studies will be discussed.

2 Biological Background and Rationale

Growth factors regulate cellular proliferation, differentiation, apoptosis, migration, and invasion by binding to their cognate receptors, which are expressed on the surface of specific cells. These receptors contain an extracellular ligand-binding domain, a single transmembrane domain, and an intracellular tyrosine kinase catalytic domain. As summarized in Fig. 1, the binding of growth factor leads to the activation of the receptor tyrosine kinase which results in the activation of the G protein Ras. Activated Ras initiates the kinase cascade which begins with Raf. Raf phosphorylates and activates MEK1

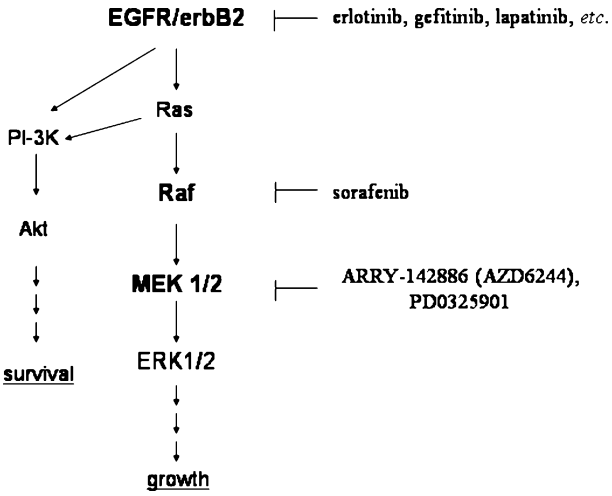


Fig. 1 Targets of interest (*bold*) that are reviewed in this chapter

and 2, which in turn phosphorylate and activate ERK1 and 2. ERK1/2 proceed to phosphorylate other downstream proteins, including transcription factors, which will determine the overall cellular response, such as growth. In addition to Raf/MEK/ERK, other downstream signaling modules can also be activated, such as the PI-3 kinase/Akt pathway, usually associated with the propagation of survival signals, which is the subject of another chapter (Garcia-Echeverria et al., in this volume).

There are more than 70 members of the tyrosine kinase family of growth factor receptors. Overexpression and/or mutation of many of these receptors, such as EGFR, ErbB2, KDR (VEGFR2), PDGFR, IGF-1R, MET, and RET, have been identified and implicated in human cancers [1–4]. The EGFR and ErbB2 signaling pathways will be highlighted here. There are four members of the ErbB family of growth factor receptors: EGFR (epidermal growth factor receptor, ErbB1, HER-1), ErbB2 (HER-2, neu), ErbB3 (HER-3), and ErbB4 (HER-4). EGF, TGFα, and other EGF-related peptide growth factors bind to EGFR, whereas heregulin and other neuregulins bind to ErbB3 and ErbB4. There are no known ligands for ErbB2, but it does contain a functional kinase domain. In contrast, ErbB3 lacks an active catalytic domain. Consequently, in order to compensate for their missing functions, ErbB2 and ErbB3 participate in heterodimers with other ErbB family members.

Overexpression of ErbB2 has been observed in cancer, particularly in breast cancer [5]. Trastuzumab, a monoclonal antibody directed against ErbB2 for treating ErbB2-positive breast cancer, was the first agent to validate growth factor receptors as molecular targets for therapeutic intervention [6]. Overexpression of EGFR has also been detected in many human cancers, including more than 65–85% of non-small cell lung cancer (NSCLC), and has

been associated with poor prognosis [2]. Deletion or point mutations that result in increased EGFR activity have been detected in human tumors as well. For example, ca. 50% of human glioblastomas express a form of EGFR that is missing part of the extracellular region (EGFRvIII), which results in ligand-independent kinase activity [7]. Recently, NSCLC patients who responded favorably to an EGFR inhibitor were shown to have mutations in the EGFR catalytic domain that can lead to increased and prolonged kinase activity [8, 9]. The identification of receptor overexpression and overactive mutant forms has led to a significant effort toward the development of small-molecule inhibitors of the ErbB family receptor tyrosine kinases.

Activation of the ErbB receptors, usually through ligand binding, leads to the formation of homo- and heterodimers and the transphosphorylation of tyrosine residues that subsequently serve as docking sites for signaling proteins, such as the p85 subunit of PI-3 kinase, Src, PLC-γ, STAT proteins, and Grb2. Membrane-associated Ras, a G protein with intrinsic GTPase activity, exists in either an active GTP-bound form or an inactive GDP-bound form. When SOS, a GTP–GDP nucleotide exchange factor, is recruited to the receptor complex through its association with Grb2, it catalyzes the exchange of GDP for GTP, resulting in an increase in the active GTP-bound form of Ras.

Ras, a historical proto-oncogene, is frequently mutated in many human cancers, including 90% of pancreatic cancers, 50% of colorectal cancers, 30% of lung cancers, and 15–30% of melanomas [10–12]. There are three Ras genes that encode four family members: K-Ras (two alternatively spliced isoforms), H-Ras, and N-Ras. Mutations are most commonly found in K-Ras [13]. These mutations result in impaired GTP hydrolysis, which shifts the equilibrium toward GTP-bound active Ras, and results in constitutive intracellular signaling.

In order for Ras to function properly, the protein must be localized to the plasma membrane. This is achieved by the addition of a farnesyl group to a cysteine residue near the carboxy terminus, which then acts as a tether to the cellular membrane. Farnesyl transferase, the enzyme that adds the farnesyl moiety to Ras, is also a target for small-molecule intervention. This class of inhibitors is the subject of another chapter (Angibaud et al., in this volume).

The binding of GTP to Ras induces a conformational change, resulting in the unveiling of a high-affinity binding region for downstream effector proteins. Although Raf is the best studied effector protein, Ras also interacts with the p110 subunit of PI-3 kinase, ralGEF proteins, AF-6 and RASSF adaptor proteins, and the IMP E3 ubiquitin ligase [14]. Interestingly, mutations in Ras and B-Raf appear to be mutually exclusive, suggesting that, at least in the context of human cancer, Raf may be the most relevant effector in Ras-dependent oncogenic signaling. It is not surprising that K-Ras, the most prevalent mutated form of Ras, is also the most potent activator of Raf [15]. Ras induces the translocation of Raf to the plasma membrane, where it can be phosphorylated and activated by other kinases in the vicinity, such as PAK, Src, and JAK.

There are three members of the Raf family: A-Raf, B-Raf, and C-Raf (Raf-1). C-Raf was first identified as the cellular homolog of the *v-Raf* oncogene and has been the subject of extensive study over the last two decades. However, in recent years, the focus has shifted to B-Raf after it was discovered that point mutations in B-Raf are prevalent (approximately 65%) in human melanomas [16]. Subsequently, point mutations in B-Raf have been identified in other cancers including 40–70% of papillary thyroid cancers, 60% of low-grade ovarian tumors, and 4–16% of colorectal cancers [17]. The most prevalent point mutation in B-Raf, V600E, results in a constitutively active B-Raf. Presumably, the negatively charged glutamate mimics the phosphorylation of a neighboring serine or threonine residue required for activation. Conversely, mutations in A-Raf and C-Raf are rare in human cancer [18, 19]. Unlike A-Raf and C-Raf, B-Raf is primed for activation because the other critical residues that need to be phosphorylated are either already constitutively phosphorylated or are replaced by a negatively charged aspartate residue.

The two principal substrates for Raf are MEK1 and MEK2, which are dual-specificity kinases that can phosphorylate both serine/threonine and tyrosine residues. MEK1 and 2 share 80% sequence identity in the catalytic domain with 100% identity within the ATP binding site. The role of these enzymes in oncogenesis has not been differentiated. As such, the discussions that follow will generally not distinguish them. Interestingly, the enzymes do play different roles in development, as the MEK2 knockout is viable while the MEK1 knockout is lethal [20, 21].

Upon activation, MEK1/2 phosphorylate and activate the serine/threonine kinases ERK1 and 2. As studies have yet to define distinct roles of ERK1 and 2 in cancer, they will also be grouped together in the discussions that follow. ERK1/2 translocate to the nucleus where they phosphorylate transcription factors, resulting in specific changes in gene transcription that ultimately influence cellular responses. Although MEK1/2 are not frequently mutated in human cancers, elevated ERK1/2 phosphorylation is detected in numerous cancers, which reflects the convergence of various oncogenic signals (e.g., overexpressed/mutated EGFR, mutated Ras, or mutated B-Raf) at this point [22].

From the identification of abnormally active signaling proteins to the use of molecularly targeted agents, the role of the EGFR/ErbB2–Raf–MEK1/2–ERK1/2 pathway in growth factor signaling and oncogenesis has been clearly established.

3 Structural Biology

Despite more than 10 years of clinical interest in EGFR signaling, there are remarkably few structural biology reports for targets in this central path-

way. X-ray crystal structures of EGFR complexed with two inhibitors (erlotinib [23] and lapatinib [24]) have been described in the literature and deposited with the Protein Data Bank. Available structures of B-Raf are limited to low-resolution complexes with sorafenib [25], and only cocrystals of MEK with two related allosteric inhibitors have been reported [26]. Although few in number, these crystal structures reveal some of the more unusual examples of induced fit and allosteric binding available for kinases. It is also important to note that none of these structures was available at the time that the inhibitors were first disclosed. The structural biology is insightful in evaluation of advanced compounds, and these complexes are undoubtedly in use by several researchers in devising and optimizing new chemical matter.

3.1 EGFR

The first reported X-ray structures of the catalytic domain of EGFR consist of unbound enzyme and a single complex with erlotinib (Fig. 2) [23]. The standard kinase fold is evident, wherein a smaller N-proximal lobe consisting largely of β-sheet is connected to a helix-rich C-proximal lobe via a single hinge-like strand that interacts with ATP and ATP-competitive inhibitors [27, 28]. Ligand binding does not alter the structure; the backbone RMSD of the two structures is 0.6 Å and the side change positions are comparable.

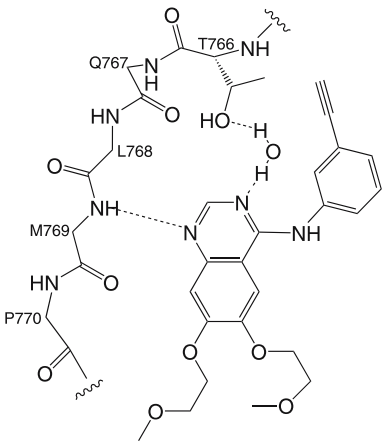


Fig. 2 X-ray structure of erlotinib and the catalytic domain of EGFR

The binding mode of the aminoquinazoline inhibitor, erlotinib, is consistent with that which had been observed previously for the intracellular kinases CDK2 and p38 [29]. The N1 and C8 edge of the heterocycle is oriented toward the hinge, with N1 accepting a hydrogen bond from Met769. N3

participates in a water-mediated hydrogen bond to the side chain of Thr766, and the aniline NH serves as a conformational device that directs the phenyl group into the gatekeeper pocket of the enzyme.

There are two distinguishing features of the EGFR family of receptor tyrosine kinases. First, they do not require phosphorylation for catalytic activity [30]. Second, an intracellular dimerization motif is presumed to exist between the catalytic domain and the C-terminus [31, 32]. The apo and binary X-ray structures of EGFR offer plausible explanations for both of these observations: most kinases require phosphorylation on their activation loop in order to adopt a stable fold that is sterically and catalytically capable of binding a substrate. Crystal structures of both the non-phosphorylated and phosphorylated forms are available for a few kinases (e.g., IRK [33], Lck [34]). In the non-phosphorylated state, disorder of the activation loop is evident and appears to adopt many random conformations. In contrast, phosphorylated structures present a consistent loop conformation that is closely wrapped against the C-lobe. The apo structure of EGFR reveals an activation loop that is comparable to that observed in phosphorylated kinases such as Lck and IRK [23]. This competent loop structure likely owes its stabilization to the presence of Glu848, whose interaction with Tyr845 is strikingly similar to pTyr394 in phosphorylated Lck. The Glu848 carboxylate can mimic the phosphate interaction with Arg812, which precedes the catalytic Asp and whose interaction appears important in bringing the catalytic elements into proximity. The interdomain angle in the EGFR structures is also comparable to that seen in phosphorylated kinases such as Lck and IRK. These observations are consistent with the fact that Tyr845 can be mutated to a Phe without detriment to activity [30].

The expression construct used for the X-ray structure (residues 672–998) includes a motif that is implicated in regulation of oligomerization and transphosphorylation in EGFR family complexes [35]. This Leu955-Val-Ile (LVI) motif has been postulated to contact another protein directly, as mutations (particularly at Leu955) reduce transphosphorylation. In this structure, the LVI motif is associated with the C-lobe, and Leu955 in particular is buried in a hydrophobic pocket. Loss of this contact likely leads to a significant conformational change and availability of the carboxyl terminus as a substrate.

The original structure of EGFR in complex with erlotinib revealed the general binding mode for quinazoline inhibitors, but could not offer a rationale for the differences in inhibition profiles between them. For example, erlotinib and gefitinib are EGFR-selective, while the structurally related lapatinib is a dual inhibitor of EGFR and ErbB2 (vide infra). Additionally, the erlotinib X-ray complex was generated by soaking the inhibitor into the preformed apo crystals. The recent structure of EGFR cocrystallized with lapatinib reveals significant differences that provide insights into inhibitor behavior [24].

The overall fold of the domain and binding mode of the aminoquinazoline is conventional. However, the C-helix has been displaced by approximately 10 Å at its N-terminus, and the activation loop is severely disordered. The structure does retain the DFG sequence that precedes the activation loop in a conventional, closed conformation, and the residues within Van der Waals contact of the inhibitor retain positions comparable to those observed in the erlotinib complex. The larger benzyloxy substituent is accommodated by movement of Met742 of the C-helix, and a pivot of the side chain of Phe832 (DFG sequence). The Met742 reposition is enabled by the overall shift of the more distal regions of the helix that contains it. Lapatinib appears to have captured and stabilized a state of the enzyme that is unable to bind or phosphorylate substrate. Lapatinib has also been shown to have a very slow off-rate, which is consistent with the significant reorganization required to disengage the deeply buried benzyloxy substituent (Fig. 3).

Erlotinib and gefitinib are apparently able to diffuse readily into (and out of) the preformed pockets of EGFR, while lapatinib requires capture or formation of an inactive state. The ability of lapatinib to inhibit ErbB2 would indicate that a lower population of the catalytically relevant conformation of ErbB2 is available compared to EGFR. In fact, ErbB2 possesses an Asp residue (i.e., a shorter side chain) in its activation loop in the position equivalent to the phosphate-mimicking Glu848 in EGFR, which would be consistent with a decreased ability to stabilize the active conformation of the enzyme.

The LVI motif retains its conformation and interactions in the lapatinib-bound EGFR structure, but the C-terminal residues of the construct are in a significantly different orientation compared to the erlotinib/EGFR structure. In the original apo and erlotinib-bound structures, residues Glu961–Gln996 interact with a neighboring molecule in the crystal, hence they are unlikely to be in a physiologically relevant orientation. In the lapatinib struc-

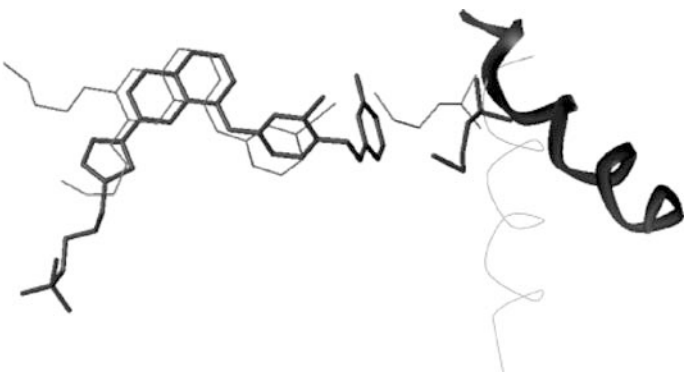


Fig. 3 Comparison of erlotinib (*thin lines*) with lapatinib (*thick lines*). The C-helix is portrayed as a backbone ribbon, and the side chain of Met742 is explicitly shown

ture, most of this sequence is not modeled, but ten residues (Ser971–Glu980) are clearly associated as a helical motif passing across the ATP pocket near the hinge. This association is reminiscent of kinases where the C-terminus is intact, e.g., AKT [36] and PKA [37]. In fact, Met978 is within Van der Waals contact of the C8–C9 edge of lapatinib. It is likely that residues from the C-terminus that extend beyond what is considered generally to be solvent-exposed space contribute to potency and selectivity profiles for aminoquinazoline inhibitors.

3.2 B-Raf

In an even more striking example of induced fit, the X-ray structure of B-Raf in complex with the recently approved sorafenib [25] reveals the same general binding mode that was disclosed previously for both imatinib bound to Abl [38] and for BIRB796 in complex with p38 [39]. The authors disclosed two cocrystal structures with sorafenib: wild-type B-Raf and the activating mutant V600E. The structures are low resolution (3.9 and 4.0 Å, respectively), but the similarity of the binding modes and retention of interactions compared to imatinib and BIRB796 increase confidence in the accuracy of the salient features (Fig. 4).

The pyridinyl amide makes two hydrogen-bonding contacts to the main chain of Cys531, while the central aryl ring occupies the gatekeeper pocket—

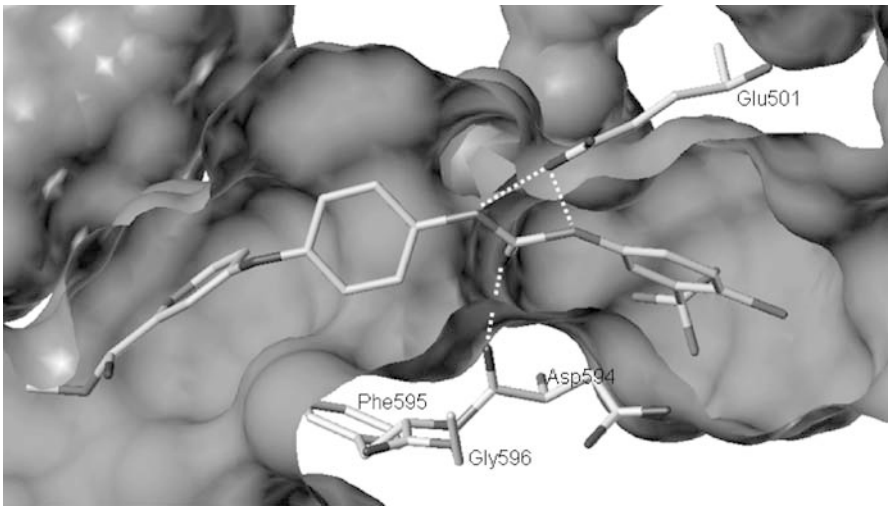


Fig. 4 The binding site for sorafenib is illustrated as a Connolly surface, with Glu501 and the displaced DFG sequence explicitly displayed. Hydrogen bonds from the aryl urea moiety are shown with *dotted lines*

which is accessible owing to a small threonine at the beginning of the hinge. The aryl urea fits into a pocket that is formed between the C-helix and a displaced DFG sequence of the activation loop. The activation loop is severely disordered, and the inhibitor appears to trap an inactive conformation with a blocked substrate binding site. The urea forms hydrogen bonds with the side chain of Glu501 and the main chain NH of Asp594. The terminal aryl ring occupies a hydrophobic pocket that has been vacated by the displaced Phe595 of the DFG sequence, which in turn forms a new edge–face contact with the central aryl ring of sorafenib. This general binding mode has emerged over the last few years as a potential source of efficacy via its combination effect of blocking ATP and substrate binding, preventing a kinase's own activation, and possessing a slow off-rate [40].

3.3 MEK1 and MEK2

To date, the only MEK1/2 kinase inhibitors to enter clinical development have shown inhibition that is not ATP competitive. This unique mechanism of inhibition of MEK1/2 has enabled compounds such as ARRY-142886 and PD0325901 to achieve excellent enzymatic and cellular selectivity for, and potency against, MEK1/2.

Publication of the X-ray crystal structures of the catalytic domains of both MEK1 and MEK2, in complex with MgATP and various small-molecule inhibitors, have provided key insights into the binding features that give rise to the kinetics of inhibition [26]. The key binding interactions of the ternary complex of an N-terminally truncated (unphosphorylated) human MEK1 with MgATP and the anthranilic acid type inhibitor PD318088 are highlighted in Fig. 5.

This mode of inhibition bears little resemblance to the typical hinge region H-bond donor–acceptor arrangement found in the vast majority of ATP-competitive kinase inhibitors. As revealed in the 2.4-Å-resolution X-ray structure, the B-ring of PD318088 fills a narrow hydrophobic pocket formed by residues Leu118, Ile126, Val127, Phe129, Ile141, Met143, Phe209, and Val211. Additionally, at the base of this pocket, Phe209 forms an edge-to-face interaction with the B-ring (the center-to-center ring distance is 5.4 Å). The allosteric pocket is separated from the MgATP binding site by the side chains of Lys97 and Met143. Given the hydrophobic nature of this pocket, it is not surprising that substitution of the B-ring with relatively large halogen atoms leads to increased potency [41]. In particular, 4-iodo substitution is near optimal for the B-ring of the PD318088 series of compounds, and it has been suggested that it participates in an electrostatic interaction with the backbone carbonyl oxygen of Val127, which is at a distance of 3.1 Å. The two fluorine atoms of the 3,4-difluoro substitution of the A-ring of PD318088 appear to play dual roles. The 3-fluoro group fills a small hydrophobic pocket

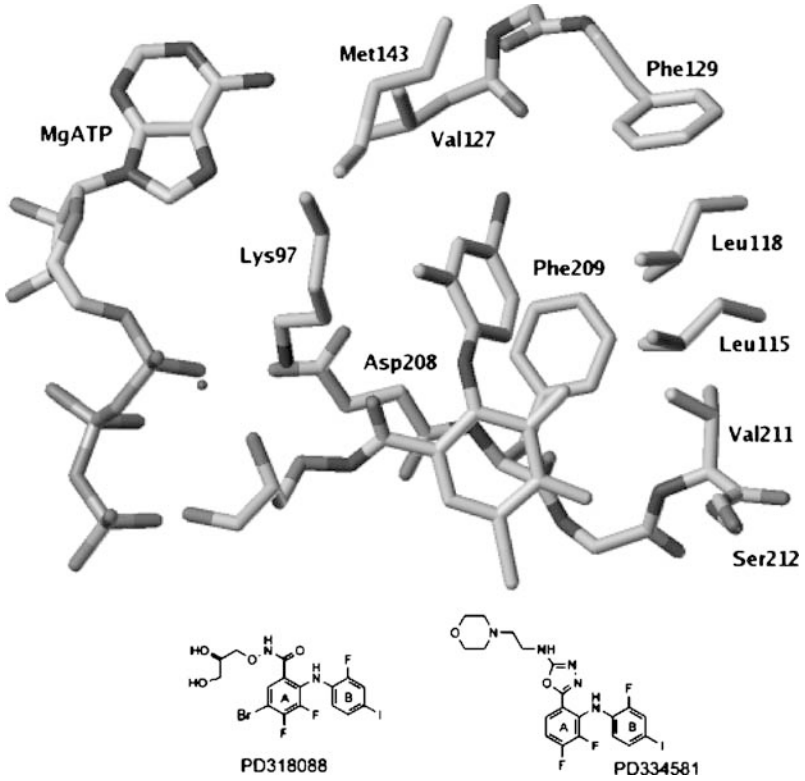


Fig. 5 The binding site for PD318088 with MgATP and MEK1

formed by Leu115, Leu118, and Val211. The 4-fluoro substitution, on the other hand, is within 3.5 Å of the amide N of Ser212 and 3.4 Å of the N of Val211, and appears to form a weak hydrogen bond, or electrostatic interaction. The hydrophobic A and B rings of the PD318088 series of inhibitors contribute to the bulk of the potency of this class of compounds, and form the core of the pharmacophore. The remaining inhibitor interactions are primarily H-bonding in nature. Both oxygen atoms of the hydroxamate interact with the amine portion of Lys97 (the catalytic lysine), and form a bifurcated H-bond, while the diol of the side chain interacts with the terminal phosphate of ATP and Lys97.

While no MEK apo structures have been published, comparisons to the catalytic domains of similar kinases reveal a number of differences versus the tertiary structure of MEK1. Relative to a crystal structure of PKA, there is an outward rotation of the N-terminal portion of helix C by approximately 10 Å and the formation of a short, two-turn α-helical segment of the activation loop. Both of these changes give rise to the allosteric binding pocket which enables the unique binding mode. Inhibitors such as PD318088 stabi-

lize this conformation, which prevents the conserved Glu114 in helix C from participating in its catalytic interaction with Lys97. Interestingly, the role of Glu114 appears to have been replaced by the hydroxamate of PD318088-like compounds, which occupies a similar position and interaction with Lys97. The shift in the position of helix C and displacement of the activation loop likely preclude binding and phosphorylation of ERK1/2.

As with MEK1, the structure of MEK2 has also been solved as a ternary complex with MgATP and the oxadiazole inhibitor, PD334581. Overall, the catalytic domain of MEK2 is ca. 85% identical to that of MEK1, thus one would expect a high degree of structural similarity as well. A comparison of the 3.2-Å X-ray structure of MEK2 to MEK1 reveals ca. 0.8 Å RMSD between the α-carbon positions and preservation of the allosteric binding pocket as well. The binding of PD334581 is very similar to the mode depicted for PD318088 (Fig. 5). The 1,3,4-oxadiazole of PD334581 interacts with the catalytic lysine (Lys101 in MEK2), and the A- and B-rings occupy equivalent hydrophobic pockets as in MEK1. The most significant difference is seen in the region of the terminal phosphate of the bound MgATP. For this inhibitor, the N of the 2-amino side chain is ca. 3.1–3.7 Å from the closest phosphate oxygen, with the morpholine group extended toward the solvent-exposed region.

Despite being formed by relatively simple conformational changes in the activation loop and helix C, thus far only MEK1 and 2 have this allosteric binding site adjacent to the ATP site. Comparison of the structural similarity of the residues that make up the MEK1 allosteric binding pocket (within 5 Å of PD318088) with the superfamily of human protein kinases reveals that only MEK2 (100%) and MEK5 (81%) possess significant identity. MKK4 shows moderate similarity (70% identity) and all other kinases show only low similarity in that region [26]. The lack of significant sequence identity in the inhibitor binding pocket most likely contributes to the exquisite selectivity profile of un-competitive MEK inhibitors.

4
Inhibitors of the ErbB Family

The discussion of drugs and drug candidates that target the EGFR/ErbB2–Raf–MEK pathway will begin with the largest group, those that target ErbB receptor tyrosine kinases directly. These have been separated into subgroups: (1) selective inhibitors of single ErbB family members, (2) dual inhibitors targeting EGFR and ErbB2, and (3) inhibitors of ErbB family members and other kinases. With the exception of BMS 599626 and AEE788, all of the inhibitors reviewed here utilize an aminoquinazoline or aminoquinoline template, and their fundamental mode of interaction is expected to be comparable to that described in Sect. 3 for erlotinib and lapatinib (vide supra).

4.1
Selective Inhibitors Targeting Individual ErbB Family Members

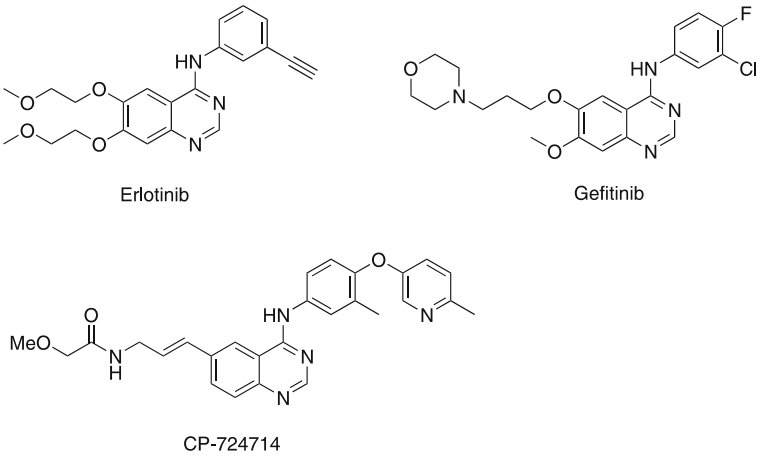


Fig. 6 Selective ErbB inhibitors

4.1.1
Erlotinib

Erlotinib is a selective and reversible ATP-competitive inhibitor of EGFR. The compound was originally discovered by Pfizer but later transferred to OSI Pharmaceuticals under a Federal Trade Commission decree following Pfizer's acquisition of Warner–Lambert in 2000. Currently, erlotinib is codeveloped by OSI, Genentech, and Roche. Erlotinib is a potent inhibitor of the EGFR enzyme (IC_{50} = 1 nM) and is selective vs ErbB2 (IC_{50} = 760 nM). Erlotinib has been screened in a panel consisting of 20 kinases, and has been found to have nearly a 100-fold window between EGFR activity and the other enzymes, with the exception of GAK, where a 40-fold selectivity was reported [42]. In cell-based models, erlotinib inhibits EGFR autophosphorylation (IC_{50} = 20 nM in HN5 head and neck squamous cell carcinoma cells) as well as cellular proliferation (IC_{50} = 100 nM in EGFR overexpressing DiFi colon carcinoma cells) [43]. Erlotinib is not a general inhibitor of proliferation since its effects on the proliferation of non-EGFR driven cell lines, such as NIH-3T3-Raf and FRE-Ras, are weak (IC_{50} = 7 and 3 μM, respectively). Furthermore, erlotinib inhibits EGF-dependent growth of FRE cells (IC_{50} = 70 nM), but does not inhibit growth that is induced by other growth factors (IC_{50} > 1 μM). Depending on the cellular context, erlotinib is capable of inducing apoptosis or cell cycle arrest in the G1 phase of the cell cycle.

Erlotinib has been evaluated in several human tumor xenograft models, with extensive emphasis placed on establishing both the mechanism of inhi-

bition in vivo and a pharmacokinetic/pharmacodynamic (PK/PD) relationship [44]. Efficacy was demonstrated in immunocompromised mice bearing small (< 50 mm^3) human tumors, HN5 and A431, by oral administration of erlotinib. Fifty percent tumor growth inhibition (TGI) was observed with doses of 7 (HN5) and 14 (A431) mg/kg/day, respectively. In an HN5 tumor xenograft study that was initiated with larger tumors of approximately 500 mm^3, complete stasis (100% TGI) was observed with oral doses of 11 mg/kg/day. Inhibition of EGFR autophosphorylation (pEGFR) in excised HN5 tumors after erlotinib treatment was measured in a dose–response study and over 24 h after a single dose of the inhibitor. Maximum inhibition of pEGFR (80%) in tumors was observed at the top oral dose of 100 mg/kg. In the dose–response study, pEGFR was examined 1 h after dosing, and a clear PD relationship was established. The ED_{50} of erlotinib for pEGFR inhibition was determined to be 9.9 mg/kg, which corresponds to an EC_{50} in plasma of 8 μM. An EC_{50} of free drug concentration of 400 nM (160 ng/ml) was determined after factoring in the plasma protein binding (95%) of erlotinib in mice. Since the human plasma protein binding was similar, a target plasma concentration was determined for clinical development. The PK/PD relationship was less evident when the time course of inhibition of pEGFR was examined. A single dose of ca. 100 mg/kg of erlotinib gave equivalent plasma levels of erlotinib at both the 12- and 24-h time points. However, the magnitude of the inhibition of pEGFR in the tumors was not equivalent, with 80 and 50% inhibition at the 12- and 24-h time points, respectively. Neither tumor drug levels nor density of EGFR receptors resolved this discrepancy. It is possible that unidentified active metabolites contribute to the in vivo activity of erlotinib in the HN5 model. In general, the role of metabolites must be considered when evaluating PK/PD relationships and is of particular importance when examining the time course of inhibition. It is possible that within 12 h of dosing, active metabolites of erlotinib are involved, while in the later stages of the dosing interval, metabolites become insignificant contributors.

Erlotinib has been approved by the FDA for second- or third-line treatment of NSCLC [45], and in combination with gemcitabine as first-line therapy for pancreatic cancer [46]. Since the majority of human studies with erlotinib have been in NSCLC, the clinical discussion will focus on this indication. When dosed orally once a day at 150 mg/day in an uncontrolled phase II study, erlotinib resulted in a 12% objective response rate in second- or third-line NSCLC patients [47, 48]. In first-line NSCLC, phase III studies of standard-of-care with or without erlotinib failed to show a clinical benefit in objective response rate, time to progression, or survival for the erlotinib-treated group. However, in a large placebo-controlled phase III trial, erlotinib provided a clear survival benefit as single agent in second- or third-line treatment of NSCLC, which subsequently led to FDA approval [45, 49]. In that trial, patients were randomized to receive erlotinib in addition to supportive

care or supportive care alone. Erlotinib treatment resulted in a 2.0 month increase in survival as compared to placebo with a hazard ratio (HR) of 0.76. Two interesting subsets of patients were identified in this pivotal trial. First, in a third of patients evaluated for EGFR status there was a clear benefit for patients with EGFR-positive tumors. Survival in this group increased to 10.7 months, compared to 3.3 months in the placebo-treated patients that were EGFR-positive. In contrast, no survival benefit was detected in the EGFR-negative subset when compared to placebo. Second, the HR for never smokers was 0.42 vs 0.87 for current or former smokers. While the number of patients in these subsets was too low to make definitive conclusions, the results were convincing enough for the FDA to recommend follow-up studies [45].

At the recommended dose of 150 mg, erlotinib is rapidly absorbed (T_{max} 2–4 h) in patients with an elimination half-life of ca. 18 h, which supports once-daily dosing [50, 51]. The maximum concentration attained is variable, 2.12 + 1.52 μg/ml, while the steady-state trough concentrations are less so, at 1.17 + 0.50 μg/ml. Roughly linear exposure was observed over the 20 to 200 mg/day dose range. Erlotinib has an oral bioavailability of 59% in fasted patients and 100% in fed patients. It is primarily metabolized by CYP3A4, with generation of an active desmethyl species as the major metabolite. Clinical studies with both inhibitors and inducers of CYP3A4 have demonstrated influence on the levels of erlotinib. The CYP3A4 inducer rifampin decreased the exposure to erlotinib by 67%, while the CYP3A4 inhibitor ketoconazole increased exposure by 67%.

As described previously, significant preclinical evaluation of erlotinib in human tumor xenograft models established an EC_{50} for pEGFR inhibition of 8 μM total and 400 nM free plasma concentration [44]. Considering free plasma concentrations are similar in humans and mice, the levels required for significant inhibition of EGFR are not achieved over the dosing interval in the clinic (steady-state total plasma trough levels are ca. 2 μM in the clinic). This analysis does not account for the presence of active metabolites in either human or rodent. It is notable that the increased sensitivity of the EGFR mutants (vide supra), which correlates with objective response rate, would be expected to be inhibited by the steady-state levels of erlotinib at the recommended 150-mg dose level. Thus, it is possible that the single agent clinical activity of erlotinib is a result of the presence of sensitive mutant EGFR receptors, and that the modest activity in patients with wild-type EGFR is a result of tolerability-limited exposure. Alternatively, because it has been demonstrated with erlotinib and other targeted agents (i.e., sorafenib) that objective response rate does not correlate with survival and progression-free survival (PFS), robust inhibition of pEGFR may not be required for disease stabilization.

4.1.2 Gefitinib

Gefitinib, which was developed by AstraZeneca, was the first small-molecule inhibitor of EGFR to be approved for use as a third-line agent in NSCLC. Gefitinib is a potent and selective reversible ATP-competitive inhibitor of EGFR. The compound demonstrates an IC_{50} vs EGFR of 33 nM and excellent selectivity against ErbB2 (IC_{50} = 3.7 μM) [52]. Recently, a broader selectivity profile was reported for both gefitinib and erlotinib [42]. Their profiles were similar, with the least selectivity against GAK (erlotinib IC_{50} = 40 nM and gefitinib IC_{50} = 7 nM).

Gefitinib inhibits EGFR autophosphorylation in several human cell lines (HT29, KB, Du145, A549, and A431) with IC_{50} values ranging from 30 to 100 nM [52]. Inhibition of EGF-induced growth of KB and HUVEC cells has also been demonstrated with similar potencies. This inhibitory effect is selective for EGF-mediated growth, because gefitinib does not significantly affect the growth of KB cells in the absence of EGF (IC_{50} = 8.8 μM) or the growth of FGF- and VEGF-stimulated HUVEC cells (IC_{50} = 1–3 μM). The antiproliferative effect of gefitinib in the majority of responsive cell lines arises from cytostasis, although induction of apoptosis can also occur (e.g., MDA-MB-175, SK-Br-3, and H3255 cells) [53, 54]. In NSCLC cell lines, sensitivity to growth inhibition correlates with the inhibition of EGFR-mediated phosphorylation of AKT and ERK1/2 [55]. In addition, and consistent with clinical observation, the presence of EGFR mutations can confer increased sensitivity to gefitinib. For example, the H3255 cell line, which expresses the $EGFR^{L855R}$ mutant, is one of the most sensitive NSCLC cell lines (IC_{50} = 40 nM), whereas the wild-type EGFR-expressing cell line H1666 is much less sensitive (IC_{50} = 2 μM) [54].

Gefitinib has shown efficacy in several preclinical models [52]. For example, when dosed orally at 200 mg/kg/day it inhibited the growth of small A431 tumors (< 100 mm^3 at dosing initiation) and caused regression in large A431 tumors (600 mm^3 at dosing initiation). In excised A431 tumors, c-fos mRNA, which is a biomarker of proliferation, was completely inhibited at the 200 mg/kg/day dose. In addition, in an HT-29 colon carcinoma model, a 200 mg/kg/day oral dose resulted in modest tumor growth inhibition.

Gefitinib was one of the first selective small-molecule kinase inhibitors to be approved for the treatment of solid tumors. It was approved by the FDA as a single agent for locally advanced or metastatic non-small cell lung cancer in patients that have failed both platinum-based and docetaxel therapies [56]. At the time of its approval, there were no drugs available for third-line NSCLC. The approval of gefitinib was based on two large, uncontrolled phase II trials where ca. 10% objective response rates were observed [57, 58]. Approval was contingent on demonstrating a survival benefit in subsequent

trials. The phase III trial was designed to compare supportive care with and without gefitinib in NSCLC patients for whom chemotherapy and radiation had failed. Unfortunately, the drug showed no survival benefit [59]. Consistent with the phase II data, there was ca. 10% objective response rate in this placebo-controlled trial, but that did not translate into a significant survival benefit. Additionally, gefitinib failed to show any clinical benefit in two large phase III trials in first-line NSCLC that examined standard care with and without gefitinib [60, 61]. Because of the lack of survival benefit, AstraZeneca has withdrawn gefitinib from the market, but continues to make it available to patients that have experienced clear benefit from the drug. Currently, gefitinib is undergoing clinical evaluation in a variety of indications and combinations.

The pharmacokinetic profile of gefitinib supports once-daily dosing in patients [56, 62]. The fasted bioavailability is ca. 60%, with a moderate rate of absorption (T_{max} = 3–7 h), and an elimination half-life of 24–30 h. Exposure increases nearly linearly over a dose range of 50–500 mg. The compound has moderate binding to plasma proteins (91%) and is predominantly metabolized to an active *O*-desmethyl species by CYP3A4. As was observed with erlotinib, the CYP3A4 inducer rifampicin decreased exposure (85%) and the CYP3A4 inhibitor itraconazole increased exposure (88%). Gefitinib is a moderate inhibitor of CYP2D6 (43% at 11.2 μM). As a result, when gefitinib is dosed with the CYP2D6 substrate metoprolol, exposure of the latter is increased by 30%. Coadministration of gefitinib with the H_2-antagonist ranitidine resulted in lower exposure of the EGFR inhibitor. This result highlights the importance of pH-dependent solubility in the absorption of gefitinib. Ranitidine administration increases the pH of the stomach to ca. 5, which decreases the solubility of the weakly basic gefitinib. Since many cancer patients are currently being treated with proton-pump inhibitors, this becomes an important consideration for drug developers who are evaluating weakly basic kinase inhibitors.

Although formal preclinical PK/PD experiments have not been reported with gefitinib, significant antitumor activity was achieved in sensitive human tumor xenograft models at 200 mg/kg/day. The trough levels in mice at 200 mg/kg are ca. 2.8 μg/ml, and although the trough levels of gefitinib from the clinic have not been reported, the maximum concentration at the recommended 250 mg dose is typically 0.16 μg/ml. As with erlotinib, the recommended dose of gefitinib results in significantly less plasma concentration than the levels required for preclinical efficacy. With the limitations of preclinical models, it is impossible to conclude that these exposure comparisons explain the modest clinical activity of gefitinib. However, as additional agents progress, more comparisons to preclinical efficacy models will be made, which will allow better evaluation of their validity.

4.1.3
Erlotinib and Gefitinib: Comparison of Clinical Outcomes

As described above, erlotinib and gefitinib are similar in several respects, and have followed similar development paths, yet only one drug has demonstrated a survival benefit. Several possible explanations for the different survival outcomes have been suggested [50], and there are several facts to consider. First, in most, if not all, preclinical studies, erlotinib is approximately twofold more potent than gefitinib [47], and the exposure in patients at the recommended dose of erlotinib (150 mg/day) is significantly higher than that for the recommended dose of gefitinib (250 mg/day) [62, 63]. Patients given erlotinib clearly achieve higher exposures of active drug. Second, erlotinib was administered at its maximum tolerated dose (MTD), while gefitinib was dosed at its "optimum dose" as determined from clinical evaluation [64]. The decision to evaluate gefitinib below its MTD was supported by clinical data that showed no increase in objective response rate, but with increased incidence of adverse events, when comparing 250 and 500 mg/day. In retrospect, the clinical results from erlotinib and the anti-EGFR monoclonal antibody cetuximab, both of which demonstrate a survival benefit and a correlation between response and severity of rash [65, 66], argue for dosing these signal transduction inhibitors to the MTD. Another interesting point is that survival benefit does not correlate with response rate with these inhibitors. Both of these agents showed similar objective response rates in all trials, yet only erlotinib demonstrated a survival benefit. A relationship between EGFR expression levels and survival was demonstrated with erlotinib in the phase III setting, despite earlier trials in which both these agents failed to link EGFR expression levels with objective response rate. Finally, even though gefitinib did not establish a survival benefit, subsets of patients clearly experience impressive objective responses. The overall marginal clinical benefits observed over the last several years highlight the importance of identifying predictive markers of patients that will benefit from selective anti-EGFR therapy.

4.1.4
CP-724714

CP-724714 is a reversible ErbB2-selective inhibitor that has been advanced into phase I clinical trials by Pfizer. The compound has an in vitro IC_{50} against ErbB2 of 8 nM and inhibits ErbB2 autophosphorylation in engineered NIH-3T3 cells that express chimeric ErbB2 (IC_{50} ca. 30 nM). It also inhibits the proliferation of SK-Br-3 cells with an IC_{50} of approximately 50 nM. It is a weak inhibitor of EGFR (IC_{50} = 4300 nM), and is essentially inactive (IC_{50} > 10 000 nM) against several other kinases including PDGFR, KDR, IR, IGF-1R, c-Abl, c-Src, and c-Met. CP-724714 has demonstrated preclinical efficacy

in three human tumor xenograft models [67]. In PK/PD studies that measured pErbB2 inhibition after dosing CP-724714 in the FRE/ErbB2 transfectant mouse model, the EC_{50} was determined to be 1 μg/ml.

Phase I evaluation of CP-724714 in cancer patients is ongoing [68]. The majority of patients in this trial have breast cancer and have received trastuzumab previously. The MTD was determined to be 250 mg with the dose-limiting toxicity defined as hyperbilirubinemia and elevated liver enzymes. No objective responses have been reported for the 20 patients evaluated to date. Thirty-five percent of patients have experienced stable disease for an undetermined period of time. In contrast to trastuzumab, cardiomyopathy has not been observed in this trial.

Pharmacokinetic data reported thus far show dose-proportional exposure with a short half-life of ca. 2 h. Exposure in patients receiving 250 mg/day of CP-724714 reportedly exceeds the plasma levels required for efficacy in preclinical tumor xenograft models (50 to 60% TGI). However, robust efficacy (stasis or minor regression) in mouse models was only achieved at doses resulting in plasma exposures 40% higher than those observed in humans. From a kinetic standpoint, it appears that within 3 h, plasma concentrations in humans of CP-724714 fall below the levels required for pErbB knockdown in mouse models. This analysis, of course, neglects free fraction differences that may exist between species and the differential levels of active metabolites. Enrollment in this trial continues at the 250 mg/tid dosing level.

4.2 EGFR/ErbB2 Dual Inhibitors

4.2.1 Lapatinib

Lapatinib, discovered by GSK, is a reversible ATP-competitive dual inhibitor of EGFR and ErbB2 which is in phase III clinical trials for breast cancer. Lapatinib shows comparable in vitro potencies against both EGFR (IC_{50} = 11 nM) and ErbB2 (IC_{50} = 9 nM) [69]. It inhibits ErbB4 at higher concentrations with an IC_{50}>1 μM [24]. Gefitinib, erlotinib, and lapatinib were tested under the same assay conditions. Gefitinib and erlotinib were found to have greater EGFR potency (K_i^{app}: 0.70 and 0.40 nM, respectively, vs 3.0 nM), and much weaker ErbB2 activity (K_i^{app}: 760 and 240 nM, respectively, vs 13 nM) than lapatinib [24]. Lapatinib has been tested against a variety of tyrosine and serine/threonine kinases and has been found to be quite selective [69]. A unique feature of lapatinib is its very slow off-rate (K_{off} = 0.0023 min^{-1}) from both EGFR and ErbB2 [24]. Its slow off-rate is also evident in cells. Lapatinib-treated HN5 cells still showed 85% inhibition of p-EGFR formation 96 h postwashout as compared to gefitinib- and erlotinib-treated cells, which exhibited 40 and 0% inhibition, respectively,

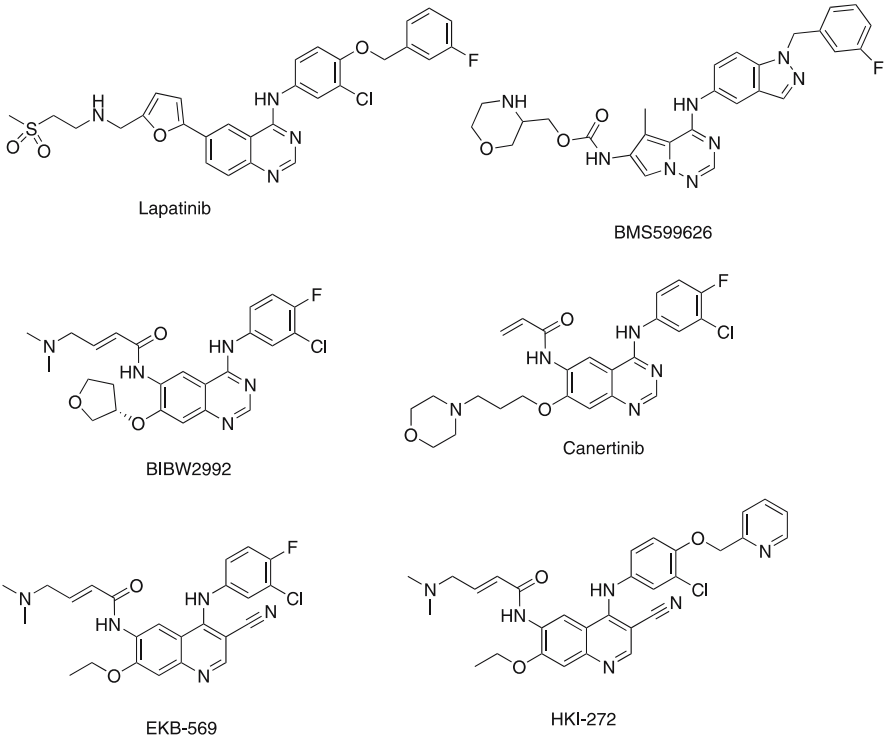


Fig. 7 Dual EGFR/ErbB2 inhibitors

at the same time point. The clinical significance of this property remains to be seen.

Lapatinib exhibits potent growth inhibition against EGFR- and ErbB2-expressing cell lines (Table 1). As expected, this compound is superior to gefitinib and erlotinib in inhibiting the proliferation of tumor cell lines that have significant expression of both ErbB2 and EGFR [69]. In cell lines that preferentially overexpress EGFR relative to ErbB2, all three compounds are essentially equipotent. Lapatinib has been shown to almost completely inhibit both pAKT and pERK1/2 production in BT-474 and HN5 cells at 1 and 5 μM, respectively [70]. Moreover, HN5 cells that are treated with lapatinib undergo cell cycle arrest rather than apoptosis.

Preclinical evaluation of lapatinib has demonstrated inhibition of tumor growth and phosphorylation of EGFR, ErbB2, and downstream signaling proteins in excised tumors from EGFR- and ErbB2-driven xenograft models [69]. In nude mice with established HN5 tumors that overexpress EGFR, twice-daily oral treatment with lapatinib at 30 mg/kg resulted in greater than 50% tumor growth inhibition. A 100 mg/kg/bid dose completely inhibited tumor growth. Similarly, in SCID mice bearing BT-474 tumors that overexpress

Table 1 Cell growth inhibition by lapatinib, gefitinib, and erlotinib [69]

Cell line	EGFR[a]	ErbB2[a]	Lapatinib IC_{50} (μM)	Gefitinib IC_{50} (μM)	Erlotinib IC_{50} (μM)
HFF (normal fibroblasts)	+	–	12	> 10	> 8.7
MCF-7	+	+	4.0	14.2	> 30
T47D	+	+	3.0	10.5	14.5
A-431	+++	+	0.16	0.08	0.10
HN5	+++	+	0.12	0.08	0.18
BT-474	+	+++	0.10	1.1	9.9
N87	+	+++	0.09	2.6	4.6
CaLu-3	ND	+++	0.13	ND	ND
HB4a	+	–	9.1	14.5	23.8
HB4a c5.2	+	+++	0.21	1.1	1.8

[a] Expression level as determined by Western blot analysis

ErbB2, 30 and 100 mg/kg/bid lapatinib resulted in 30% tumor growth inhibition and tumor stasis, respectively. In these models, lapatinib treatment inhibited both autophosphorylation and activation of downstream signaling proteins [70]. In HN5 xenografts, production of pEGFR, pAkt, and pERK1/2 was inhibited in excised tumors after five doses of lapatinib at either 30 or 100 mg/kg/bid. Likewise, in BT-474 tumors excised after five doses of 100 mg/kg/bid lapatinib, both pErbB2 and pERK1/2 formation were inhibited. These xenograft studies suggest that lapatinib can inhibit tumor growth that is driven by either EGFR or ErbB2. The corresponding plasma levels of lapatinib that are required for inhibition of the targets in vivo were not reported.

Lapatinib is currently undergoing clinical evaluation for several cancer indications. In a phase II study, doses of lapatinib escalating from 1250 to 1500 mg/day resulted in ca. 10% objective response in trastuzumab-refractory metastatic breast cancers [71]. More recently, approximately 30% objective response was observed when lapatinib was administered at 1500 mg/qd or 500 mg/bid in first-line ErbB2-positive (as determined by the FISH technique) breast cancer [72].

Single-dose pharmacokinetic analysis in healthy volunteers showed near-linear increases in exposure over the 10 to 250 mg dose range, with a half-life of 7 h [73]. In cancer patients, exposure increased with dose, albeit with significant interpatient variability [74]. In patients dosed with 1600 mg lapatinib, maximum concentrations of drug ranged from 1.36 to 3.35 μg/ml with steady-state trough values ranging from 0.28 to 1.49 μg/ml. Interestingly, the area under the curve and half-life increased upon reaching steady-

state levels in both healthy volunteers and cancer patients, even though the single dose half-life does not support accumulation. Possible explanations include a food effect, which would result in both a delay of and an increase in lapatinib absorption, or a decrease in systemic clearance because of inhibition of metabolism. Regardless of the mechanism of accumulation, the steady-state pharmacokinetic profile in patients supported once-daily dosing. As was observed for several of the agents discussed in this review, the area under the curve that is required for robust preclinical antitumor activity is significantly higher (ca. 120 μg h/ml) than that achieved in the clinic (29 μg h/ml) [74, 75].

Unlike other compounds with EGFR inhibitory activity, the DLT of lapatinib has not been reached upon once-daily dosing [74]. While mild to moderate rash and diarrhea have been observed (generally grade 1 and 2), they have not limited dosing. Interestingly, increasing the dose from 500 to 1600 mg/day did not increase the incidence of moderate rash (grade 1/2 rash observed in 5 of 13 patients @ 500 mg vs 3 of 13 patients @ 1600 mg). With twice-daily dosing, the severity of diarrhea became dose-limiting.

4.2.2 BMS 599626

BMS 599626 is a pyrrolotriazine-based dual EGFR/ErbB2 inhibitor discovered by BMS that has recently entered into phase I clinical trials [76–78]. To date, very little data have been disclosed regarding this compound. BMS 599626 inhibits both EGFR (IC_{50} = 40 nM) and ErbB2 (IC_{50} = 40 nM), and is inactive (IC_{50}>10 μM) against a limited panel of kinases reported thus far (Met, FAK, p38, MAPKAP Kinase2, and IGF-1R). BMS 599626 inhibits the growth of BT-474 cells (IC_{50} = 860 nM) and Sal2 cells (salivary gland carcinoma IC_{50} = 460 nM). This compound also inhibits ErbB2, ERK1/2, and AKT activation in N87 cells. BMS 599626 is reported to have good oral bioavailability in dogs (49%), monkeys (31%), and mice (83%), and has demonstrated antitumor activity in models driven by either EGFR (GEO colon carcinoma) or ErbB2 (N87 gastric carcinoma). Although dose–response relationships were established in these models, robust antitumor activity required 240 mg/kg/qd.

Phase I clinical evaluation of BMS 599626 is ongoing [79, 80]; however, no efficacy data have been reported to date. Dose escalation has proceeded to 660 mg/day and is ongoing since a MTD has not been reached. The pharmacokinetic profile in both healthy volunteers and cancer patients supports once-daily dosing with a half-life of ca. 20 h. An area under the curve of ca. 2.4 μg h/ml with a maximum concentration of ca. 0.18 μg/ml was achieved at steady state after 100-mg dosing in patients. In mice at a dose that resulted in modest antitumor activity (ca. 50% TGI), significantly higher plasma levels were achieved (AUC ca. 14.5 μg h/ml and C_{max} ca. 5.0 μg/ml) [76].

4.2.3
ARRY-334543

ARRY-334543, discovered by Array BioPharma, is a quinazoline whose structure has not been fully disclosed [81]. It is a potent, ATP-competitive, selective inhibitor of EGFR and ErbB2 receptor tyrosine kinases (IC_{50} = 2 nM and 7 nM, respectively). ARRY-334543 demonstates residual activity against Src family members (e.g., 82% inhibition of Lck at 1 μM), but does not appreciably inhibit a panel of greater than 100 kinases examined to date. ARRY-334543 shows good activity in cell-based assays. It inhibits EGFR autophosphorylation with an IC_{50} of 36 nM in A431 cells, and inhibits ErbB2 autophosphorylation (IC_{50} = 43 nM) in BT-474 cells. It also inhibits AKT activation in BT-474 cells (IC_{50} = 44 nM).

ARRY-334543 shows robust efficacy in several human tumor xenograft models, including BT-474, MDA-MB-453, H1650, Calu3, and A431 [81, 82]. Target inhibition in vivo or PK/PD relationships have not been reported. ARRY-334543 recently entered phase I trials; no data have been reported.

4.2.4
Canertinib

Canertinib, discovered by Parke-Davis, was the first irreversible pan-inhibitor of the ErbB family to enter clinical trials. It is a potent ATP-competitive compound that exhibits time-dependent inhibition. The compound binds covalently to the Cys773 residue of EGFR in the ATP-binding site [83]. Canertinib inhibits EGFR, ErbB2, and ErbB4 with IC_{50} values of 0.8, 19, and 7 nM, respectively [84]. Furthermore, canertinib has a comparable selectivity profile to those of gefitinib and erlotinib, with activity against only two non-ErbB family kinases (GAK and EphA6: IC_{50} values of 44 and 72 nM, respectively) [42].

It also inhibits ligand-dependent autophosphorylation of EGFR in A431 cells, and ErbB2, ErbB3, and ErbB4 in MDA-MB-453 cells with IC_{50} values ranging from 5 to 14 nM [84]. It is notable that upon binding, canertinib stimulates receptor ubiquitination, which leads to the ultimate degradation of the receptor via a mechanism mediated by HSP-90.

It has demonstrated activity in several mouse xenograft models, including A431 epidermoid carcinoma, H125 NSCLC, MDA-MB-468 mammary carcinoma, and SF767 glioblastoma xenografts [31]. Efficacy in these models ranged from regressions (A431 and SF767) to moderate tumor growth inhibition (H125). Examination of excised A431 tumors after dosing canertinib showed inhibition of EGFR phosphorylation.

Canertinib appears to have been evaluated in more phase I and II trials than the other inhibitors discussed in this review [84–87]. It has been studied under a wide variety of oral dosing schedules. Moreover, canertinib

has also been dosed by intravenous administration [88]. After oral administration, canertinib is rapidly absorbed and eliminated, with a half-life of ca. 2 h. Tolerated doses of 250 mg result in maximum concentrations approaching 0.20 μg/ml. Neither a high-fat diet nor multiple days of dosing led to increases in canertinib exposure. Depending on the turnover of the ErbB receptors, half-life may be less important in determining the dosing frequency with an irreversible inhibitor. Despite its broad clinical experience, little efficacy has been reported. Of the nearly 200 treated patients, only one partial response in a squamous cell skin cancer patient was reported. Approximately 28% of patients experienced stable disease of variable duration. Phase II single agent and combination clinical studies are still under way.

4.2.5
BIBW 2992

BIBW 2992 is an irreversible ATP-competitive EGFR/ErbB2 inhibitor that is under development by Boehringer Ingelheim. The compound inhibits EGFR and ErbB2 in vitro with IC_{50} values of 0.50 and 14 nM, respectively, and does not potently inhibit a small set of non-ErbB family kinases (IR, KDR, HGFR, c-Src). Of the principal EGFR/ErbB2 inhibitors in clinical development, BIBW 2992 appears to be the most potent compound in cell-based assays when examining inhibition of pEGFR, pErbB2, and cell proliferation (Table 2). In preclinical models, BIBW 2992 has shown tumor stasis or regression at 20 mg/kg/day in models of epidermal, ovarian, and gastric carcinomas [89].

Phase I clinical evaluation of BIBW 2992 is under way [90–92]. An MTD of 70 mg/day on a 14-days-on/14-days-off schedule, with DLTs consisting mainly of rash and diarrhea has been reported. Overall, 84 patients have received BIBW 2992 on a 14-day-on/14-day-off or continuous schedule, with one partial response (adenocarcinoma of the lung) and 36% stable disease rate reported. Clinical pharmacokinetic data have not yet been reported.

4.2.6
HKI-272

HKI-272 (Wyeth) is also an irreversible ATP-competitive inhibitor of EGFR/ErbB2, and is currently in phase I clinical trials. This compound replaced an earlier irreversible entrant, EKB-569, which demonstrated good potency against EGFR (IC_{50} = 80 nM), but weaker activity against ErbB2 (IC_{50} = 1230 nM) [93, 94]. HKI-272 inhibits both ErbB2 (IC_{50} = 59 nM) and EGFR (IC_{50} = 92 nM) in vitro. HKI-272 is reported to be very selective against a series of tyrosine and serine/threonine kinases. Only KDR (IC_{50} = 800 nM) was significantly inhibited at concentrations less than 1 μM.

HKI-272 inhibits autophosphorylation of ErbB2 in BT474 cells (IC_{50} = 5 nM), and EGFR in A431 cells (IC_{50} = 3 nM). Accordingly, the levels of pERK1/2, pAkt, and CyclinD1 are also decreased (IC_{50} = 2–9 nM). The improved potency that is observed in cells is probably a result of the time-dependent inhibition profile of HKI-272, as the cells were exposed to inhibitor from 3 to 12 h, while the enzymatic assays were incubated with inhibitor for 1 h. HKI-272 inhibits the growth of cell lines that either overexpress EGFR (e.g., A431, IC_{50} = 81 nM) or ErbB2 (e.g., BT-474, IC_{50} = 2 nM). Notably, cell lines that exhibit weak expression of EGFR and ErbB2, and are thus less likely to be driven by ErbB signaling (e.g., MDA-MB-435, SW620, and 3T3 cells), are less sensitive to growth inhibition (IC_{50} ca. 700 nM).

HKI-272 has shown activity in animal models: daily doses of 40 mg/kg result in greater than 75% tumor growth inhibition in xenograft models of both BT-474 breast carcinoma and A431 epidermal carcinoma [93]. Reports of the phase I evaluation of HKI-272 are not yet available.

4.3 Direct Comparison of Select EGFR/ErbB2 Inhibitors

ErbB inhibitors in development differ in several aspects, including potency, selectivity, and mechanism of inhibition. Fortunately, there are data that compare erlotinib, gefitinib, lapatinib, canertinib, HKI-272, and BIBW 2992 directly in enzymatic and cellular studies, as shown in Tables 2 and 3 [89, 95].

Although there are differences in enzymatic and cellular potencies against EGFR, all five compounds show an increase in potency when tested against the mutant EGFR (L858R) that was identified in NSCLC patients that are most responsive to erlotinib and gefitinib (Table 3). Similarly, there is a loss of potency when tested against the mutant EGFR (T790M) that has been associated with resistance to small-molecule inhibitors of EGFR. Interpretation of the T790M data is straightforward from the structural biology information. All

Table 2 Comparison of EGFR/ErbB2 inhibitors [89]

Compound	EGFR IC_{50} (nM)	ErbB2 IC_{50} (nM)	A431 EC_{50} (nM)	NIH-3T3 ErbB2 EC_{50} (nM)	N87 EC_{50} (nM)	BT-474 EC_{50} (nM)	
Assay	Enzyme	Enzyme	pEGFR	pErbB2	pErbB2	pErbB2	Growth
Gefitinib	3	1100	35	2300	541	3710	1070
Erlotinib	2	238	5	734	468	930	829
Canertinib	0.3	30	22	85	288	184	66
Lapatinib	3	15	105	171	101	99	52
BIBW 2992	0.5	14	13	71	48	35	12

Table 3 Activity against EGFR wild-type and mutant-containing cell lines [95]

Compound	H1666 (wt-EGFR) EC_{50} (nM)		H3255 (L858R mutant EGFR) EC_{50} (nM)		H1975 (T790M mutant EGFR) EC_{50} (nM)	
	Growth	pEGFR	Growth	pEGFR	Growth	pEGFR
Gefitinib	187	72	5	11	> 4000	> 4000
Erlotinib	110	87	40	52	> 4000	> 4000
Canertinib	198	127	1	5	101	79
Lapatinib	534	1424	63	3698	> 4000	> 4000
HKI-272	172	201	7	18	172	579
BIBW 2992	60	7	0.7	6	99	93

of these inhibitors are expected to occupy the "gatekeeper" pocket of EGFR that is made accessible by the small threonine residue; mutation to a large and hydrophobic methionine clearly blocks entry to this space. The cause for the increase in potency against the L858R mutant is not clear.

In terms of clinical efficacy, the most important attribute that differentiates the inhibitors may be the relative potencies for EGFR vs ErbB2, because EGFR-related toxicities appear to be dose-limiting in humans.

4.4 Multi-Kinase Inhibitors

4.4.1 AEE788

AEE788 is the second pyrrolopyrimidine inhibitor of the ErbB family that Novartis has progressed into the clinic. The first one was PKI166, which was discontinued due to elevated, but reversible, liver enzyme levels (17% of patients experienced grade 3 levels of elevated transaminases) [96]. In AEE788, the phenol of PKI166 is replaced with a methylene-linked *N*-ethylpiperazine moiety that may have been designed in an effort to increase solubility and de-

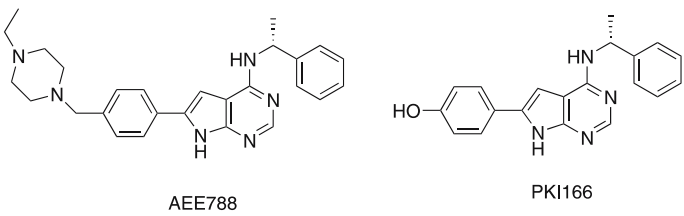


Fig. 8 Multi-kinase inhibitors

crease hepatic toxicity. Although AEE788 is described as an inhibitor of KDR as well as of EGFR/ErbB2, it is more potent at inhibiting the latter. The in vitro IC_{50} values against EGFR and ErbB2 are 2 and 6 nM, respectively, while the in vitro potency of AEE788 against KDR, Flt1, c-Abl, c-Src, c-Fms, and PDGFRβ is at least an order of magnitude weaker (IC_{50} values are 77, 59, 52, 61, 60, and 320 nM, respectively) [97].

In cellular models, AEE788 is a more potent inhibitor of EGFR autophosphorylation (A431, IC_{50} = 11 nM) than ErbB2 autophosphorylation (BT-474, IC_{50} = 220 nM) [97]. Its activity against KDR autophosphorylation in CHO transfectants is weaker still (IC_{50} = 960 nM). AEE788 exhibits antiproliferative effects in many different cell lines at sub-micromolar levels, including NCI-H596, MK, SK-BR-3, and BT-474. As expected, AEE788 does not potently inhibit the proliferation of MCF-7 (IC_{50} = 2500 nM) or T24 cells (IC_{50} = 4526 nM), because neither of these cell lines is driven by EGFR or ErbB2 activation. In contrast, AEE788 inhibits the proliferation of VEGF- and EGF-driven HUVEC cell proliferation (IC_{50}: 43 and 155 nM, respectively) but does not inhibit serum- or bFGF-driven HUVEC proliferation at concentrations up to 1 μM.

AEE788 inhibits the growth of two human tumor xenografts that overexpress EGFR [97]. In the NCI-H596 adenosquamous lung carcinoma model, tumor stasis was observed following a 50 mg/kg oral dose three times a week. Using the same dosing regimen, the growth of DU145 human prostate carcinoma tumors was moderately inhibited. Examination of excised tumors from the NCI-H596 model after five daily doses of AEE788 at 30 mg/kg showed complete inhibition of pEGFR for 72 h beyond the last dose. Activity of AEE788 in an ErbB2-driven cell line was determined using a NeuT/ErbB2 GeMag syngeneic orthotopic mouse tumor model. Oral doses of AEE788 at 50 mg/kg three times a week resulted in approximately 60% tumor growth inhibition. Inhibition of the autophosphorylation of ErbB2 after five daily treatments of AEE788 was established in this model, with inhibition lasting ca. 24 h after the final dose. The duration of inhibition of the targets in vivo by AEE788, particularly the inhibition of pEGFR formation for 72 h following the last dose, was surprising.

Pharmacokinetic data may explain these observations [97]. In a separate study, analysis of AEE788 following a single 100 mg/kg oral dose in nude mice showed high levels of AEE788 in the plasma over 24 h (AUC 58.5 μmol h/l), with even higher levels found in tumor tissue (AUC 1337 nmol h/g). The ratio of drug in tumor tissue to plasma was ca. 20-fold and 50-fold at 6 and 24 h, respectively. This accumulation of inhibitor in tumor tissue may explain the long-lasting inhibition of pEGFR formation in excised tumors. However, these data are difficult to interpret, as there is no way to determine if the drug found in the tumor is available for action. If the drug is freely permeable and available, the free drug levels in plasma should approximate the free drug levels in the tumor. The property of importance is the concentration

of free fraction in the cytosol, which is undetermined. Regardless of the explanation, the ability of AEE788 to inhibit pEGFR for several days following administration may allow for intermittent dosing in the clinic.

AEE788 is currently in phase I/II clinical testing, where it has been dosed orally in more than a hundred patients [98–100]. Two phase I trials have been completed: one study enrolled patients with a wide variety of solid tumors, while the other focused on recurrent glioblastoma multiforme (GBM). In both trials, DLTs were observed at 550 mg/day and consisted of diarrhea, fatigue, and anorexia. The MTD was determined to be 400 mg/day, and an intermediate dose is under evaluation. Mild to moderate rash was also observed at doses above 150 mg. AEE788 was not associated with changes to electrocardiographs; however, grade 3 and 4 reversible increases in liver enzyme levels were reported. As mentioned previously, the first-generation pyrrolopyrimidine, PKI166, was discontinued because of reversible, elevated liver enzyme levels. One patient with angiosarcoma who received 400 mg/day AEE788 experienced a partial response. Overall, ca. 35% of patients in the AEE788 trials have had stable disease of varying duration.

Pharmacokinetic analysis in cancer patients shows that steady-state levels are reached within 15 days and that the effective half-life is greater than 24 h [99]. The exposures in patients are variable, with an AUC after administration of 400 mg of approximately 2.0 μg h/ml, and maximum and trough concentrations of approximately 0.13 and 0.06 μg/ml, respectively. AEE788 is metabolized by CYP3A4 to an active species, AQM674, the structure of which has not been disclosed. The plasma levels of AEE788 increase superproportionally, wherein a 22-fold increase in dose results in a 58-fold increase in exposure. Over the same dose range, the formation of metabolite AQM674 increases nearly linearly with dose. The ratio of parent to metabolite increases with dose, which suggests saturation or inhibition of metabolite formation. This is consistent with CYP3A4-mediated formation of AQM674, because AEE788 is an inhibitor of CYP3A4 (IC_{50} = 3.6 μM).

4.4.2 XL647

XL647 was discovered by Exelexis, and is another potent inhibitor of both EGFR (IC_{50} = 0.3 nM) and ErbB2 (IC_{50} = 16.1 nM). XL647 is currently undergoing phase I clinical trials; although its full structure has not been disclosed, it is known to be a quinazoline-based inhibitor. This compound also inhibits KDR (IC_{50} = 1.5 nM) and EphB4 (IC_{50} = 1.4 nM) in cell-free assays. Moreover, XL647 inhibits PDGFR and FGFR1 at moderate levels (IC_{50}: 346 and 855 nM, respectively), but does not inhibit IRK (IC_{50} > 26 000 nM). In cell assays, XL647 inhibits the autophosphorylation of EGFR (IC_{50} = 1 nM) and EphB4 (IC_{50} = 3 nM). It also inhibits the autophosphorylation of ErbB2 and KDR, but less potently with IC_{50} values of 84 and 70 nM,

respectively. XL647 inhibits VEGF-induced tubule formation in endothelial cells, but does not inhibit PDGFR autophosphorylation at concentrations up to 1200 nM [101].

The preclinical pharmacokinetic profile of XL647 is interesting. The compound is only 26% orally bioavailable in mice despite having low plasma clearance (615 ml/h/kg, corresponding to an extraction ratio of 12%) and high solubility (ca. 19 mg/ml). Similarly, the compound has moderate oral bioavailability in rats (46%) despite low plasma clearance (1214 ml/h/kg, corresponding to an extraction ratio of 30%). These data may indicate that the compound has limited permeability. Conversely, XL647 has high oral bioavailability in dog (72%) despite high plasma clearance that is essentially equal to hepatic blood flow. The compound may either be saturating the first pass effect or the clearance could have an extra-hepatic component. Compounds that exhibit low permeability are often better absorbed in dogs due to leakier junctions in the intestines. In all three species (mouse, rat, and dog), the steady-state volume of distributions (19, 18, and 24 l/kg) are very high and the half-lives are correspondingly long (11, 22, and 12 h) [101].

Extensive in vivo studies that examine tumor growth inhibition, pharmacodynamic measurements of target inhibition including EGFR, ErbB2, KDR, and EphB4, and establishment of PK/PD relationships have been reported in several xenograft models with XL647 [101]. Dose–response relationships have been established in models of breast (MDA-MB-231 and BT-474), colon (HT29), NSCLC (Calu6), prostate (PC3), and epidermal (A431) cancers. Additionally, 100 mg/kg XL647 dosed for 7 days resulted in a significant decrease in microvessel density in excised tumors, which supports an antiangiogenic effect [101]. This inhibitor displays a multi-kinase profile in the preclinical setting.

XL647 is currently in phase I clinical trials [102]. The MTD has not yet been defined, although two DLTs have been observed: one QTc prolongation at 3.12 mg/kg/day (ca. 220 mg/day) and one grade 3 diarrhea at 7.0 mg/kg/day (ca. 500 mg/day). One partial response (NSCLC) has been observed early in the dose escalation (0.06 mg/kg or 4.2 mg/day). Twenty-three percent of patients have experienced stable disease in the trial.

Exposures at the highest dose level reported (3.12 mg/kg) were variable, with an AUC at day 1 that ranges from less than 5.0 to close to 15 μg h/ml [102]. The variability is also evident in maximum concentrations, which range from less than 0.05 to nearly 0.30 μg/ml. After eight daily doses, XL647 accumulates with 2.8- to 3.2-fold increases in AUC and 2.5- to 4.2-fold increases in maximum concentrations. Accumulation was expected as the elimination half-life of XL674 is ca. 70 h. As the dose escalation continues, it will be interesting to see if the exposure level reaches the reported EC_{50} for inhibition of the multiple targets of this inhibitor that were derived from preclinical rodent models (EGFR, 360 nM, 0.18 μg/ml; KDR, 600 nM, 0.30 μg/ml; EphB4, 1200 nM, 0.60 μg/ml; ErbB2, 1800 nM, 0.90 μg/ml).

Table 4 Preclinical EGFR and ErbB2 activity of XL647 [101]

Assay	A431 EGFR driven	BT-474 ErbB2 driven
Inhibition of cellular autophosphorylation IC_{50} (nM)	1	84
In vivo inhibition of receptor phosphorylation EC_{50} (μM)	0.72	3.6
In vivo inhibition of receptor phosphorylation ED_{50} (mg/kg)	2.8	7
Tumor growth inhibition ED_{50} (mg/kg)	3.8	35
Mouse xenograft—100 mg/kg	Partial tumor regression	Tumor stasis

AEE788 and XL647 clearly show profiles of multiple kinase inhibition in vitro and in preclinical models. However, closer examination of the pharmacology suggests that these "multi-kinase" inhibitors are functionally selective. As mentioned above, AEE788 is most potent at inhibiting EGFR (IC_{50} = 11 nM), followed by moderate activity against ErbB2 (IC_{50} = 220 nM) and with weak activity against KDR (IC_{50} = 1 μM) in cellular assays of autophosphorylation [97]. Thus, the concentration required to inhibit EGFR autophosphorylation is 20-fold and 100-fold less than that required for ErbB2 and KDR, respectively. If clinical dosing is limited by an event that is driven by EGFR signaling (i.e., rash and diarrhea), then the concentrations required to affect ErbB2 and KDR activity may never be reached. The same is true for XL647, as can be seen from an examination of its activities against EGFR- and ErbB2-driven effects across biological systems (Table 4) [101]. If the rodent models are not susceptible to the EGFR-driven toxicities that are observed in humans, as seems likely, then the preclinical data that demonstrate multi-kinase inhibition may be misleading. Close evaluation of the clinical data will determine whether these drugs work through the inhibition of multiple kinases or if dose-limiting toxicities and/or pharmacokinetics reveal them to be a new generation of EGFR-selective inhibitors.

5 Inhibitors of Raf

Sorafenib is a reversible ATP-competitive inhibitor of multiple kinases developed by Bayer and Onyx. It was originally described as a C-Raf inhibitor, but has since been reported to inhibit B-Raf, p38α, KDR, and a several other

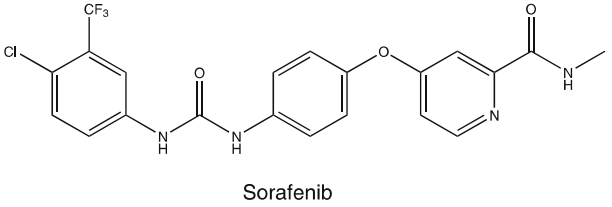


Fig. 9 Structure of sorafenib

kinases (Table 5) [103–105]. The activity against the mutant B-Raf^{V600E} has generated interest, because it has been reported recently that over 60% of melanomas have mutations in B-Raf, and most of these contain the V600E point mutation. The inhibitory potency of sorafenib against B-Raf^{V600E} is essentially equivalent to that of wild-type B-Raf, which is not unexpected since the mutant residue is located in the activation loop and is not in direct contact with the inhibitor.

Sorafenib has been shown in MDA-MB-231 cells to inhibit both MEK1 phosphorylation (IC_{50} = 40 nM) and ERK1/2 phosphorylation (IC_{50} = 100 nM), while having no affect on the Akt pathway. However, its ability to inhibit pERK1/2 production in other cell lines (HCT-116, DLD-1, Colo205, BxPC-3, and LOX) has been less impressive (IC_{50} = 1000–4000 nM), despite the presence of Ras and B-Raf mutations. In contrast, sorafenib inhibits PDGFRβ autophosphorylation (IC_{50} = 10 nM) in HAoSMC cells and also inhibits KDR autophosphorylation in HUVEC cells (IC_{50} = 100 nM) and 3T3-VEGFR2 transfectant cells (IC_{50} = 100 nM). Inhibition of the cellular growth of MDA-MB-231 and HCT116 cells is moderate (IC_{50} = 2.6 and 4.6 μM, respectively), although the compound has higher activity in the PDGFR-dependent cell line HAoSMC (IC_{50} = 280 nM), which suggests that the inhibition of non-Raf targets may be the driving mechanism of action for sorafenib [105].

Sorafenib has demonstrated significant antitumor activity against a range of mouse xenograft models from breast, colon, and lung carcinomas that contain either Ras or B-Raf mutations [105]. MDA-MB-231 is the most sensitive

Table 5 Enzymatic activity of sorafenib [103–105]

Kinase	IC_{50} (nM)	Kinase	IC_{50} (nM)
C-Raf	6	mVEGFR3	12
B-Raf wt	22	mPDGFRβ	57
B-Raf^{V600E}	38	Flt-3	58
KDR	90	c-Kit	68
mVEGFR2	6	P38α	38

model, with regressions observed at the top dose of 60 mg/kg/qd. Treatment with 30 and 60 mg/kg/qd sorafenib resulted in tumor stasis in HT-29, Colo-205, DLD-1, and A549 models.

Closer examination of the results from HT-29, A549, and NCI-H460 xenograft models illustrates the difficulty of pinpointing the mechanism of antitumor activity in these preclinical models with a multi-kinase inhibitor [105]. In the HT-29 xenograft model, tumors were excised 3 h after the last of five daily 30 and 60 mg/kg doses of sorafenib. Examination of pERK1/2 levels in these tumors showed complete inhibition of pERK1/2 formation as compared to the control tumors, with no effect on total ERK1/2 protein levels. As there was no pharmacokinetic component reported with the pharmacodynamic data, it can only be speculated that the plasma drug concentrations at the 3-h time point were greater than 5 μM, as those concentrations were required to completely inhibit pERK1/2 production in HT-29 cells. These results support the view that the activity of sorafenib in this model arises from inhibition of ERK1/2 phosphorylation. However, significant inhibition of microvessel area (MVA) and microvessel density (MVD) was also seen in the excised tumors, which indicates that sorafenib has an antiangiogenic effect. This antiangiogenic effect could be attributed to disruption of MEK/ERK signaling [41], or from direct inhibition of KDR. With the lack of pharmacokinetic and excised tumor data at the dosing interval (24 h), it is not possible to determine the mechanism of the antitumor effects of sorafenib in the HT-29 xenograft model. Additionally, the activity of sorafenib against both A549 and NCI-H460 xenografts does not correlate with cellular MEK/ERK studies. Both of these cell lines express high basal levels of pERK, which sorafenib was unable to inhibit at concentrations as high as 15 μM. While no studies were reported on excised tumors from these models, it is reasonable to assume that the antitumor effects observed are attributable to the inhibitory activities of sorafenib on protein kinases other than those of the Raf family. Undoubtedly, sorafenib is a multi-kinase inhibitor whose preclinical effects are caused by inhibition of its multiple targets. It is also clear that extrapolation from the clinical results of sorafenib to future selective Raf kinase inhibitors is inappropriate.

Phase I dose-ranging studies (50 to 800 mg) of sorafenib in 69 patients demonstrated the compound to be generally tolerated up to 400 mg bid [106]. Dose-related toxicities included skin rash, diarrhea, fatigue, vomiting, hypertension, and hand–foot syndrome. At doses above 400 mg bid (the MTD), unacceptable incidents of diarrhea and hand–foot syndrome were reported. Of the 45 patients that were evaluated for efficacy in phase I, one patient with hepatocellular carcinoma had a partial response, and 25 had stable disease, while 18 patients had progressive disease. Based on these results, phase II studies were designed with increased progression-free survival (PFS) as the primary endpoint. Since sorafenib inhibits multiple targets, the phase II trial included all comers, who were treated with 400 mg bid for 12 weeks. At the

end of this period, patients were assessed for tumor progression. Responders and nonresponders were assigned randomly to either a treated or a placebo control group (responders, who were defined as patients that experienced a > 25% regression in lesions, continued in the treated group); a total of 484 patients were enrolled, 202 of which had RCC. During the 12-week enrollment phase, the RCC patients were evaluated for efficacy [107]. From this population of patients, 144 showed disease stabilization; eight experienced a partial response. From the randomization phase, the PFS was found to be 24 weeks, compared to only 6 weeks in the control group.

Given the high occurrence of B-Raf mutations in melanoma lesions [107] and the reported activity of sorafenib against B-Raf in vitro, its efficacy was evaluated in 20 patients with stage IV refractory melanoma [108]. During the course of the 12-week study (400 mg bid), 15 patients experienced disease progression prior to the study endpoint. One patient had a partial response while three had stable disease. Five patients experienced grade 3 skin toxicity, and two experienced hypertension that necessitated treatment. Sorafenib was not effective in treating melanoma as a single agent.

Based on the efficacy of sorafenib in treating RCC in the phase II setting, a phase III study was initiated that targeted this indication in patients that were refractory to previous treatments, and had overall survival and PFS as the endpoints [109]. From the 796 patients that were enrolled into the study, 384 were administered 400 mg bid sorafenib, while the remainder received a placebo. Objective evaluation of 574 patients revealed disease stabilization in 261 (78%) of the treated group vs 186 (55%) in the placebo control group. Seven partial responses were observed in the sorafenib group vs none in the placebo group; disease progression was found in 29 (9%) of the sorafenib group, compared to 102 (30%) of the control group. As in the phase II studies, there was a significant increase in the PFS from 12 weeks in the placebo to 24 weeks in the sorafenib group. Based on interim analysis, patients who received sorafenib experienced a 39% survival advantage over patients that received the placebo, although the data did not reach statistical significance. Upon the completion of the interim analysis, the benefit of sorafenib treatment was deemed sufficient to allow patients that received the placebo to cross over to the treatment arm. The approval of sorafenib for RCC was based on PFS.

Exposure to sorafenib in patients is extremely high and variable [106, 110]. Absorption is slow and highly variable, with T_{max} ranging from ca. 2 to 8 h in single-dose studies. After both single and multiple doses, exposure does not increase with dose, nor does it appear to be related to dose (Table 6). Significant accumulation is observed, with C_{max} increasing 3.8-fold and AUC increasing 5.7-fold after multiple doses. The elimination half-life of sorafenib is consistent and ranges from 24 to 30 h, while food intake did not significantly affect any parameter. Once per day dosing should be supported with a 24- to 30-h half-life; however, comparisons of once per day and twice per

Table 6 Multiple-dose pharmacokinetic parameters of sorafenib in cancer patients [106, 110]

Dose	C_{max} (μg/ml) Mean	%CV [a]	AUC (μg h/ml) Mean	%CV [a]
100	2.31	54	23.8	43
200	2.84	88	16.1	83
400	9.35	44	71.7	43
600	9.81	51	79.0	52
800	7.21	28	44.9	30

[a] %CV is the coefficient of variance, also known as "relative standard deviation"

day schedules demonstrated a twofold greater AUC and higher C_{min} levels. Apparently, twice-daily dosing partially alleviates issues related to saturation of absorption. The lack of a dose–response relationship in exposure, and the high variability, makes it difficult to correlate dose/exposure to either adverse events or efficacy in patients.

6 Inhibitors of MEK

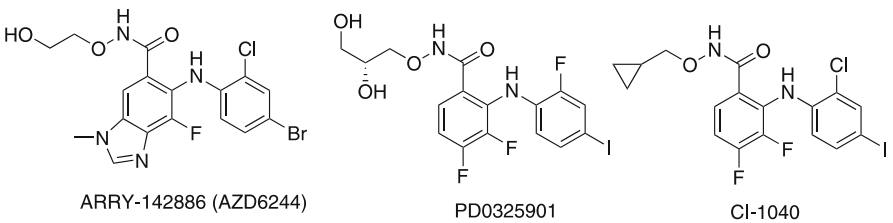


Fig. 10 MEK inhibitors

6.1 ARRY-142886 (AZD6244)

ARRY-142886 (AZD6244) is a potent, highly selective, ATP-uncompetitive MEK1/2 inhibitor [111]. ARRY-142886 was discovered at Array BioPharma and is currently in phase I clinical trials. The compound has high activity against constitutively active MEK (IC_{50} = 12 nM) and is highly selective (IC_{50} >10 μM) vs a panel of approximately 40 kinases. Although ARRY-142886 inhibits EGF-stimulated phosphorylation of ERK1/2 in A431 cells, it does not inhibit EGF-stimulated phosphorylation of ERK5 or MEK1/2. Moreover, the

compound does not inhibit p38 or Akt in HeLa cells at concentrations up to 50 μM. The compound inhibits ERK1/2 phosphorylation with an IC_{50} < 40 nM in HT-29, Malme-3M, SK-MEL-28, MiaPaCa2, and BxPC-3 cells [112]. ARRY-142886 also inhibits the proliferation of several cell lines that contain Ras and B-Raf mutations; it does not inhibit the growth of Malme-3 cells (IC_{50} >10 μM), which are the normal counterparts of Malme-3M melanoma cells. In responsive cell lines, ARRY-142886 induces a G1/S growth arrest (HT-29) and, depending on cellular context, is also able to induce apoptosis (Malme-3M and SK-MEL-2). The cellular activity of ARRY-142886 is summarized in Table 7.

The inhibitor has excellent pharmacokinetics in both mice and rats, with high bioavailabilities (82 and 74%, respectively) [113]. Additionally, the potential for drug–drug interactions after administration of ARRY-142886 is low as it does not inhibit any of the major cytochrome p450 isoforms below 20 μM.

In preclinical models, ARRY-142886 treatment results in either tumor regression or stasis in xenograft models of colorectal, non-small cell lung, pancreatic, breast, and melanoma cancers. Most of these cell lines contain either the $B\text{-}Raf^{V600E}$ or K-Ras mutations. Complete inhibition of pERK1/2 formation in excised tumors from both HT-29 and BxPC3 studies was achieved 4 h after an oral dose of 20 mg/kg/day ARRY-142886 [114]. In a separate HT-29 study, a PK/PD relationship was established [115]. Twelve hours after a single 30 mg/kg oral dose of ARRY-142886, approximately 80% inhibition of ERK1/2 phosphorylation was observed with a corresponding plasma concentration of about 0.60 μg/ml. In the same study at 24 h, ERK1/2 phosphorylation was

Table 7 Cellular activity of ARRY-142886 [111, 112]

Cell line	Stimulation	Study	IC_{50} (nM)
HT-29	None	pERK	< 8
Malme-3M	None	pERK	≤ 8
BxPC-3	None	pERK	< 8
HT-29	None	Cell viability	190
Malme-3M	None	Cell viability	50[a]
SK-MEL-2	None	Cell viability	270[a]
SK-MEL-28	None	Cell viability	76
Malme-3	None	Cell viability	No effect
BT474	None	pERK5	No effect
A431	EGF	pMEK1,2/pERK5	No effect
HeLa	10% FBS	pS6	No effect
HeLa	Anisomycin	pHsp27	No effect

[a] Caspase 3/7 activation observed—indication of apoptosis induction

inhibited by approximately 65% with ca. 0.10 μg/ml of ARRY-142886. Similarly, in a BxPC3 xenograft study, EC_{50} and EC_{90} (near C_{min}) on tumor growth inhibition were determined to be 0.18 and 0.34 μg/ml, respectively [116]. The EC_{90} levels were achieved following a 6 mg/kg/day dose where pERK1/2 levels were inhibited by about 90% in excised tumors. In a third BxPC3 study, ARRY-142886 was able to shrink large tumors after a dosing holiday [114]. After demonstrating complete tumor growth inhibition at 100 and 50 mg/kg/day for 21 days, a 7-day dosing holiday followed, in which tumor size in the 100 mg/kg/day group remained static and those in the 50 mg/kg/day group grew to ca. 500 mg. Continued treatment resulted in significant regression of the low-dose group to levels equivalent in size to predose animals (200 mg). Preclinical efficacy studies with ARRY-142886 established antitumor activity, proof of inhibition of target in vivo, and PK/PD relationships in two models (Table 8).

Preliminary results from the initial phase I study suggested dose-limiting toxicities of rash and diarrhea, with prolonged stable disease the best clinical response reported [117]. The rapid absorption and half-life of ARRY-142886 in cancer patients supports twice-daily dosing. A trend toward increasing exposure with dose was observed over a 50 to 300 mg dose range. An evaluation of pERK1/2 inhibition in PBMC from treated patients determined an EC_{50} of 0.17 μg/ml [118]. This agrees well with preclinical PK/PD studies in excised tumors from both HT-29 and BxPC3 models. Encouragingly, the trough levels of ARRY-142886 at both the 100 and 200 mg/bid doses (0.20 and 0.39 μg/ml, respectively) exceed the concentrations necessary to inhibit 50% of ERK1/2 phosphorylation in PBMC from treated patients and in excised tumors from preclinical xenograft models.

Table 8 Preclinical efficacy of ARRY-142886 [113–116]

Cancer type	Model	Mutational status	Activity (mg/kg/day)
Colorectal	HT-29	B-Raf^{V600E}	Cytostatic—ED_{50} 20–40
	HCT116	Mutant Ras	Cytostatic—$ED_{90} < 20$
NSCL	A549	Mutant Ras	Cytostatic—$ED_{90} < 20$
	Calu6	Mutant Ras	Cytostatic—$ED_{90} < 20$
Pancreatic	BxPC-3	Unknown	Regressions @ 3–12 EC_{50} 0.18 μg/ml EC_{90} 0.34 μg/ml
	PANC-1	Unknown	Cytostatic—$ED_{90} < 20$
	MIA PaCa-2	Mutant Ras	Regressions @ ∼ 40
Breast	MDA-MB-231	Wild-type Raf/Ras	Cytostatic—$ED_{90} < 20$
	Zr-75-1	Wild-type Raf/Ras	Cytostatic—ED_{50} 20–40
Melanoma	LOX	B-Raf^{V600E}	Regressions @ < 20

6.2 PD0325901

Pfizer has reported data for PD0325901, which is a second-generation, potent, highly selective inhibitor of MEK that is currently in phase I/II clinical trials. This compound replaced CI-1040, which was terminated in phase II trials due to poor efficacy and exposure [119]. PD0325901 is an improvement over CI-1040 in nearly every relevant aspect [120, 121]. PD0325901 exhibits a K_i for activated MEK1 of 1.1 nM and 0.79 nM for activated MEK2. The compound is also very potent (K_i = 0.90 nM) in the more biologically relevant Raf-activated MEK assay. PD0325901 is highly selective (IC_{50}>10 μM in a panel of 27 kinases), which is not surprising because the compound is not competitive with ATP or ERK1/2. PD0325901 inhibits ERK1/2 phosphorylation in C26 mouse colon carcinoma cells with an IC_{50} of 0.34 nM, whereas CI-1040 is much less potent (IC_{50} = 82 nM). PD0325901 is much more soluble at pH 6.5 than CI-1040 (190 vs < 1 μg/ml, respectively) and inhibits CYP3A4 less than CI-1040 (IC_{50} > 40 μM vs 5 μM, respectively). The rat oral bioavailability is similar between the two compounds (77% vs 85% at 10 mg/kg); however, the dog oral bioavailability is much better for PD0325901 (103% vs 5%).

PD0325901 has shown activity in a range of xenograft models at its MTD of 25 mg/kg/qd [122]. In a syngenic mouse C26 model, a single 3 mg/kg dose was able to inhibit pERK1/2 production in excised tumors by 75% 10 h postdose, with recovery of pERK1/2 levels to control values at 24 h, while a single 25 mg/kg dose was able to inhibit ERK1/2 phosphorylation by 75% at 24 h. Extensive PK/PD modeling determined both the EC_{50} for inhibition of ERK1/2 phosphorylation in excised tumors and the EC_{50} for tumor growth inhibition in four responsive xenograft models (Table 9) [123]. In addition to showing that 0.10 μg/ml plasma concentrations of PD0325901 are sufficient for significant inhibition of the target in vivo, this work demonstrates that inhibition of the target in vivo is correlated with antitumor effects in several models. This study also demonstrated that inhibition of ERK1/2 phosphorylation declined with declining concentrations of PD0325901 and in a rapid fashion. This extensive preclinical work could be coupled with predictions

Table 9 PK/PD relationships of PD0325901 in mouse xenograft models [123]

Model	pERK1/2 response in excised tumors (EC_{50} [μg/ml])	Tumor growth inhibition (EC_{50} [μg/ml])
BxPC-3	0.041	0.009
HT-29	0.054	0.116
Mia PaCa-2	0.005	0.023
Colo-205	0.012	0.049

of human pharmacokinetics to direct the clinical program. For example, it may be reasonable to set an initial target drug minimum concentration of 0.10 μg/ml. These data also suggest that a dosing interval should be chosen that will result in 24-h coverage, if complete and continuous inhibition of pERK1/2 is necessary for efficacy.

PD0325901 is currently in phase II clinical trials [119]. In phase I studies, 41 patients received drug with the MTD reported as less than 20 mg/bid. The DLTs were rash, congestive heart failure, and syncope. Two melanoma patients achieved partial responses, albeit at dose levels (20 mg/bid) above the MTD, and eight patients experienced stable disease. In paired pre- and post-treatment biopsies, doses above 2 mg/bid resulted, on average, in greater than 80% inhibition of ERK1/2 phosphorylation. Unfortunately, tumor tissue inhibition of ERK1/2 phosphorylation did not correlate with clinical response. Although the complete pharmacokinetic profile has not been disclosed, doses above 15 mg/bid resulted in steady-state levels of PD0325901 maintained above those predicted from preclinical models for significant inhibition of ERK1/2 phosphorylation (> 0.27 μg/ml). The activity in melanoma is encouraging: the prevalence of B-Raf mutations gives a clear biological rationale for MEK inhibition in this disease. Most important, current treatment options for patients with malignant melanoma are poor.

7 Biomarkers

In this emerging era of molecular-targeted therapies, biomarkers are becoming more useful and perhaps even necessary for the success of new drugs. In this context, the two classes of biomarkers that are most relevant are pharmacodynamic biomarkers, which are used to confirm drug activity and mechanism of action, and predictive biomarkers, which are used to predict clinical response to targeted therapies.

Among the compounds that have been discussed in this chapter, the analysis of biomarkers has been most extensive and advanced for gefitinib and erlotinib. Skin has been used as a surrogate tissue in several studies because it is more readily attainable and is a known site of EGF action and EGFR expression. In phase I and phase II studies of gefitinib and erlotinib, skin biopsies from pretreated and treated patients were analyzed for steady-state levels of protein biomarkers by immunohistochemistry [124–128]. Decreases in pEGFR and pERK1/2, as well as an increase in the level of the cell cycle inhibitor p27 KIP-1, were observed in 25–90% of patients. However, as reported for the gefitinib trial, there was no correlation between biomarker readout and dose, plasma concentration, rash, or objective response [124]. These studies demonstrate that the biological activity and mechanism of action of EGFR inhibitors can be verified using skin biop-

sies, but that activity in accessible surrogate tissue may not predict antitumor activity.

What is ultimately important is whether the compound is distributed to the site of the tumor and able to inhibit the target of interest. Obtaining paired tumor biopsies, however, is challenging on several levels, from patient accrual to the quality of the tumor biopsy for subsequent analysis. Although there have been studies addressing biomarkers for EGFR inhibitors in tumors, the actual number of tumor pairs that can be evaluated has been restrictive. In a phase I/II trial of gefitinib in colorectal cancer, pre- and posttreatment biopsies from liver metastases were analyzed for levels of pEGFR, pERK1/2, and pAkt [129]. From 11 paired biopsies, only one of the pretreatment tumors had detectable pEGFR, one had detectable pERK1/2, and two had detectable pAkt. Although the number of samples was limited, inhibition of each phosphobiomarker was seen. More significantly, detectable pERK1/2 in neighboring tumor stromal fibroblasts was inhibited in five of nine patients, which demonstrated that the compound did reach the site of the tumor. In a phase II gefitinib trial in breast cancer, examination of 16 available paired tumor biopsies showed that four tumors had detectable baseline pEGFR and eight tumors had detectable baseline pERK1/2 [125]. Decreases in each phosphoprotein in these tumors were observed with treatment. However, inhibition of pERK1/2 levels did not correlate with an increase in p27 KIP-1, emphasizing that, in addition to demonstrating inhibition of the target, it is equally important to select tumors that will respond to that inhibition. Although tumor biomarker information is limited at this time, these results do suggest that gefitinib is distributed to the tumor and behaves as expected; a correlation to objective response is still lacking.

There has been recent promise of a predictive biomarker for EGFR inhibitors with the identification of EGFR mutations in approximately 80% of patients that respond to gefitinib or erlotinib [8, 9, 54]. The presence of EGFR mutations correlates strongly with the clinical response rates. This correlation is further upheld when patients are stratified into various subgroups: nonsmokers vs smokers, adenocarcinoma vs other cancers, female vs male, and East Asian vs US populations [9, 54, 130]. These EGFR mutations are somatic, often heterozygous, and comprise both point and deletion mutations in the catalytic domain. Characterization of several EGFR mutants in cellular studies suggests that most, but not all, lead to not only a greater response to ligand but also a longer duration of the response [8, 9]. Most importantly, the EGFR mutants are 10–100-fold more sensitive to inhibition by gefitinib and erlotinib [8, 9]. The presence of a mutation may confer a greater tumor response rate to EGFR inhibition because (1) the increased activity of the receptor results in an increased propensity for the tumor to be driven by EGFR, and (2) the mutant may exhibit increased sensitivity to inhibition by gefitinib and erlotinib. This increased sensitivity has significant clinical consequences, since plasma levels of compound can be limiting.

The analysis of the biomarker data for sorafenib is more difficult to interpret due to limited data and the broad activity of this compound [17]. Clinical biomarker studies have been focused on the activity of sorafenib against Raf. Although MEK1/2 are the downstream targets of Raf, phosphorylation of ERK1/2 has been the principal biomarker because the available reagents are superior and ERK1/2 are accepted as the only known substrates of MEK1/2. In a phase I trial, the levels of PMA-induced pERK1/2 in CD7+ T cells were shown to decrease after 21 days of sorafenib dosing [106, 131]. Neither plasma concentrations nor the postdose time point for the samples was reported, making these observations less meaningful. The analysis of pERK1/2 in paired tumor biopsies has been reported for one melanoma patient who experienced a partial response [110]. The levels of pERK1/2 were shown to decrease after the second cycle of treatment (1 cycle = 7 days on, 7 days off), and correlated with a decrease in tumor activity as measured by glucose uptake.

The utility of baseline pERK1/2 as a predictive biomarker for tumor response was evaluated in a phase II trial for sorafenib where pretreatment tumors were analyzed for pERK1/2 levels [132]. Three patients that showed moderate to high staining of pERK1/2 were partial responders, including the melanoma patient described above. One patient did not respond and had low basal levels of pERK1/2. Another prime candidate for a predictive biomarker is the mutational status of B-Raf. The analysis of mutant B-Raf and tumor response has been reported for only a combination study of sorafenib with carboplatin and paclitaxel in melanoma patients [17]. No correlation was seen in this context. It is difficult to draw conclusions from all these observations, since sorafenib may be acting through its activity against kinases other than Raf. Unfortunately, the analysis of biomarkers for these other targets in the clinical setting has not been reported. These studies emphasize the difficulty of using biomarkers for agents that target multiple kinases.

The measurement of pERK1/2 inhibition has proven to be a useful pharmacodynamic marker for measuring the activity of MEK1/2 inhibitors in the clinical setting. Inhibition of PMA-treated pERK1/2 in PBMCs has been reported for both CI-1040 and ARRY-142886 [117, 118, 133]. In both cases, a dose-dependent relationship was demonstrated between plasma concentration and inhibition. Pre- and postdose biopsies from patients dosed with CI-1040 and PD0325901 have shown inhibition of ERK1/2 phosphorylation in the tumor, ranging from 40 to 100% inhibition [119, 133]. However, no correlation with tumor response has been reported. The lack of correlation could be because dependency on the MEK1/2 pathway will vary among tumors, but also because the degree of inhibition was either not high enough or not sustained long enough.

The utilization of pERK1/2 as a predictive biomarker for MEK inhibitors is still unclear. In the phase II trial for CI-1040, archived tumors that dated from months to years before treatment were analyzed for pERK1/2 [134].

Despite the limitations of the samples, the data did suggest a correlation between elevated baseline pERK1/2 levels and stable disease. The mutational status of B-Raf is another logical candidate for a predictive biomarker, especially since B-Raf mutations have been shown to confer increased sensitivity to MEK1/2 inhibitors using in vitro and in vivo models [135]; nothing has yet been reported.

An interesting observation from the analysis of many clinical studies is that many inhibitors of the EGFR–Raf–MEK1/2–ERK1/2 pathway share the dose-limiting toxicities of rash and diarrhea. Even administration of EGFR antibodies, such as cetuximab, can lead to rash, which suggests that this side effect is pathway-based. These adverse events are not life-threatening and may be better tolerated than the side effects of existing chemotherapeutic agents. In fact, this side effect may actually be informative as a predictive biomarker, as there are data that correlate objective response to the severity of the rash [66].

8 Conclusions and Outlook

The last decade of research and development on small-molecule inhibitors of growth factor signaling has led to many advances, but there is still much more to accomplish. Initially, the rationale to target the EGFR/ErbB2–Raf–MEK pathway relied primarily on overexpression of the growth factor receptors in human cancer. Since then, research in the laboratory and the clinic has established more relationships between this pathway and uncontrolled cell growth. Several of these relationships were discovered after small-molecule inhibitors were identified. Some of the excitement over the MEK inhibitors derives from the prevalence of B-Raf mutations in certain cancers that leads to constitutive activity of MEK and ERK. Interestingly, B-Raf mutations were identified after a MEK inhibitor was already in human trials. Likewise, EGFR activating mutants were not found until erlotinib and gefitinib were in phase III trials. Clinical experience has shown that target overexpression alone is not as powerful a validation as was first thought. Much more useful, and elusive, is a correlation between the target and tumorigenesis, such as Bcr-Abl in CML. The hope is that in the near future, even stronger correlations to disease progression can be made and used to select responsive patients to EGFR/ErbB2–Raf–MEK inhibitors.

There was a time not long ago when researchers were united in the belief that potent, selective kinase inhibition was crucial for the development of safe drugs and that it would be very difficult, if not impossible, to achieve. Conventional wisdom held that the high concentration of cytosolic ATP (in the millimolar range) precluded the identification of nanomolar ATP-competitive kinase inhibitors in cell-based assays. Some of these beliefs were grounded

in the fact that human kinases share a highly similar ATP binding site, and that there were thousands of human kinases. Since then, as a result of the human genome project, we know that the number of kinases is actually at least tenfold lower. Evident from the experimental drugs covered here is the fact that structural biology, molecular modeling, and medicinal chemistry have aided the discovery and development of selective, potent, small-molecule ATP-competitive inhibitors of many kinases. Additionally, allosteric inhibitors with exquisite selectivity have been discovered. Furthermore, the approvals of sorafenib, sutent, and imatinib have dispelled the notion that selectivity is required for the development of safe therapeutics.

The development of multi-kinase inhibitors has generated a new debate. With some safety concerns alleviated, some believe that multi-kinase inhibition in a single agent will lead to greater efficacy [136]. Others think that a selective inhibitor is preferred as nearly all cancer chemotherapy is and will continue to be poly-pharmacy, and a selective inhibitor will allow for greater flexibility, and possibly less overlap of adverse events, in devising and testing combination therapy. For example, within the field of growth factor inhibitors, it is unclear if optimal efficacy and therapeutic index are achieved by one agent that equally and continuously inhibits multiple targets, or if varying degrees and duration of inhibition of the targets of interest are desirable. The latter is only achievable with multiple selective agents. In addition, selective inhibitors allow for easier biomarker development and correlation. The final word on multi-kinase versus selective inhibitors will only come from significant clinical evaluation.

Not long ago, many clinicians and researchers thought that targeted kinase inhibitors, unlike traditional cytotoxic chemotherapy, would demonstrate efficacy well below toxic doses, as was observed in animal models. While it is true that the toxicities with targeted kinase inhibitors are less severe than those of many cytotoxic drugs, they still exist and limit dosing. Retrospectively, preclinical safety studies predicted the gastrointestinal adverse events observed with most agents, but did not predict the unique rash observed with EGFR and MEK inhibitors. With the recent realization that rash may be a mechanism-based side effect of these agents, evaluation of rash treatment options is still in its infancy. In the future, hopefully, this rash will become more manageable, potentially allowing higher dosing of EGFR and MEK inhibitors. Interestingly, the selective ErbB2 inhibitors CP-714724 and trastuzumab are not limited by skin toxicities. Until quite recently, many believed an optimal biological dose, below the MTD, existed with signal transduction inhibitors. Today, there is a growing consensus to dose targeted signal transduction inhibitors to their MTD in an attempt to demonstrate robust clinical efficacy.

Finally, these new inhibitors are forcing oncology researchers and clinicians to rethink the relationship between objective response rate and survival/progression-free survival. As a result, more and more phase II trials

are designed with treatment arms comparing the new therapy to the standard of care. This design allows a time to progression efficacy measurement not available from a single treatment arm phase II study, where the only efficacy measure is objective clinical response. Successful comparator phase II trials will give clinicians more confidence going forward into the large, placebo-controlled phase III trials required to demonstrate statistically significant progression-free survival.

This is an interesting time to be in the field of anticancer drug research and development, as the discovery and evaluation of new selective and multitargeted small-molecule inhibitors reshape our thoughts about treating cancer patients. While EGFR/ErbB–Raf–MEK targeted drugs have not been a panacea, there is no question that advances in this field have had a positive effect on the study and treatment of cancer. Many of these advances have only been made possible by progressing small-molecule inhibitors into the clinic. There is no question that as data from these cutting-edge inhibitors feed back to researchers in the laboratory, it will enable them to make the next breakthrough. The development and clinical use of targeted therapies will not be straightforward, but such agents have the potential of improved efficacy and a higher therapeutic index than current chemotherapeutic approaches.

References

1. Blume-Jensen P, Hunter T (2001) Nature 411:355
2. Choong NW, Ma PC, Salgia R (2005) Expert Opin Ther Targets 9:533
3. Porter AC, Vaillancourt RR (1998) Oncogene 17:1343
4. Robertson SC, Tynan J, Donoghue DJ (2000) Trends Genet 16:368
5. Hynes NE, Lane HA (2005) Nat Rev Cancer 5:341
6. Harries M, Smith I (2002) Endocr Relat Cancer 9:75
7. Shelton JG, Steelman LS, Abrams SL, Bertrand FE, Franklin RA, McMahon M, McCubrey JA (2005) Expert Opin Ther Targets 9:1009
8. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA (2004) N Engl J Med 350:2129
9. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, Mardis E, Kupfer D, Wilson R, Kris M, Varmus H (2004) Proc Natl Acad Sci USA 101:13306
10. Bos JL (1989) Cancer Res 49:4682
11. Fransen K, Klintenas M, Osterstrom A, Dimberg J, Monstein HJ, Soderkvist P (2004) Carcinogenesis 25:527
12. Gray-Schopfer VC, da Rocha Dias S, Marais R (2005) Cancer Metastasis Rev 24:165
13. Friday BB, Adjei AA (2005) Biochim Biophys Acta 1756:127
14. Repasky GA, Chenette EJ, Der CJ (2004) Trends Cell Biol 14:639
15. Voice JK, Klemke RL, Le A, Jackson JH (1999) J Biol Chem 274:17164
16. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S,

Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA (2002) Nature 417:949
17. Beeram M, Patnaik A, Rowinsky EK (2005) J Clin Oncol 23:6771
18. Emuss V, Garnett M, Mason C, Marais R (2005) Cancer Res 65:9719
19. Lee JW, Soung YH, Kim SY, Park WS, Nam SW, Min WS, Kim SH, Lee JY, Yoo NJ, Lee SH (2005) APMIS 113:54
20. Belanger LF, Roy S, Tremblay M, Brott B, Steff AM, Mourad W, Hugo P, Erikson R, Charron J (2003) Mol Cell Biol 23:4778
21. Giroux S, Tremblay M, Bernard D, Cardin-Girard JF, Aubry S, Larouche L, Rousseau S, Huot J, Landry J, Jeannotte L, Charron J (1999) Curr Biol 9:369
22. Hoshino R, Chatani Y, Yamori T, Tsuruo T, Oka H, Yoshida O, Shimada Y, Ari-i S, Wada H, Fujimoto J, Kohno M (1999) Oncogene 18:813
23. Stamos J, Sliwkowski MX, Eigenbrot C (2002) J Biol Chem 277:46265
24. Wood ER, Truesdale AT, McDonald OB, Yuan D, Hassell A, Dickerson SH, Ellis B, Pennisi C, Horne E, Lackey K, Alligood KJ, Rusnak DW, Gilmer TM, Shewchuk L (2004) Cancer Res 64:6652
25. Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, Jones CM, Marshall CJ, Springer CJ, Barford D, Marais R (2004) Cell 116:855
26. Ohren JF, Chen H, Pavlovsky A, Whitehead C, Zhang E, Kuffa P, Yan C, McConnell P, Spessard C, Banotai C, Mueller WT, Delaney A, Omer C, Sebolt-Leopold J, Dudley DT, Leung IK, Flamme C, Warmus J, Kaufman M, Barrett S, Tecle H, Hasemann CA (2004) Nat Struct Mol Biol 11:1192
27. Huse M, Kuriyan J (2002) Cell 109:275
28. Noble ME, Endicott JA, Johnson LN (2004) Science 303:1800
29. Shewchuk L, Hassell A, Wisely B, Rocque W, Holmes W, Veal J, Kuyper LF (2000) J Med Chem 43:133
30. Gotoh N, Tojo A, Hino M, Yazaki Y, Shibuya M (1992) Biochem Biophys Res Commun 186:768
31. Christensen JG, Vincent PW, Klohs WD, Fry DW, Leopold WR, Elliott WL (2005) Mol Cancer Ther 4:938
32. Landau M, Fleishman SJ, Ben-Tal N (2004) Structure 12:2265
33. Hubbard SR (1997) Embo J 16:5572
34. Zhu X, Kim JL, Newcomb JR, Rose PE, Stover DR, Toledo LM, Zhao H, Morgenstern KA (1999) Struct Fold Des 7:651
35. Penuel E, Akita RW, Sliwkowski MX (2002) J Biol Chem 277:28468
36. Yang J, Cron P, Good VM, Thompson V, Hemmings BA, Barford D (2002) Nat Struct Biol 9:940
37. Bossemeyer D, Engh RA, Kinzel V, Ponstingl H, Huber R (1993) Embo J 12:849
38. Mol CD, Fabbro D, Hosfield DJ (2004) Curr Opin Drug Discov Devel 7:639
39. Pargellis C, Tong L, Churchill L, Cirillo PF, Gilmore T, Graham AG, Grob PM, Hickey ER, Moss N, Pav S, Regan J (2002) Nat Struct Biol 9:268
40. Knight ZA, Shokat KM (2005) Chem Biol 12:621
41. Wallace EM, Lyssikatos J, Blake JF, Seo J, Yang HW, Yeh TC, Perrier M, Jarski H, Marsh V, Poch G, Goyette Livingston M, Otten J, Hingorani G, Woessner R, Winski SL, Anderson DA, Lee P, Winkler J, Koch K (2005) Abstracts of the AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapy, Philadelphia, 14–18 Nov 2005:Abstr B77

42. Fabian MA, Biggs WH III, Treiber DK, Atteridge CE, Azimioara MD, Benedetti MG, Carter TA, Ciceri P, Edeen PT, Floyd M, Ford JM, Galvin M, Gerlach JL, Grotzfeld RM, Herrgard S, Insko DE, Insko MA, Lai AG, Lelias JM, Mehta SA, Milanov ZV, Velasco AM, Wodicka LM, Patel HK, Zarrinkar PP, Lockhart DJ (2005) Nat Biotechnol 23:329
43. Moyer JD, Barbacci EG, Iwata KK, Arnold L, Boman B, Cunningham A, DiOrio C, Doty J, Morin MJ, Moyer MP, Neveu M, Pollack VA, Pustilnik LR, Reynolds MM, Sloan D, Theleman A, Miller P (1997) Cancer Res 57:4838
44. Pollack VA, Savage DM, Baker DA, Tsaparikos KE, Sloan DE, Moyer JD, Barbacci EG, Pustilnik LR, Smolarek TA, Davis JA, Vaidya MP, Arnold LD, Doty JL, Iwata KK, Morin MJ (1999) J Pharmacol Exp Ther 291:739
45. Cohen MH, Johnson JR, Chen YF, Sridhara R, Pazdur R (2005) Oncologist 10:461
46. Moore MJ (2005) Semin Oncol 32:5
47. Perez-Soler R (2004) Clin Cancer Res 10:4238s
48. Perez-Soler R (2004) Clin Lung Cancer 6 Suppl 1:S20
49. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van Kooten M, Dediu M, Findlay B, Tu D, Johnston D, Bezjak A, Clark G, Santabarbara P, Seymour L (2005) N Engl J Med 353:123
50. Siegel-Lakhai WS, Beijnen JH, Schellens JH (2005) Oncologist 10:579
51. Tang PA, Tsao MS, Moore MJ (2006) Expert Opin Pharmacother 7:177
52. Wakeling AE, Guy SP, Woodburn JR, Ashton SE, Curry BJ, Barker AJ, Gibson KH (2002) Cancer Res 62:5749
53. Campiglio M, Locatelli A, Olgiati C, Normanno N, Somenzi G, Vigano L, Fumagalli M, Menard S, Gianni L (2004) J Cell Physiol 198:259
54. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE, Meyerson M (2004) Science 304:1497
55. Ono M, Hirata A, Kometani T, Miyagawa M, Ueda S, Kinoshita H, Fujii T, Kuwano M (2004) Mol Cancer Ther 3:465
56. Cohen MH, Williams GA, Sridhara R, Chen G, McGuinn WD Jr, Morse D, Abraham S, Rahman A, Liang C, Lostritto R, Baird A, Pazdur R (2004) Clin Cancer Res 10: 1212
57. Kris MG, Natale RB, Herbst RS, Lynch TJ Jr, Prager D, Belani CP, Schiller JH, Kelly K, Spiridonidis H, Sandler A, Albain KS, Cella D, Wolf MK, Averbuch SD, Ochs JJ, Kay AC (2003) Jama 290:2149
58. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard JY, Nishiwaki Y, Vansteenkiste J, Kudoh S, Rischin D, Eek R, Horai T, Noda K, Takata I, Smit E, Averbuch S, Macleod A, Feyereislova A, Dong RP, Baselga J (2003) J Clin Oncol 21: 2237
59. Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, Thongprasert S, Tan EH, Pemberton K, Archer V, Carroll K (2005) Lancet 366:1527
60. Giaccone G, Herbst RS, Manegold C, Scagliotti G, Rosell R, Miller V, Natale RB, Schiller JH, Von Pawel J, Pluzanska A, Gatzemeier U, Grous J, Ochs JS, Averbuch SD, Wolf MK, Rennie P, Fandi A, Johnson DH (2004) J Clin Oncol 22:777
61. Herbst RS, Giaccone G, Schiller JH, Natale RB, Miller V, Manegold C, Scagliotti G, Rosell R, Oliff I, Reeves JA, Wolf MK, Krebs AD, Averbuch SD, Ochs JS, Grous J, Fandi A, Johnson DH (2004) J Clin Oncol 22:785
62. Swaisland HC, Smith RP, Laight A, Kerr DJ, Ranson M, Wilder-Smith CH, Duvauchelle T (2005) Clin Pharmacokinet 44:1165

63. Hidalgo M, Siu LL, Nemunaitis J, Rizzo J, Hammond LA, Takimoto C, Eckhardt SG, Tolcher A, Britten CD, Denis L, Ferrante K, Von Hoff DD, Silberman S, Rowinsky EK (2001) J Clin Oncol 19:3267
64. Wolf M, Swaisland H, Averbuch S (2004) Clin Cancer Res 10:4607
65. Perez-Soler R, Chachoua A, Hammond LA, Rowinsky EK, Huberman M, Karp D, Rigas J, Clark GM, Santabarbara P, Bonomi P (2004) J Clin Oncol 22:3238
66. Perez-Soler R, Saltz L (2005) J Clin Oncol 23:5235
67. Jani JP, Barbacci G, Bhattacharya S, Boos C, Campbell M, Clark T, Coleman K, Currier N, Emerson EO, Floyd E, Goodwin P, Gerdin K, Harriman S, Iwata K, Kath J, Kwan T, Liu Z, Mairs E, Miller P, Morris J, Pustilnik L, Rafidi K, Ricter D, Rossi AM, Soderstrom E, Steyn S, Su C, Szwec R, Thompson C, Wagner L, Wang H, Winter S, Xiao J, Zhao X, Moyer JD (2004) Proc Am Assoc Cancer Res 45:Abstr 4637
68. Munster PN, Tolcher AW, Britten CD, Gelmon K, Moulder S, Minton S, Mita M, Guo F, Noe D, Pierce KJ, Denis L, Letrent SP (2004) Eur J Cancer Suppl 2:Abstr 334
69. Rusnak DW, Lackey K, Affleck K, Wood ER, Alligood KJ, Rhodes N, Keith BR, Murray DM, Knight WB, Mullin RJ, Gilmer TM (2001) Mol Cancer Ther 1:85
70. Xia W, Mullin RJ, Keith BR, Liu LH, Ma H, Rusnak DW, Owens G, Alligood KJ, Spector NL (2002) Oncogene 21:6255
71. Blackwell K, Kaplan EH, Franco SX, Marcom PK, Maleski J, Sorensen M, Berger MS (2004) Ann Oncol 15 Suppl 3:Abstr 1030
72. Gomez HL, Doval DC, Chavez MA, Ang PC, Nag S, Chow LW, Berger M, Westlund R, Newstat B, Stein S, Stanislaus MA, Sledge GW (2005) 28th Annual San Antonio Breast Cancer Symposium, 8–11 Dec 2005:Abstr 1071
73. Bence AK, Anderson EB, Halepota MA, Doukas MA, DeSimone PA, Davis GA, Smith DA, Koch KM, Stead AG, Mangum S, Bowen CJ, Spector NL, Hsieh S, Adams VR (2005) Invest New Drugs 23:39
74. Burris HA III, Hurwitz HI, Dees EC, Dowlati A, Blackwell KL, O'Neil B, Marcom PK, Ellis MJ, Overmoyer B, Jones SF, Harris JL, Smith DA, Koch KM, Stead A, Mangum S, Spector NL (2005) J Clin Oncol 23:5305
75. Mullin RJ, Alligood KJ, Allen PP, Crosby RM, Keith BR, Lackey K, Gilmer TM, Griffin RJ, Murray DM, Tadepalli SM (2001) Proc Am Assoc Cancer Res 42:Abstr 4579
76. Gavai AV, Fink BE, Tokarski JS, Fairfax D, Martin G, Grubb L, Fu Z, Kim SH, Leavitt K, Mastalerz H, Mitt T, Hunt JT, Kadow JF, Du K, Han WC, Norris D, Goyal B, Vyas DM, Yu C, Oppenheimer S, Zhang H, Lee FY, Wong TW, Vite GD (2005) 229th ACS National Meeting, San Diego, 13–17 Mar 2005:Abstr MEDI021
77. Wong TW, Lee F, Oppenheimer S, Yu C, Huang F, Zhang H, Fink B, Gavai A, Vite G (2005) Proc Am Assoc Cancer Res 46:Abstr 3395
78. Gavai AV, Fink BE, Tokarski JS, Fairfax D, Martin G, Grubb L, Fu Z, Kim SH, Leavitt KJ, Mastalerz H, Mitt T, Hunt JT, Kadow JF, Du K, Han WC, Norris D, Goyal B, Vyas DM, Yu C, Oppenheimer S, Zhang H, Lee F, Wong TW, Vite GD (2005) Proc Am Assoc Cancer Res 46:Abstr 2539
79. Garland LL, Pegram M, Song D, Mendelson D, Parker KE, Martell RE, Gordon MS (2005) 41st Annual Meeting American Society of Clinical Oncology (ASCO), Orlando, 13–17 May 2005:Abstr 3152
80. Soria JC, Cortes J, Armand JP, Taleb A, Van Bree L, Lopez E, Song S, Zeradib K, Vazquez F, Martell RE, Baselga J (2005) 41st Annual Meeting American Society of Clinical Oncology (ASCO), Orlando, 13–17 May 2005:Abstr 3109
81. Miknis G, Wallace E, Lyssikatos J, Lee P, Zhao Q, Hans J, Topalov G, Buckmelter A, Tarlton G, Ren L, Tullis J, Bernat B, Pieti Opie L, von Carlowitz I, Parry J, Morales T,

Perrier M, Woessner R, Pheneger T, Hoffman K, Borton C, Winkler J, Koch K (2005) Proc Am Assoc Cancer Res 46:Abstr 3399
82. Pheneger TM, Woessner R, Lyssikatos J, Miknis G, Anderson D, Winski S, Lee PA (2005) AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapy, Philadelphia, 14–18 Nov 2005:Abstr A247
83. Smaill JB, Rewcastle GW, Loo JA, Greis KD, Chan OH, Reyner EL, Lipka E, Showalter HD, Vincent PW, Elliott WL, Denny WA (2000) J Med Chem 43:1380
84. Allen LF, Lenehan PF, Eiseman IA, Elliott WL, Fry DW (2002) Semin Oncol 29:11
85. Nemunaitis J, Eiseman I, Cunningham C, Senzer N, Williams A, Lenehan PF, Olson SC, Bycott P, Schlicht M, Zentgraff R, Shin DM, Zinner RG (2005) Clin Cancer Res 11:3846
86. Calvo E, Tolcher AW, Hammond LA, Patnaik A, de Bono JS, Eiseman IA, Olson SC, Lenehan PF, McCreery H, Lorusso P, Rowinsky EK (2004) Clin Cancer Res 10: 7112
87. Campos S, Hamid O, Seiden MV, Oza A, Plante M, Potkul RK, Lenehan PF, Kaldjian EP, Varterasian ML, Jordan C, Charbonneau C, Hirte H (2005) J Clin Oncol 23:5597
88. Simon G, Olson S, Langevin M, Eiseman I, Mahany J, Helmke W, Garrett C, Lush R, Lenehan P, Sullivan D (2004) Eur J Cancer Suppl 2:Abstr 281
89. Solca F, Baum A, Guth B, Colbatzky F, Blech S, Amelsberg A, Himmelsbach F (2005) AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapy, Philadelphia, 14–18 Nov 2005:Abstr A244
90. Plummer R, Vidal L, Perrett R, Shaw H, Pilkington M, Hanwell J, Temple G, Fong P, Amelsberg A, Calvert H, De Bono J (2005) AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapy, Philadelphia, 14–18 Nov 2005:Abstr A105
91. Eskens FA, Mom CH, Planting A, Gietema JA, Amelsberg A, Huisman H, Verweij J, de Vries EG (2005) AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapy, Philadelphia, 14–18 Nov 2005:Abstr A235
92. Marshall JL, Lewis NL, Amelsberg A, Briscoe J, Hwang J, Malik S, Cohen R (2005) AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapy, Philadelphia, 14–18 Nov 2005:Abstr B161
93. Rabindran SK, Discafani CM, Rosfjord EC, Baxter M, Floyd MB, Golas J, Hallett WA, Johnson BD, Nilakantan R, Overbeek E, Reich MF, Shen R, Shi X, Tsou HR, Wang YF, Wissner A (2004) Cancer Res 64:3958
94. Tsou HR, Overbeek-Klumpers EG, Hallett WA, Reich MF, Floyd MB, Johnson BD, Michalak RS, Nilakantan R, Discafani C, Golas J, Rabindran SK, Shen R, Shi X, Wang YF, Upeslacis J, Wissner A (2005) J Med Chem 48:1107
95. Solca F, Klein C, Schweifer N, Baum A, Rudolph D, Amelsberg A, Himmelsbach F, Beug H (2005) AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapy, Philadelphia, 14–18 Nov 2005:Abstr A242
96. Traxler P (2003) Expert Opin Ther Targets 7:215
97. Traxler P, Allegrini PR, Brandt R, Brueggen J, Cozens R, Fabbro D, Grosios K, Lane HA, McSheehy P, Mestan J, Meyer T, Tang C, Wartmann M, Wood J, Caravatti G (2004) Cancer Res 64:4931
98. Dumez H, Clement P, Takimoto CH, van Oosterom AT, Mita A, Parker K, Rowinsky EK, Salazar R, Martinelli E, Baselga J (2004) Eur J Cancer Suppl 2:Abstr 387
99. Mita A, Takimoto CH, Martinelli E, Dumez H, DiLea C, Mietlowski W, Tabernero J, Dugan M, Isambert N, Van Oosterom AT (2004) Eur J Cancer Suppl 2:Abstr 412

100. Reardon D, Cloughesy T, Conrad C, Prados M, Xia J, Mietlowski W, Dugan M, Mischel P, Friedman H, Yung A (2005) 41st Annual Meeting American Society of Clinical Oncology (ASCO), Orlando, 13–17 May 2005:Abstr 3063
101. Joly A (2004) Eur J Cancer Suppl 2:Abstr 134
102. Wakelee HA, Adjei AA, Halsay J, Lensing JL, Dugay JD, Hanson LJ, Reid JM, Hutchison SA, Piens JR, Sikic BI (2005) AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapy, Philadelphia, 14–18 Nov 2005:Abstr A261
103. Lyons JF, Wilhelm S, Hibner B, Bollag G (2001) Endocr Relat Cancer 8:219
104. Wilhelm S, Chien DS (2002) Curr Pharm Des 8:2255
105. Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, Chen C, Zhang X, Vincent P, McHugh M, Cao Y, Shujath J, Gawlak S, Eveleigh D, Rowley B, Liu L, Adnane L, Lynch M, Auclair D, Taylor I, Gedrich R, Voznesensky A, Riedl B, Post LE, Bollag G, Trail PA (2004) Cancer Res 64:7099
106. Strumberg D, Richly H, Hilger RA, Schleucher N, Korfee S, Tewes M, Faghih M, Brendel E, Voliotis D, Haase CG, Schwartz B, Awada A, Voigtmann R, Scheulen ME, Seeber S (2005) J Clin Oncol 23:965
107. Ratain MJ, Eisen T, Stadler WM (2005) J Clin Oncol 23:Suppl Abstr 4544
108. Ahmad T, Marais R, Pyle L, James M, Schwartz B, Gore M, Eisen T (2004) J Clin Oncol 22:Suppl Abstr 7506
109. Escudier B, Szczylik C, Eisen T, Stadler WM, Schwartz B, Shan M, Bukowski RM (2005) Proc Am Soc Clin Oncol 23:Suppl Abstr LBA4510
110. Clark JW, Eder JP, Ryan D, Lathia C, Lenz HJ (2005) Clin Cancer Res 11:5472
111. Lyssikatos J, Yeh T, Wallace E, Marsh V, Bernat B, Gross S, Evans R, Colwell H, Parry J, Baker S, Ballard J, Morales T, Smith D, Brandhuber B, Winkler J (2004) Proc Am Assoc Cancer Res 45:Abstr 3888
112. Yeh T, Wallace E, Lyssikatos J, Winkler J (2004) Proc Am Assoc Cancer Res 45:Abstr 3889
113. Wallace E, Yeh T, Lyssikatos J, Winkler J, Lee P, Marlow A, Hurley B, Marsh V, Bernat B, Evans R, Colwell H, Parry J, Baker S, Ballard J, Morales T, Smith D, Brandhuber B, Gross S, Poch G, Litwiler K, Hingorani G, Otten J, Sullivan F, Blake J, Rizzi J, Pheneger T, Goyette M, Koch K (2004) Proc Am Assoc Cancer Res 45:Abstr 3891
114. Lee P, Wallace E, Yeh T, Poch G, Litwiler K, Pheneger T, Lyssikatos J, Winkler J (2004) Proc Am Assoc Cancer Res 45:Abstr 3890
115. Lee PA, Wallace E, Yeh TC, Poch G, Hunt D, Pheneger T, Woessner R, Cartlidge S, Klinowska T, Winkler J (2004) Eur J Cancer Suppl 2:Abstr 368
116. Winkler JD, Lee PA, Wallace E, Poch G, Litwiler K, Pheneger T, Lyssikatos J, Perrier M, Eckhardt SG, Yeh TC (2004) Eur J Cancer Suppl 2: Abstr 342
117. Chow LQM, Eckhardt SG, Reid J, Molina J, Hanson L, Piens J, Hariharan S, Basche M, Gore L, Diab S, O'Bryant C, Grolnic S, Hippert B, Doyle MP, Maloney L, Gordon G, Brown S, Litwiler K, Poch G, Adjei AA (2005) AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapy, Philadelphia, 14–18 Nov 2005:Abstr C162
118. Doyle MP, Yeh TC, Brown S, Morrow M, Lee PA, Hughes AM, Cartlidge S, Wallace E, Lyssikatos J, Eckhardt SG, Winkler JD (2005) 41st Annual Meeting American Society of Clinical Oncology (ASCO), Orlando, 13–17 May 2005:Abstr 3075
119. Lorusso P, Krishnamurthi S, Rinehart JR, Nabell L, Croghan G, Varterasian M, Sadis SS, Menon SS, Leopold J, Meyer MB (2005) 41st Annual Meeting American Society of Clinical Oncology (ASCO), Orlando, 13–17 May 2005:Abstr 3011
120. Kaufman MD, Barrett SD, Flamme CM, Warmus J, Smith YD, Cheriyan M, Zhang L, Tecle H, Sebolt-Leopold J, Valik H, Gowan R, Van Becelaere K, Merriman R, Przybra-

nowski S, Ohren J, Whitehead C, Leopold WR, Dobrusin E, Bridges A (2004) Proc Am Assoc Cancer Res 45:Abstr 2477
121. Dudley DT (2004) Cambridge Healthcare International 2nd Annual Protein Kinase Targets: Strategies for Drug Development, Boston, MA, 9–10 June 2004
122. Sebolt-Leopold JS, Merriman R, Omer C, Tecle H, Bridges A, Klohs W, Loi C-M, Valik H, Przybranowski S, Meyer M, Leopold WR (2004) Proc Am Assoc Cancer Res 45:Abstr 4003
123. Koup JR, Liu J, Loi C-M, Howard C, Van Becelaere K, Przybranowski S, Walton J, Sebolt-Leopold J, Merriman R (2004) Proc Am Assoc Cancer Res 45:Abstr 5409
124. Albanell J, Rojo F, Averbuch S, Feyereislova A, Mascaro JM, Herbst R, LoRusso P, Rischin D, Sauleda S, Gee J, Nicholson RI, Baselga J (2002) J Clin Oncol 20:110
125. Baselga J, Albanell J, Ruiz A, Lluch A, Gascon P, Guillem V, Gonzalez S, Sauleda S, Marimon I, Tabernero JM, Koehler MT, Rojo F (2005) J Clin Oncol 23:5323
126. Baselga J, Rischin D, Ranson M, Calvert H, Raymond E, Kieback DG, Kaye SB, Gianni L, Harris A, Bjork T, Averbuch SD, Feyereislova A, Swaisland H, Rojo F, Albanell J (2002) J Clin Oncol 20:4292
127. Malik SN, Siu LL, Rowinsky EK, deGraffenried L, Hammond LA, Rizzo J, Bacus S, Brattain MG, Kreisberg JI, Hidalgo M (2003) Clin Cancer Res 9:2478
128. Tan AR, Yang X, Hewitt SM, Berman A, Lepper ER, Sparreboom A, Parr AL, Figg WD, Chow C, Steinberg SM, Bacharach SL, Whatley M, Carrasquillo JA, Brahim JS, Ettenberg SA, Lipkowitz S, Swain SM (2004) J Clin Oncol 22:3080
129. Daneshmand M, Parolin DA, Hirte HW, Major P, Goss G, Stewart D, Batist G, Miller WH Jr, Matthews S, Seymour L, Lorimer IA (2003) Clin Cancer Res 9:2457
130. Johnson BE, Janne PA (2005) Cancer Res 65:7525
131. Hilger RA, Kredtke S, Scheulen ME, Seeber S, Strumberg D (2004) Int J Clin Pharmacol Ther 42:648
132. Belenchia R, Broggi M, Georgelos K, McNabola A, Novicki E, Rowley B, Trombley S, Wilkie D, Wilhelm S, Taylor I (2004) Proc Am Assoc Cancer Res 45:Abstr 3677
133. Lorusso PM, Adjei AA, Varterasian M, Gadgeel S, Reid J, Mitchell DY, Hanson L, DeLuca P, Bruzek L, Piens J, Asbury P, Van Becelaere K, Herrera R, Sebolt-Leopold J, Meyer MB (2005) J Clin Oncol 23:5281
134. Rinehart J, Adjei AA, Lorusso PM, Waterhouse D, Hecht JR, Natale RB, Hamid O, Varterasian M, Asbury P, Kaldjian EP, Gulyas S, Mitchell DY, Herrera R, Sebolt-Leopold JS, Meyer MB (2004) J Clin Oncol 22:4456
135. Solit DB, Garraway LA, Pratilas CA, Sawai A, Getz G, Basso A, Ye Q, Lobo JM, She Y, Osman I, Golub TR, Sebolt-Leopold J, Sellers WR, Rosen N (2006) Nature 439:358
136. Hopkins AL, Mason JS, Overington JP (2006) Curr Opin Struct Biol 16:127

Top Med Chem (2007) 1: 133–168
DOI 10.1007/7355_2006_003

Published online: 13 December 2006

Farnesyl Protein Transferase Inhibitors: Medicinal Chemistry, Molecular Mechanisms, and Progress in the Clinic

D. W. End[1] · L. Mevellec[2] · P. Angibaud[2] (✉)

[1]Early Development, Johnson & Johnson Pharmaceutical Research & Development, Springhouse, PA 19477, USA

[2]Department of Medicinal Chemistry, Johnson & Johnson Pharmaceutical Research & Development, Campus de Maigremont, BP615, 27106 Val de Reuil, France
pangibau@prdfr.jnj.com

1 Introduction and Historical Overview 135

2 Biochemistry of Farnesyl Protein Transferase 136

3 Downstream Effectors 138
3.1 Ras Proteins 138
3.2 Rho Proteins 141
3.3 Nuclear Proteins and G2/M Growth Delay 142
3.4 Upregulation of Transforming Growth Factor β (TGF-β) Receptor Type II (R II) 144
3.5 Additional Mechanisms: Rheb, Modulation of Survival, and Host–Tumor Interactions 144
3.6 FTase Knockout Mice 146

4 Biomarkers 146

5 Farnesyl Transferase Inhibitors 147
5.1 CAAX Peptidomimetics 147
5.2 FPP Competitive Compounds 148
5.3 CAAX Peptide Competitive Heterocyclic Inhibitors 149

6 Clinical Experience 157

7 Further Developments 157

8 Conclusion and Perspectives 159

References 160

Abstract Over a decade has passed since the first report describing farnesyl protein transferase (FTase) and tetrapeptide inhibitors triggered a search for small-molecule inhibitors that could be developed as oral therapeutics. There are now several farnesyl protein inhibitors (FTIs) in various phases of clinical development and at least two compounds have entered phase III. The published data suggest some disappointing activity in the major solid tumors, with more promising activities emerging from studies of hemato-

logical malignancies and glioblastoma. The current compounds emerged from various research strategies including modeling around the CAAX motif peptide substrate and the farnesyl pyrophosphate (FPP) substrate, as well as high-throughput screening campaigns. The interaction of inhibitors in the active site of the FT enzyme can be accurately described thanks to the publication of the X-ray structure as well as excellent mechanistic work. Published structure–activity data have revealed an interesting convergence on imidazole pharmacophores. The original hypothesis that drove development of FTIs anticipated therapy targeted specifically at the farnesylated Ras oncoproteins and cancers with *ras* gene mutations. As experience with the newer potent FTIs grew, data emerged to suggest that multiple downstream effectors contribute to the antitumor activity of FTIs. The mechanism(s) of action of FTIs and the full therapeutic activity of the class remain areas of active investigation.

Keywords Farnesyl transferase inhibitor · Protein prenylation · Ras protein · Acute myeloid leukemia

Abbreviations

AKAP13	A-kinase anchoring protein 13
AML	Acute myelogenous leukemia
CENP-E	Centromere-associated protein-E
CENP-F	Centromere-associated protein-F
CNS	Central nervous system
ECG	Electrocardiogram
FPP	Farnesyl pyrophosphate
FTase	Farnesyl protein transferase
FTI	Farnesyl protein transferase inhibitor
GGTase	Geranylgeranyl protein transferase
Grb2β	Growth factor receptor binding protein-2β
GTP	Guanosine triphosphate
HMGCoA	Hydroxymethylglutaryl-CoA
H-*ras*	Harvey *ras* gene
N-*ras*	Neural *ras* gene
K-*ras*	Kirsten *ras* gene
HDJ-2	Human DNAJ-2 heat shock protein
HUVEC	Human umbilical vein endothelial cell
mTOR	Mammalian target of rapamycin
NF-κ-B DNA	Nuclear factor kappa B deoxyribonucleic acid
NMR	Nuclear magnetic resonance
PI3-kinase/Akt-3	Phosphoinositide-3-kinase/serine–threonine kinase Akt-3
PGGT	Protein geranylgeranyl transferase
QTc	Duration of the QT interval corrected for changes in the heart rate
RabGGTase	Rab geranylgeranyl transferase
RCE	Ras CAAX endoprotease
Rheb	Ras homolog enriched in brain
SAR	Structure–activity relationship
SH2/SH3	Sequence homology-2/sequence homology-3
SOS	Son of sevenless
TGF-β	Transforming growth factor β
THQ	Tetrahydroquinoline

1 Introduction and Historical Overview

Interest in the posttranslational modification of proteins by prenylation grew out of studies in the early 1980s, which sought to understand how cholesterol-lowering statin HMGCoA reductase inhibitors blocked cell proliferation in vitro [1, 2]. The growth arrest was not due to cholesterol depletion, since neither exogenous cholesterol nor cholesterol intermediates could reverse the effects. Only mevalonic acid reversed the effects of HMGCoA reductase inhibitors. By tracking the fate of radiolabeled mevalonate in cells treated with HMGCoA reductase inhibitors, the presence of proteins covalently labeled with the radiotracer revealed a novel mechanism for the posttranslational modification of proteins involving an isoprenoid intermediate of cholesterol synthesis (Fig. 1) [3].

Nuclear lamin proteins and the Ras proteins were later shown to be modified by this prenylation reaction [4–6]. The prenyl molecule involved was found to be a 15-carbon farnesyl moiety attached to a C-terminal cysteine by a thioether bond [5, 6]. Since these farnesylated targets were involved in cell division and signal transduction associated with cell proliferation, the findings suggested an important role for this pathway in tumor cell

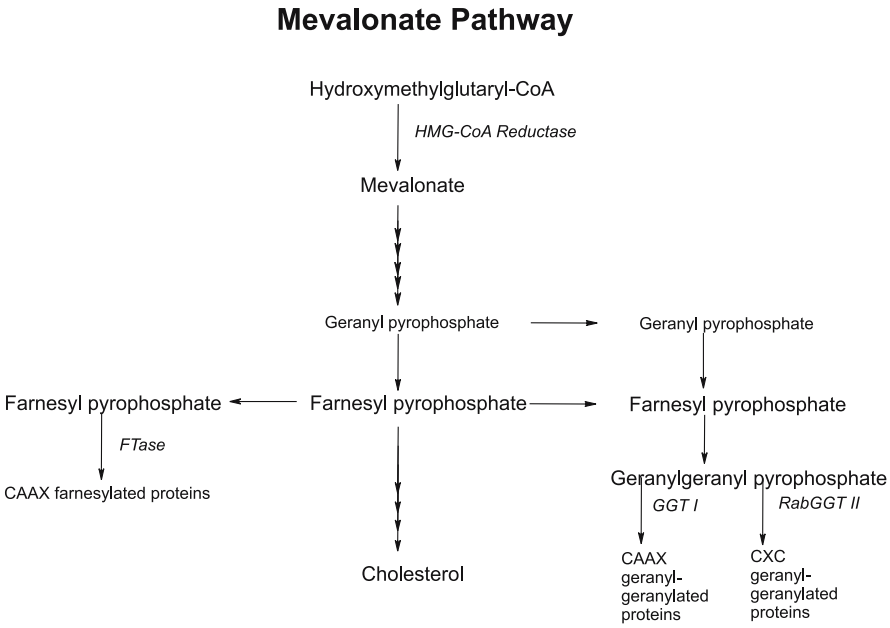


Fig. 1 Relationship of posttranslational protein isoprenylation pathways to the mevalonate pathway of cholesterol biosynthesis

growth. In particular, attention focused on the well-characterized *ras* oncogene CAAX protein product [7–9]. However, the level of isoprenoid depletion required to effect these changes in protein prenylation using HMGCoA reductase inhibitors required high micromolar concentrations of the inhibitors in vitro [10]. Also, the lack of selectivity for protein prenylation made them poorly suited for use in animal studies or in patients. In 1990, the report of the purification of the mammalian farnesyl protein transferase and characterization of CAAX tetrapeptide inhibitors launched a number of drug discovery programs hoping to find cancer chemotherapy selective for the tumors with *ras* mutations [11, 12].

The 1990s witnessed an intensive effort to develop small-molecule inhibitors of FTase, which eventually provided proof of concept for this target in preclinical models. Although the original hypothesis envisioned inhibitors that selectively depleted farnesylated Ras proteins from tumor cell membranes, the emerging data on FTIs indicated mechanisms which were subtle and more complex (for a review, see [13]).

A few successful candidates entered phase I oncology clinical trials in 1997. By 1999, the safety of chronic administration of FTIs was established, and demonstration of biological activity with the first hints of therapeutic activity in patients were presented in the following year [14–17]. Although the compounds entering into clinical development were extremely potent and selective FTase enzyme inhibitors, the downstream effectors contributing to the antitumor effects following FTase inhibition remain under investigation to this day. Searches of available genomic data have revealed over 140 CAAX motif proteins as suitable substrates for the enzyme. Several of these CAAX proteins are under investigation as downstream effectors of FTIs. Gene expression microarrays are also revealing interesting transcriptional changes triggered by FTIs, not only in cell cultures but also in patients receiving FTIs as experimental therapy [18–20]. Although complexity intervened in the development of this targeted FTI therapy, the new genomic tools may help redefine the targets of FTIs and allow optimization in therapy.

2
Biochemistry of Farnesyl Protein Transferase

FTase catalyzes the covalent attachment of a farnesyl moiety via a thioether linkage to the proteins bearing a C-terminal amino acid sequence known as the CAAX motif (Fig. 2) [12, 21]. The farnesyl moiety is derived from farnesyl pyrophosphate (FPP), a 15-carbon isoprenyl intermediate in the mevalonate pathway of cholesterol biosynthesis. The binding of FPP to the enzyme has relatively high affinity (K_d = 1–10 nM), and FPP binding must precede the binding of the peptide substrate for successful catalysis [22, 23].

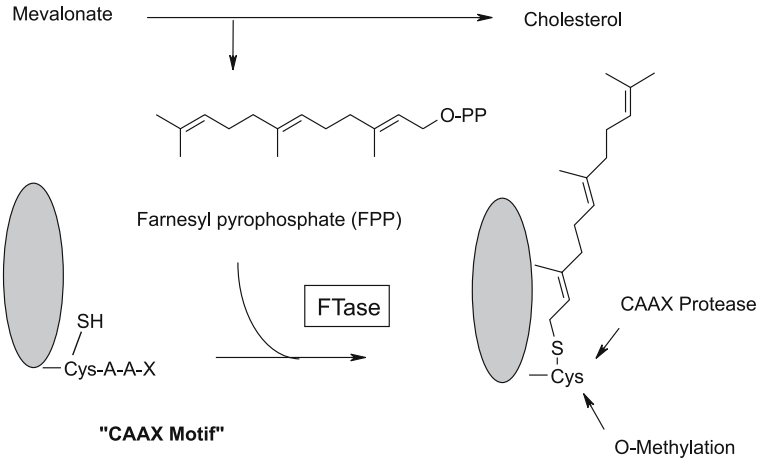


Fig. 2 Schematic representation of the farnesyl protein transferase (FTase) reaction

This high affinity is reflected in the extensive depletion of isoprenoid intermediates, which is required to inhibit farnesylation via inhibition of HMG-CoA reductase. The FTase active site is unusual in the sense that a portion of the isoprene backbone of the FPP substrate actually contributes to the binding of the CAAX peptide substrate [24]. The four carboxy-terminal amino acids of protein substrates provide the major recognition sequence for the peptide substrate binding cleft [12, 24]. This "CAAX" motif refers to the reactive cysteine residue (C), followed by two aliphatic amino acids (A) and completed by the C-terminal amino acid X, which is critical to recognition by the enzyme. The X amino acid in preferred substrates of FTase is methionine, serine, or glutamine. If the X-terminal amino acid is a leucine, the peptide is a substrate for geranylgeranyl protein transferase type I (GGTase I), a parallel and sometimes redundant posttranslational pathway [25]. Specificity is not absolute and there are some important exceptions to this.

The structure and mechanism of catalysis of FTase were well defined in the late 1990s from several X-ray crystallography and elegant biochemical studies [24, 26–30]. The enzyme is a heterodimer of α and β subunits [31, 32]. The β subunit contains binding sites for both the farnesyl pyrophosphate and the CAAX protein substrates. A catalytic zinc (Zn^{2+}) identified in the active site of the β subunit participates in the binding and activation of the CAAX protein substrates [28]. The Zn^{2+} is coordinated to the enzyme in a distorted tetrahedral geometry and surrounded by hydrophobic pockets [24, 27]. Upon binding of the CAAX peptide, the thiol of the cysteine displaces water and is activated for a nucleophilic attack via thiolate on the C-1 carbon atom of farnesyl pyrophosphate [30].

In addition to protein farnesylation, two other pathways exist for protein prenylation. As mentioned above, GGTase I also utilizes the C-terminal

C-A-A-X recognition motif, and is a heterodimer very similar to FTase [25, 32]. FTase and GGTase I share a common α subunit, which functions to enhance catalysis, but have distinct β subunits, which contain the substrate binding domains. GGTase I recognizes protein substrates containing leucine in the X-position and attaches a 20-carbon geranylgeranyl moiety to the thiol of cysteine [25]. However, there are CAAX motif proteins containing a C-terminal methionine, which can function as substrates for either FTase or GGTase I (vide infra). These exceptions to the rule have had some important implications to the mechanism of FTIs.

Following geranylgeranylation or farnesylation, modified CAAX proteins undergo proteolytic cleavage of the AAX, followed by carboxy O methylation [33, 34]. The Ras CAAX endoprotease or RCE is currently the target of several drug discovery programs. All three reactions are required for full activation or membrane localization of the proteins.

A third protein prenylation enzyme, geranylgeranyl protein transferase type II (GGTase II), is structurally and catalytically different from both FTase and GGTase I [35]. This enzyme is also known as the Rab geranylgeranyl transferase, since its activity is restricted to proteins of the Rab family bearing CXC or CC terminal motifs. Rab proteins participate in the trafficking of intracellular membrane vesicles. Since this enzyme has no known role in tumorigenesis and has been linked to the human genetic X-chromosome-linked disease choroideremia, the enzyme has not directly been a target for drug discovery [36].

3
Downstream Effectors

3.1
Ras Proteins

The initial hypothesis driving the development of FTIs for cancer therapy focused on the Ras proteins. The compounds as a class were discussed as Ras-selective therapy because Ras protein requires posttranslational processing via the farnesylation pathway to attach to the plasma membrane to function in signal transduction and cell transformation. Plasma membrane localization brings the Ras proteins into closer proximity to the initiation point for cell signaling via multimeric signaling complexes of membrane receptors and the SH2/SH3 domain adapter proteins Grb2 and SOS [37]. Raf and related effectors downstream of Ras also require the farnesylated membrane-associated form of Ras for recruitment to the membrane and subsequent activation [38, 39]. In early studies in *ras*-transfected cell lines, a series of peptidomimetic FTIs selectively reversed the malignant morphology induced by transfection of the activated *v-ras* or mutant H-*ras* gene [40, 41]. As ex-

perience with FTIs increased and more potent FTIs became available, the role of Ras proteins in mediating the antitumor effects of this class of agent became less certain. First, the FTI antiproliferative activity did not correlate with the presence or absence of *ras* mutations. Although FTIs clearly reversed the cell transformation induced in cell lines transfected with the activated mutant H-*ras* gene, the compounds were equally active in some cell lines bearing wild-type *ras* [42, 43]. Also, the different H-Ras, N-Ras, and K-Ras isoforms were found to behave differently with respect to modification by prenyl transferases and their responses to FTIs. The K-RasB protein was found to be resistant to FTIs [44–46]. This had broad implications in the therapeutic utility of FTIs, since mutations in the K-*ras* gene were the most frequently observed activating *ras* mutation in human cancers [7, 47–50]. Oncogenic mutations in the H-*ras* gene or the N-*ras* gene are quite rare in human malignancy [7, 51]. With isolated enzyme, the K-Ras protein demonstrated a higher affinity for FTase, which decreased the potency of FTIs competitive for the CAAX binding site [44]. In intact tumor cells, the mutant, activated K-Ras isoform was associated with resistance to FTIs due to alternative geranylgeranylation via GGTase I [45, 46]. K-Ras was one of the rare farnesylated proteins which could also undergo geranylgeranylation. The geranylgeranylated K-Ras protein was still active in supporting malignant transformation resulting in a redundancy in the prenylation of this important oncogene product. In separate studies the N-Ras isoform was also reported to undergo alternative geranylgeranylation to an active form [46]. However, FTIs did inhibit the growth of human tumor cell lines bearing K-*ras* and N-*ras* mutations [52, 53]. Observations that FTIs inhibited tumor cell growth while geranylgeranylated K-Ras was still functioning and that FTIs inhibited tumor cell lines bearing wild-type *ras* provided compelling evidence forcing investigation into alternatives to the "Ras-selective" hypothesis of FTI action.

The discovery of the alternative geranylgeranylation of K-Ras suggested that inhibitors of GGTase I might have a role in Ras-targeted therapy. Because the widely distributed and functionally important γ subunit of signal transducing G-proteins are geranylgeranylated, inhibition of GGTase I was originally felt to present toxicity risks [53]. The GGTase I inhibitor GGTI-297 was reported to have interesting antitumor activity without gross toxicity to the tumor-bearing mice. Inhibition of K-RasB prenylation in intact cells and tumors was reported for the combination of GGTI-297 and an FTI (FTI-276) (Fig. 3) [54].

However, neither agent alone was capable of inhibiting the growth of tumors bearing K-*ras*B mutations. These findings suggested that substrates of GGTase I, which are distinct from K-*ras*B, may also participate in tumor growth and the antitumor effects of this class of compound. In contrast to these earlier findings, a recent report cited severe in vivo toxicity for the combination of an FTI and GGTase I inhibitors [55]. In mice, continuous 72-hour

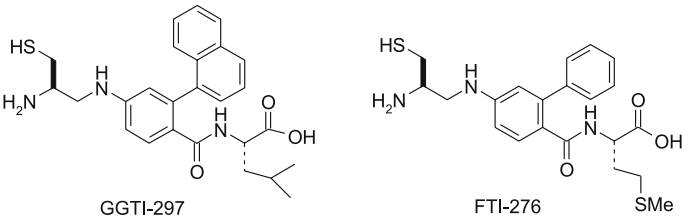


Fig. 3 Structure of GGTI-297 and FTI-276

infusion of an FTI and a GGTI produced severe myelosuppression with 100% lethality at doses which only reduced geranylgeranylation by 30%. However, two combined FTase/GGTase I inhibitors have been tested in man, L-778,123 and AZD-3409, without producing the lethality observed in the mouse studies (Fig. 4).

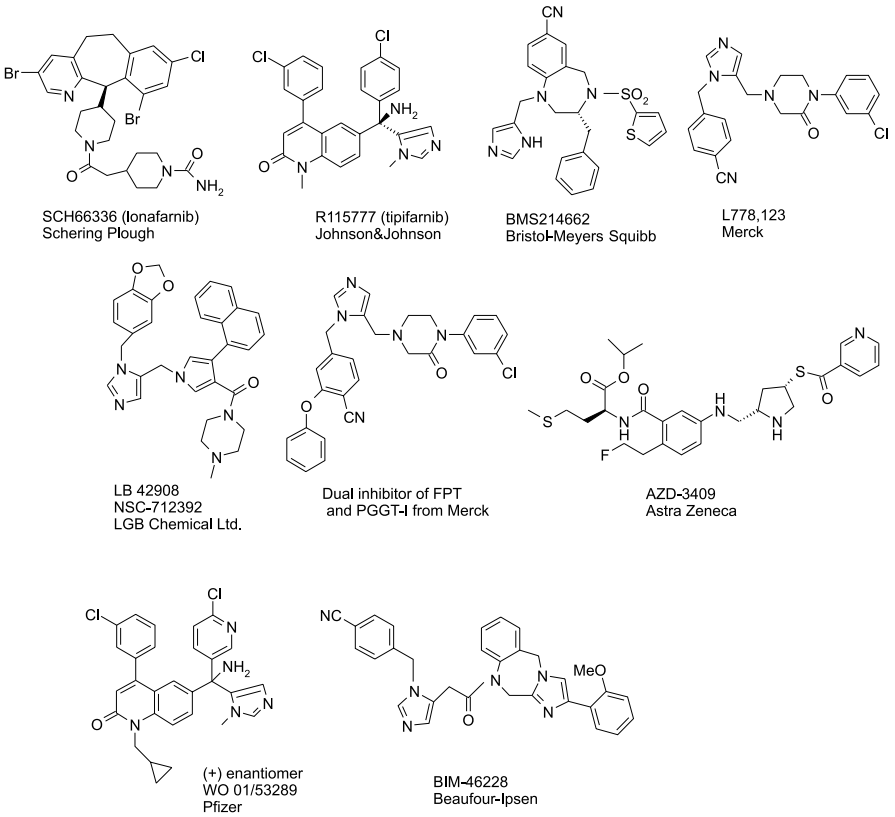


Fig. 4 CAAX competitive heterocyclic farnesyl protein transferase inhibitors. The inhibitors shown in the first row are reported to be in phase I, II, or III clinical development

3.2 Rho Proteins

A compelling set of data supports the role of RhoB as an important downstream effector following FTase inhibition [56]. RhoB is a 21 K_d GTP binding protein which regulates cytoskeletal functions related to cell shape and motility, and which participates in *ras* transformation [57, 58]. These functions are consistent with some of the profound morphological and cytoskeletal effects produced by FTI treatment of *ras*-transformed cells in culture. RhoB is required for *ras* transformation since transfection with a dominant-negative *rhoB* blocked transformation induced by activated *ras* in rat fibroblasts [58]. Although the RhoB CAAX motif contains the terminal leucine (L), which should direct the molecule to be exclusively geranylgeranylated by GGTase I, the unique CKVL CAAX motif allows the RhoB protein to be either farnesylated or geranylgeranylated [59, 60]. Treatment of intact cells with the FTI L-739,749 (Fig. 5) was shown to induce an alternative processing of RhoB via PGGT I with a gain of geranylgeranylated RhoB and concomitant reduction of the farnesylated RhoB [61, 62].

The accumulation of geranylgeranylated RhoB was associated with antiproliferative effects. In further support of this observation, transfection of RhoB with CAAX motifs that restricted prenylation to geranylgeranylation quite consistently reproduced many of the effects of FTI treatment, including reversal of the transformed phenotype as well as induction of apoptosis [63, 64]. Cells from RhoB knockout mice were also shown to lose apoptotic responses to the DNA-damaging agents doxorubicin and radiation [65]. The sensitizing effects of FTIs to the DNA-damaging agents were also lost. In separate studies, transfection of the exclusively farnesylated RhoB isoform endowed NIH3T3 cells with radioresistance, an effect that was reversed by treatment with an FTI [66]. Also, both FTIs and expression of geranylgeranylated RhoB were shown to reverse the malignant phenotype of inflammatory breast cancer cells overexpressing the RhoC or mammary epithelial cells transfected with RhoC, a Rho family protein linked to the aggressive phenotype of inflammatory breast cancer [67]. The data provided further support for RhoB farnesylation in the actions of FTIs. However, additional recent studies have shown that expression of either farnesylated RhoB or geranylgeranylated RhoB in human epithelial tumors produced similar tumor-suppressive activity [68]. The farnesylated RhoB reduced anchorage-independent growth and induced apoptosis. Transfected cells also did not produce tumors in nude mice. Thus, the role of RhoB in the effects of FTIs in human tumors is uncertain. Although it provides an attractive and encompassing mechanism for the actions of FTIs, the RhoB hypothesis may be limited to certain tumor cell lineages. Recent studies have demonstrated some additional downstream effectors required for the apoptotic effects of FTIs and geranylgeranylated RhoB. Bin 1 is a tumor suppressor gene that

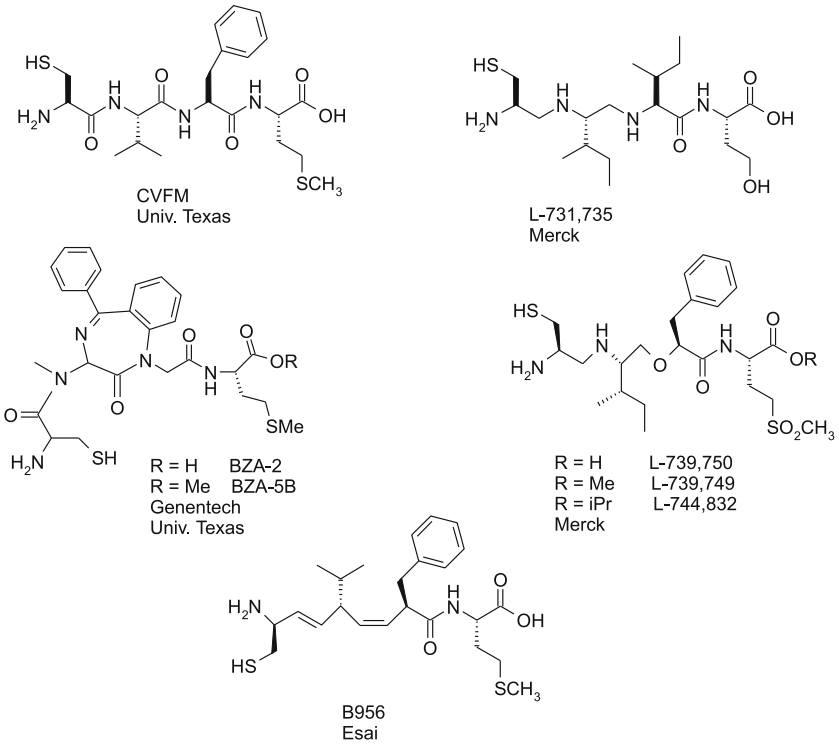


Fig. 5 CAAX peptidomimetic FTIs

can induce apoptosis when transfected into transformed but not normal cells and Bin 1 acts downstream of RhoB in triggering FTI-induced apoptosis [69]. Bin 1 can be deleted in some tumor types. Hence, Bin 1 status may be an important modulator of antitumor responses to FTIs. Bin 1 status has not been monitored in clinical trials. Additionally, the cell cycle regulatory protein Cyclin B1 also modulated the apoptic response to FTIs and geranylgeranylated RhoB [70]. Downregulation of Cyclin B1 via geranylgeranylated RhoB was required to trigger the apoptosis induced by the FTI L-744,832 in vitro (Fig. 5). Upregulation of RhoB abolished FTI-induced apoptosis in vitro and endowed tumor xenografts with FTI resistance in vivo. Cyclin B1 can be overexpressed in some human tumors. Again, Cyclin B1 status has not been evaluated in FTI clinical trials.

3.3 Nuclear Proteins and G2/M Growth Delay

Several farnesylated nuclear proteins have been identified. The nuclear lamins A, B, and C function in the assembly and reorganization of the nu-

clear membrane following mitosis. The nuclear lamin B was initially shown to incorporate radiolabel from exogenously supplied [^{14}C]mevalonate [4, 71]. Both prelamin A and B but not lamin C were later shown to contain CAAX motifs (CSIM and CAIM, respectively), which directed them to posttranslational modification by farnesylation [72]. Unlike other CAAX proteins, prelamin A undergoes a unique endoproteolytic cleavage of C-terminal farnesylated peptide resulting in a "mature", functional lamin A protein [73]. In the presence of FTIs the prelamin A accumulates in the nucleoplasm of cells, and prelamin A specific antibodies have allowed some investigators to monitor prelamin A as a biological marker for the effects of FTIs in patients [74]. Mutational analyses and studies with HMGCoA reductase inhibitors revealed that farnesylation was required for assembly of nuclear lamin A complexes [10, 72]. However, when cells were treated with the peptidomimetic FTI BZA-5B (Fig. 5) for prolonged periods of time at concentrations of compound which completely inhibited farnesylation monitored as the incorporation of [^{3}H]mevalonate, no disruption of nuclear lamin assembly into lamina or alterations in nuclear morphology were observed [75]. These unexpected results suggested that lamins A and B were not involved in the antiproliferative effects of FTIs. This might be due to redundancy in the function of nuclear lamins or to retention of residual function in unfarnesylated lamins A and B. While posttranslational processing of nuclear lamins may not be relevant to cancer therapy, recent studies have shown potential utility of FTIs in Hutchinson–Gilford syndrome or progeria, a rare genetic disease that presents with a phenotype resembling premature aging [76–79]. The disease has been linked to mutations in lamin A that prevent its proper processing and nuclear membrane localization after farnesylation. This induces a disrupted nuclear morphology that can be reversed in cell culture by several different FTIs. No in vivo preclinical data or clinical studies have been reported yet.

Additional reports provided some interesting findings from studies of the mechanism for accumulation of cells in G2/M following treatment with FTIs [80, 81]. In a search of protein databases for CAAX motif proteins, two centromere-associated proteins (CENPs) were found to have FTase-directed CAAX motifs. CENP-E and CENP-F (mitosin) contained CKTQ and CKVQ motifs which made them FTase substrates with isolated enzyme and in intact cells. The proteins could not be geranylgeranylated. The proteins were shown to be selectively expressed during mitosis, wherein they contributed to the alignment and segregation of chromosomes required for proper cell division [82–85]. Treatment of human tumor cell lines with the FTI SCH 66336 (lonafarnib) (Fig. 4) inhibited the farnesylation of CENP-E and CENP-F. Inhibition of CENP-E and CENP-F was correlated with a delay in the alignment of chromosomes and accumulation of cells in prometaphase.

While G2/M growth arrest and delay is an attractive mechanism for an antitumor agent, it remains to be determined whether this in vitro response

mediates all of the antitumor effects observed in animal tumor models or in the clinic. It has been suggested that this mechanism contributes to the synergistic interaction observed between taxanes and FTIs [86, 87]. It is also interesting to note that transfection of the geranylgeranylated RhoB produced a G2/M growth delay in some p53-deficient cell lines, suggesting that different prenylated effectors may converge to contribute to FTI-induced alterations of the cell cycle [63].

3.4 Upregulation of Transforming Growth Factor β (TGF-β) Receptor Type II (R II)

The TGF-β family of peptide growth factors (TGF-β 1,2,3, activins, and bone morphogenetic proteins) exert a variety of tissue-selective differentiating effects and prominent antiproliferative effects, which may contribute to an ambient tumor-suppressive activity in mammals [88]. Disruption of the growth-suppressive activity of TGF-β via downregulation of TGF-β receptor signaling is an early event in a variety of tumors including pancreatic cancer, colon cancer, head and neck cancer, breast cancer, and myeloid leukemia [89–93]. The ligand occupied TGF-β receptor is a heterotetramer of TGF-β receptor I (RI) and TGF-β receptor II (RII) subunits [88]. Therefore, it is very interesting that two independent studies have described an upregulation of TGF-β RII in pancreatic tumor cell lines treated with FTIs [94, 95]. Upregulation of TGF-β RII was associated with restoration of the growth-suppressive activity of TGF-β and signal transduction down to the level of transcriptional activation. FTI-induced restoration of TGF-β RII signaling was also linked to an induction of radiosensitization in pancreatic tumor cell lines [96].

3.5 Additional Mechanisms: Rheb, Modulation of Survival, and Host–Tumor Interactions

Some recent studies have suggested that the farnesylated protein Rheb (Ras homolog enriched in brain) GTP binding protein contributes to the cellular effects of FTIs [97]. Rheb can activate the mTOR (mammalian target of rapamycin) pathway, which in itself is a pathway targeted for cancer therapeutics. Rheb activation of mTOR results in S6 phosphorylation that can be inhibited by the FTI lonafarnib. FTI inhibition of the mTOR/S6 kinase pathway appeared to be linked to the ability of FTIs to enhance the sensitivity of MCF-7 breast cancer cells to tamoxifen and paclitaxel. In addition to these cellular events investigated primarily in cell culture and related to tumor cell proliferation, it must be recognized that FTIs produce effects on the malignant phenotype which are dependent on the growth environment. It is rare to find a tumor cell line responding to FTIs by induction of apoptosis when

cells are grown as monolayer cultures. However, FTIs can produce significant apoptotic events in more complex growth environments and in tumor-bearing hosts. FTIs induce apoptosis, or more specifically anoikis, when cells are denied substrate [98]. A similar profound induction of apoptosis was reported for C32 melanoma cells when grown as subcutaneous tumors in nude mice [43]. When the same C32 melanoma cell line was grown as monolayer cultures, only an antiproliferative effect was observed. Similar events have been suggested in several H-*ras* transgenic tumor models wherein profound tumor regressions have been noted following treatment with FTIs [99, 100]. The apoptotic events appear to be derived from an inhibition of H-Ras activation of the PI3-kinase/Akt-3 pathway [101]. Furthermore, activation of the PI3-kinase/Akt survival pathway can block FTI-induced anoikis [102]. The findings suggest the possibility that a small pool of H-Ras protein that is highly sensitive to FTIs can be sufficiently depleted to produce a significant reduction in survival signaling. RhoB may also be involved in survival signaling and apoptosis in as much as deletion of RhoB prevented the induction of apoptosis produced by doxorubicin, radiation, and FTIs [64]. Transfection with RhoB constructs that are exclusively geranylgeranylated also suppressed Akt activity, demonstrating that the geranylgeranylation of RhoB is sufficient to account for the effects of FTIs on the Akt survival pathway [103]. As is consistent with the concept of the malignant phenotype, cell transformation and the effects of FTIs on cell transformation involve more than cell proliferation. FTIs seem to impair tumor cell survival mechanisms, which function in unfavorable environments.

In four multiple myeloma cell lines, tipifarnib inhibited the PI3-kinase/AKT phosphorylation survival pathway in a concentration-dependent manner. A concentration-related increase in apoptosis was observed in three out of the four cell lines. Levels of phospho-AKT expression correlated with resistance to tipifarnib induction of apoptosis, with the more resistant cell lines showing higher levels of phospho-AKT and incomplete inhibition [104]. The PI3-kinase pathway may be particularly relevant for the effects of FTIs in acute myelogenous leukemia (AML), since a Ras-dependent activation of the PI3-kinase pathway was linked to the constitutive NF-κ-B DNA binding activity observed in 16 out of 22 (73%) AML cases [105]. In AML cells in culture, both a PI3-kinase inhibitor and an FTI suppressed the constitutive activation of NF-κ-B. The activated NF-κ-B in the AML cells was linked to a reduced apoptotic response. Similarly, the inhibition of NF-κ-B functions by FTIs was confirmed in several different cell lines including the Jurkat human T-cell lymphoma [106]. The inhibition of NF-κ-B activation by a variety of stimuli, including cigarette smoke and phorbol 12-myristate 13-acetate, appeared to involve modulation of Ras function. Taken together, recent evidence points to an important role for inhibition of the NF-κ-B survival pathway in the actions of FTIs in AML.

3.6
FTase Knockout Mice

Recently, results from studies of FTase knockout mice have been reported [107]. The FTase knockout was incompatible with embryo survival. The recovered embryonic tissue was disorganized and displayed reduced proliferation with increased apoptosis. Embryonic fibroblast recovered from the FTase knockout mice proliferated at a slower rate and displayed a flattened morphology, findings consistent with the phenotype observed for FTI-treated cells in culture. In order to generate mature mice lacking FTase enzyme, conditional knockouts were successfully bred. The mice did not display any obvious pathological phenotype, except some subtle defects were seen such as delayed wound healing, slightly smaller spleens, and a defect in erythroid maturation. This was consistent with the FTIs being fairly well tolerated but did not reproduce the dose-limiting toxicities reported for FTIs in the clinic. This could be species differences or a real disconnect between genetic versus pharmacological modulation of the target. Two models of in vivo tumorigenesis were investigated. The FTase knockout background did not alter the number of tumors driven by a K-*ras* mutation consistent with the observations that the K-Ras can be alternatively prenylated via geranylgeranylation. In a skin tumor model involving H-*ras* mutations, the FTase knockout did not alter the incidence of chemically induced tumors but did reduce the progression of early tumors. The findings do provide genetic evidence for a role of FTase in proliferation and tumor progression.

4
Biomarkers

Since FTIs were originally anticipated to specifically target the Ras proteins and other well-defined biochemical targets, a series of biomarkers or ex vivo biochemical correlates were examined in various phase I studies. It was anticipated that a biologically optimal dose could be defined using pharmacodynamic endpoints. Most studies utilized surrogate tissues such as mucosal epithelial cells or peripheral blood lymphocytes because of the ethical and technical difficulties of serial biopsies from solid tumors [16, 108–110]. At least one study did perform paired serial biopsies of tumor samples by utilizing sequential bone marrow aspirates in patients with advanced leukemia before and during treatment with the FTI tipifarnib [108]. Several biochemical endpoints were investigated including direct ex vivo measurement of FTase enzyme activity and analysis of the accumulation of unprenylated FTase substrate proteins including Ras, lamin A, and HDJ-2 [108, 109]. Quite consistently, inhibition of FTI-targeted biomarkers was demonstrated at doses that were clinically tolerated. However, inhibition of FTI-targeted biomark-

ers did not correlate with responses, and dose–response relationships were not evident. Thus, the targeted research endpoints of optimal biological dose selection or selection of patients who would be FTI responders could not be achieved. The results suggested that better knowledge of the downstream mechanisms discussed previously is needed to develop better biochemical correlates, predictive of response. At least retrospectively, the biomarker data did demonstrate that the design of dose-escalation schemes in the phase I studies of both tipifarnib and lonafarnib did minimize patient exposures to biologically inactive doses of compounds. Microarray analysis of patient bone marrow samples from tipifarnib AML clinical studies revealed an interesting predictor of FTI-responsive patients. The expression of the lymphoid blast crisis genes AKAP13 (A-kinase anchoring protein 13) was highly predictive of responses to the FTI tipifarnib in a large phase II study [20, 110].

5
Farnesyl Transferase Inhibitors

During the last decade the design of potent FTIs has been the focus at a significant number of pharmaceutical companies and academic research institutions, as tools to better understand biology or as clinical candidates, and has led to the synthesis of thousands of highly active compounds (some of them have already been cited in the preceding sections). It will not be possible to exhaustively review all these inhibitors, but we will rather focus on key examples and compounds that have reached the clinical development phase.

5.1
CAAX Peptidomimetics

The first FTIs were the CAAX tetrapeptides reported by Brown and Goldstein [12]. The tetrapeptides were valuable tools allowing for characterization of the isolated FTase enzyme, but their zwitterionic charge and instability precluded studies in intact tumor cells. In 1993, Genentech and Merck generated the first CAAX peptidomimetics which were active in intact tumor cells (Fig. 5) [40, 41]. These molecules provided an important proof of principle that inhibition of FTase in intact cells could lead to growth arrest and reversion of the *ras*-transformed phenotype, observations which supported the FTI–Ras hypothesis. Although the compounds were extremely potent with isolated enzyme, the molecules still suffered significant loss of potency in intact cells. Further directed chemical synthesis using the CAAX peptidomimetic strategy led to molecules such as B956 (Esai), L-739,750 (Merck), and FTI-276 (University of Pittsburgh) which inhibited the growth of tumors when administered parenterally in mice (Figs. 5 and 3) [111–114]. In FTI-276 for example, the Val-Ile of CVIM was replaced by 2-phenyl-4-aminobenzoic

acid giving a compound that inhibits farnesylation of H-Ras with an IC_{50} of 0.5 nM. These molecules were important in extending proof of principle to in vivo tumor models.

5.2 FPP Competitive Compounds

Rhône Poulenc Rorer synthesized an extensive series of heterocyclic FPP competitive compounds such as RPR130401 (Fig. 6) displaying antitumor activity in murine tumor models following oral administration [115–117]. RPR130401 demonstrated some interesting activity when combined with a CAAX peptidomimetic GGTase 1 inhibitor. Banyu's J-104,871 (Fig. 6) is another interesting competitive inhibitor of FPP developed from a series of squalene synthase inhibitors [118, 119]. The molecule inhibited the isolated enzyme at a concentration of 3.9 nM and inhibited the growth of tumors produced by H-*ras* transformed NIH3T3 cells at doses of 40 and 80 mg/kg administered parenterally. In cell culture studies, the investigators demonstrated an important principle that lowering the FPP pool via inhibition of HMGCoA reductase enhanced the activity of FPP competitive compounds. It is doubtful that this combination strategy would work with the CAAX com-

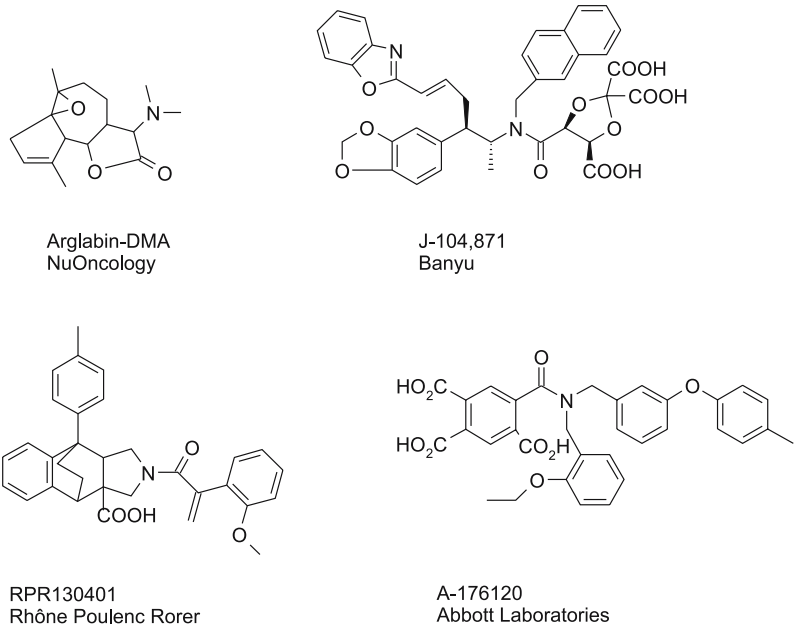


Fig. 6 Inhibitors of farnesyl protein transferase, which are competitive for farnesyl pyrophosphate binding

petitive FTIs. A-176120 (Fig. 6) from Abbott Laboratories was another FPP competitive molecule derived from squalene synthase inhibitors [120]. The compound exhibited excellent selectivity toward FTase versus squalene synthase. In addition to inhibiting tumor cell proliferation in vitro, the molecule exhibited some interesting antiangiogenic activity in human umbilical vein endothelial cell (HUVEC) cultures. The compound produced some modest increases in survival in mice bearing H-*ras* transformed NIH 3T3 cell tumors but required continuous infusion from osmotic minipumps, suggesting some problems with bioavailability. Although originally reported to be an immunostimulant, Arglabin (Fig. 6) has also been reported to be an FPP competitive FTI [121, 122]. Arglabin is a natural product isolated from *Artemisia glabella* (wormwood). The compound has been tested clinically in the country of origin, Kazakstan.

The development status of these molecules is not known. It will be interesting to note whether any differences emerge from the CAAX competitive versus FPP competitive molecules as more data become available for these compounds. Since FPP itself contributes to the CAAX peptide binding pocket, the interaction of FPP competitive FTIs with CAAX peptide competitive FTIs will be of interest. The selectivity of FPP competitive FTIs for the FTase pathway versus other biochemical pathways utilizing FPP, such as ubiquinone synthesis and the heme farnesyltransferase, has also not been reported. These other FPP reactions have important roles in mitochondrial function, which presents some risk for adverse events or possibly opportunities for modulating early apoptotic events.

5.3
CAAX Peptide Competitive Heterocyclic Inhibitors

Two orally active heterocyclic FTIs have advanced phase II/III clinical studies: tipifarnib (R115777, Zarnestra®) from Johnson & Johnson Pharmaceutical Research & Development, and lonafarnib (SCH66336, Sarasar®) from Schering-Plough (Fig. 4) [108–110, 123, 124]. Both molecules compete for the CAAX peptide binding site but at different regions within the site [125]. The tricyclic ring system appears to be the dominant pharmacophore in lonafarnib with a great deal of tolerance for substitutions in the attached tail. X-ray crystallographic studies revealed that the tricyclic pharmacophore aligns perfectly in the CAAX peptide binding site created by FPP substrate binding. In tipifarnib, the major pharmacophore is the imidazole ring, which interacts with the Zn^{2+} coordination structure required for catalysis in the active site. Both molecules were discovered using traditional screening methods of compound libraries. The Schering molecule was developed from early leads derived from the antihistamine loratadine which was rapidly transformed into SCH44342 (Fig. 7), use of which was hampered by a very short half-life in vivo [126].

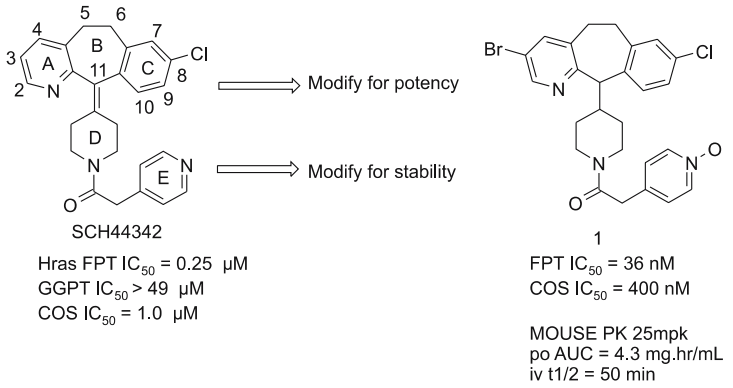


Fig. 7 Target design strategy of Schering-Plough's FTIs

In attempts to reduce metabolism and increase the potency of SCH44342, pyridine *N*-oxide and a bromine atom at C-3 on the benzocycloheptapyridine nucleus were introduced and resulted in a sevenfold potency improvement (see compound **1** in Fig. 7) [127]. Further bromination at C-10 improved cellular potency and introduction of the 1-formylamine-piperidine-4-yl-acetic acid moiety on the piperidine ring nitrogen provided lonafarnib with improved pharmacokinetics and oral bioavailability [128]. Bromination at C-10 is a key substitution, which introduces an intramolecular constraint on piperidine forcing this ring to exist in a pseudoaxial position. This restriction of mobility is translated into reduced entropy and could explain the enhanced potency of lonafarnib. The initial synthesis of lonafarnib of 19 steps contained a resolution process in the end steps. It was replaced by an elegant shorter synthesis based on an asymmetric condensation of a 4-methylpiperidinyl Grignard reagent [129]. Extensive SAR data on this series of compounds has now been published [130–133], including modifications of lonafarnib by incorporating groups such as amides, esters, ureas, and lactams on the first or the distal piperidine [132] and bridgehead modification resulting in a potent FTI **2** (Fig. 8) with improved oral metabolic stability [133].

The X-ray crystal structure of the lonafarnib:FTase complex inspired the de novo design of indolocycloheptapyridyl FTIs [134] (i.e., SCH 207758) where the β-methyl substituent on the indole ring restricts conformational mobility of the C(11) appendage. Interestingly, targeting the catalytic zinc by introducing a propylaminolimidazole amide moiety on the 2-position of the piperidine ring gave FTIs with activities in the picomolar range, as exemplified by compound **3a** (Fig. 8) [135]. Zinc chelation can also be reached by introducing a piperazine moiety from either the 5- or 6-position of the tricyclic bridgehead giving an FTI of nanomolar potency (**3b**) despite the lack of C-3 and C-10 bromine substituents [136]. A very recent paper also depicts

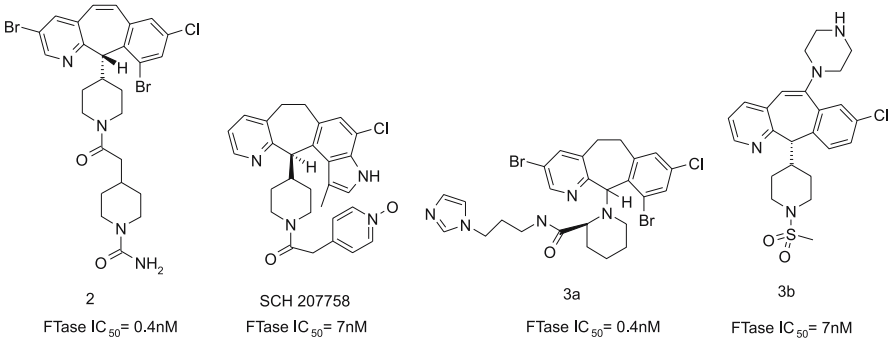


Fig. 8 Second-generation benzocycloheptapyridyl FTIs

the systematic efforts deployed toward zinc binding using specially designed libraries [131].

Tipifarnib was optimized from early antifungal imidazoles [137] and despite the fact that no crystal structure was available at the time of its discovery, key interactions were deduced from traditional medicinal chemistry programs. The putative interaction of the imidazole free nitrogen with the zinc cation, which was demonstrated retrospectively [138], is one of the key anchor points of tipifarnib in the catalytic site, together with aromatic stacking interactions that the two phenyl groups and the quinolinone backbone make with Tyr or Trp amino acids. Tipifarnib also tolerated several modifications of its quinolinone backbone while keeping intact the overall potency, giving birth to a series of azoloquinolines and quinazolines [139, 140].

It is interesting to note the convergence of independent screening programs into molecules containing the imidazole pharmacophore (Fig. 4). Also noteworthy is the absence of the hydroxamic acid moieties, the Zn^{2+} pharmacophore featured prominently in inhibitors of the zinc matrix metalloproteases [141]. The structure–activity relationships developed in independent research programs and publications point to a unique catalytic role for the FTase Zn^{2+} coordination structure, which is susceptible to coordination by imidazoles. BMS-214662 from Bristol-Myers Squibb (Fig. 4), a nonpeptidic FTI containing an unsubstituted imidazole ring, has also entered clinical phase I trials [142, 143]. SAR studies demonstrated that a hydrophobic group (thienyl here, but also phenyl or methyl) linked to N-4 by a hydrogen bond accepting group (e.g., a sulfonyl moiety) as well as 7- or 8-hydrophobic substituents were important to achieve potent enzyme inhibition. The 7-cyano moiety also provided a better solubility. BMS-214662 was originally reported to have some unique apoptosis-inducing properties not necessarily shared by other FTIs. A recent publication indicated that BMS-214662 is a RabGGTase II inhibitor and inhibition of RabGGTase II was correlated with the apoptotic activity of the molecule in cell culture [144]. These findings were unexpected given the unique C-terminal CXC motif recognized by RabG-

GTase II. Although orally available, dose-dependent gastrointestinal toxicities limited the use of this route and administration to patients was performed by intravenous infusion [145]. This may explain why Bristol-Myers Squibb has also investigated a second generation of FTIs, represented by the lead compound BMS-316810 (IC_{50} = 0.7 nM) based on the tetrahydroquinoline ring (Fig. 9). BMS-316810 showed good oral absorption properties and is orally active in a murine tumor model [146].

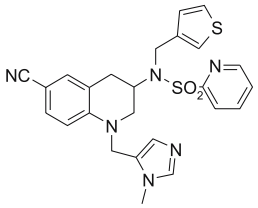


Fig. 9 Structure of BMS-316810

In an effort to find a thiol surrogate to their already potent FTI **4** (IC_{50} = 1 nM) which could function as a zinc ligand, Merck also directed its chemistry toward imidazole-containing compounds (Fig. 10). In an important discovery, attaching a 4-cyanobenzyl group to the imidazole significantly improves potency relative to the unsubstituted imidazole [147, 148], suggesting that the added cyanobenzyl group takes advantage of a novel, high-affinity aryl binding site [149]. A breakthrough for in vitro and in vivo potency of nonpeptide FTIs (i.e., L-778,123) arose from remodeling the structure of **5**, guided by a NMR-based model for the enzyme-bound conformation of a peptidomimetic FTI. The central piperazinone moiety of L-778,123 represents a constraint of the original tetrapeptide backbone also present in the 3-amino pyrrolidinone ring that has been used to generate potent FTIs (i.e., **8**) (Fig. 10) [150]. Compound **8** was found to have an unusually favorable ratio of cell potency to intrinsic potency, compared with those for other known FTIs. It exhibited excellent potency against a range of tumor cell lines in vitro and showed full efficacy in the K-*ras*B transgenic mouse model.

L-778,123 was evaluated in phase I/II as a continuous intravenous infusion but development was stopped due to compound-associated ECG abnormalities [151, 152]. The molecule is a dual FTase and GGTase I inhibitor that has a unique dual mechanism. X-ray crystallography revealed that L-778,123 competed for the CAAX peptide binding region in FTase [153]. However, inhibition of GGTase I proceeded through competition of the geranylgeranyl phosphate binding site [154]. A strategy to modify L-778,123 or other constraint analogs was to alter the linkage of the cyanophenyl and *N*-aryl rings via macrocyclization, without disrupting the other structural features, aiming to improve potency and increase the window between inhibition of Ftase

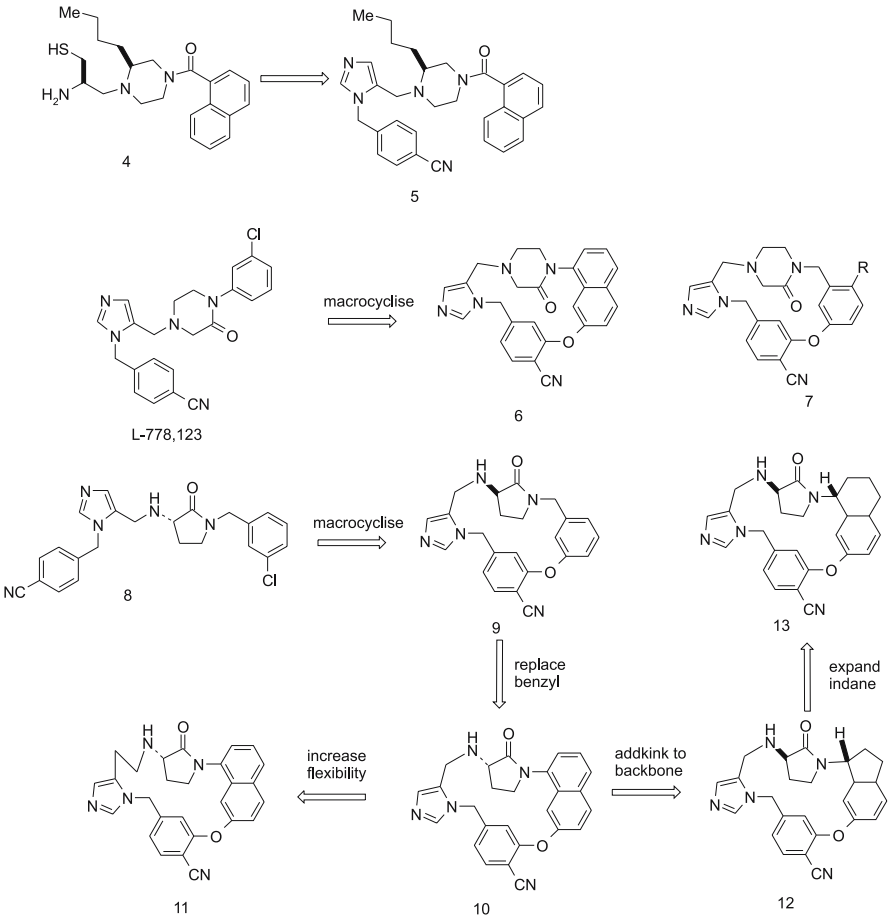


Fig. 10 Strategy developed by Merck to modify L-778,123 and other constraint analogs

and prolongation of the QTc interval in vivo. Macrocyle **6** (Fig. 10) emerged as a highly potent FTI with only moderate activity versus GGTase I. Macrocycles such as **10** (Fig. 10) combined improved pharmacokinetic properties with a reduced potential for side effects. In dogs, oral bioavailability was good to excellent, and increases in plasma half-life were due to attenuated clearance. Optimization of this 3-aminopyrrolidinone series of compounds led to significant increases in potency, providing **13** [155].

Interestingly, by replacing the naphthyl ring in **6** with a substituted benzyl group, a series of macrocyclic compounds **7** has been identified with dramatically increased GGTase I inhibition, leading to highly potent macrocyclic dual FTI-GGTIs (Fig. 10) [156]. A series of novel diaryl ether lactams (Fig. 11) have also been identified as very potent dual inhibitors of FTase and GGTase I [157].

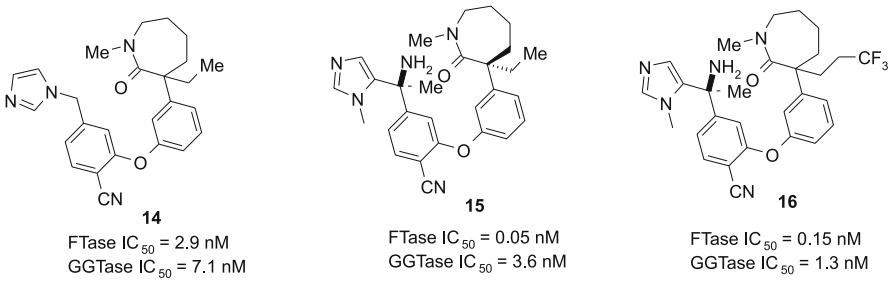


Fig. 11 Aryl cyanophenyl FTIs

Modifications to the structure of **14**, including an alternative imidazole substitution pattern and quaternization of the benzylic carbon, were aimed at limiting in vivo metabolism. Compounds **15** and **16** inhibit the prenylation of the important oncogene Ki-Ras4B in vivo. Unfortunately, doses sufficient to achieve this endpoint were rapidly lethal. Moreover, aryloxy substitution alpha to the cyano group yielded compounds with significantly improved GGTase I activity while maintaining high intrinsic FTase activity [158]. These latter analogs were used to demonstrate the potentially severe toxicity of combined FTase/GGTase I inhibition [55].

Interestingly, Abbott also employed the peptidomimetic strategy of replacing the central two amino acids of the CAAX by a biphenylene moiety but also further replaced the cysteine residue with aryl, alkyl, or heterocyclic side chains [159]. This work culminated in the discovery of the potent ABT-839 (IC_{50} = 1 nM on enzyme, IC_{50} = 16 nM on cells) (Fig. 12). They discovered that many of these amino acid containing biphenyl compounds possessed modest bioavailability and a short half-life. Therefore, Abbott researchers then looked at replacing the last amino acid, namely the methionine moiety, by a cyano group and found that they could advantageously use the interaction between an imidazole group and the Zn^{2+} cation [160]. Although compounds such as **17** showed an unacceptable pharmacokinetic profile in rats, continued efforts toward the discovery of potent, orally bioavailable FTIs gave birth to a huge series of imidazole-containing FTIs (some examples are shown in Fig. 12) [161–166]. As exemplified by recent publications, some of these efforts were based on modifications of the tipifarnib structure (Fig. 13) [167–170]. This culminated in the discovery of ABT-100 (Fig. 14), a highly selective, potent (IC_{50} on human Ftase is 0.05 nM), and orally bioavailable FTI that was reported to be near the clinical phase [171].

Looking at the X-ray crystal structure of Ftase complexed with ABT-100, it is very interesting to note that both are making similar interactions and that one can nearly superimpose tipifarnib and ABT-100 in the catalytic groove. ABT-100 showed broad-spectrum antitumor activity against a series of xenograft models similar to the FTIs in clinical development, which has led

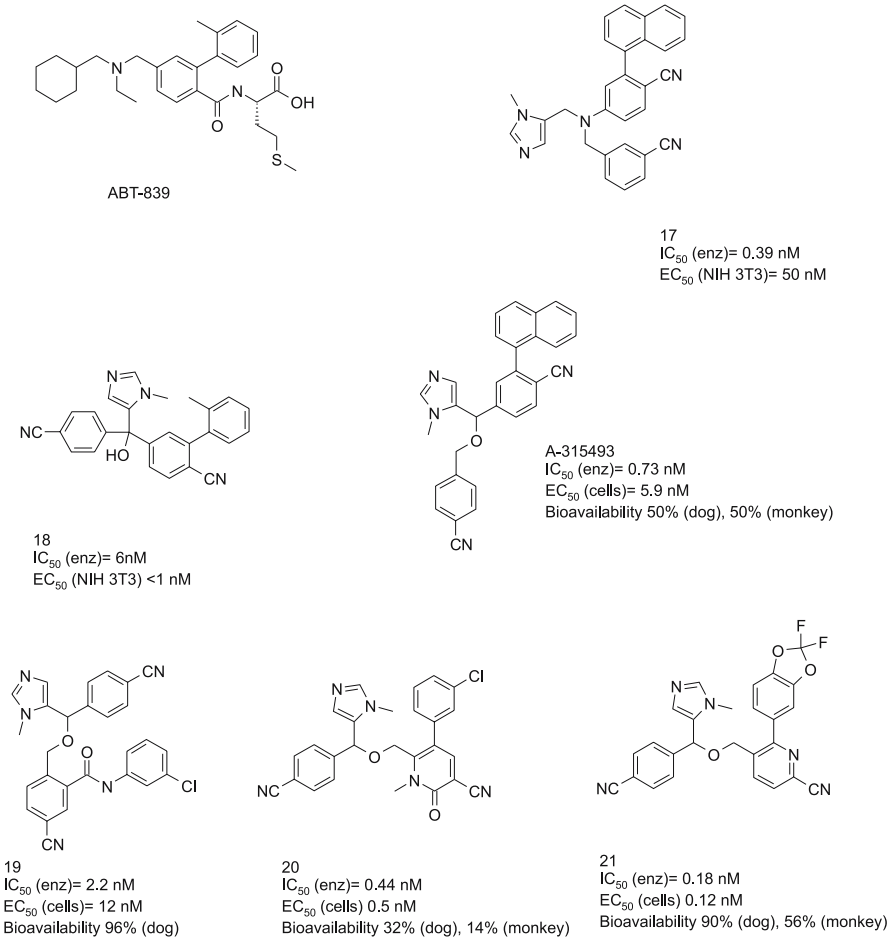


Fig. 12 Representatives of Abbott's FTIs

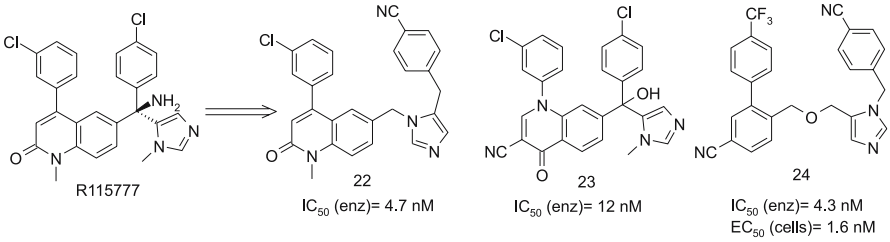
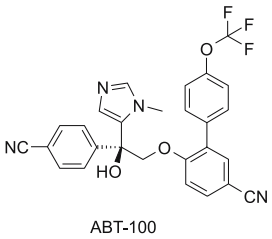


Fig. 13 Examples of Abbott's modifications of the tipifarnib structure

to the design of a kilogram-scale process [172]. However ABT-100 has been discontinued due to toxicity problems [173]. Several attempts were made to find an imidazole surrogate able to chelate zinc in a similar way, but results



ABT-100

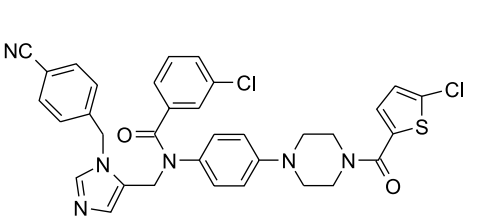
Fig. 14 Structure of ABT-100

were disappointing with perhaps the exception of the *N*-methyl-1,2,4-triazol-3-yl moiety [137, 165, 174].

BIM-46228 (Fig. 4) from Beaufour Ipsen is another interesting FTI featuring the cyanobenzyl-substituted imidazole [175]. The compound was active following oral administration in several xenograft models and reversed the radioresistance of tumor cells in culture. The development status of this compound is not known. The cyanobenzyl–imidazole moiety was also introduced with success by Pierre Fabre researchers on their CNS aminophenyl piperazine scaffold, giving compounds of nanomolar potency (Fig. 15) [176]. A group from Laboratoires Servier introduced the same moiety on an azepin-2-one based scaffold (Fig. 15) and obtained a series of FTIs exhibiting nanomolar activities on both enzyme and cells [177, 178]. However, to our knowledge no further developments were reported for these compounds.

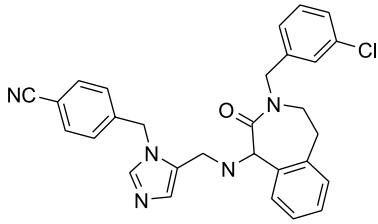
LB-42908 (Fig. 4) is another interesting phenyl-substituted FTI from LGB Chemical Ltd. The compound was submitted to the National Cancer Institute for preclinical and clinical development as NSC-712392 [179].

AstraZeneca also entered a molecule (AZD-3409) (Fig. 4) into phase I/II studies [180]. AZD-3409 is a double prodrug inhibiting both FTase and GGTase I. AZD-3409 is converted in vivo into a prodrug, the main component in plasma of dosed animals, which is further metabolized in cells to the active drug. Recently published phase I data indicate that AZD-3409 was well



25

Laboratoires Pierre Fabre
IC_{50} (enz)= 2 nM
EC_{50} (cells)= 2 nM



26

Laboratoires Servier
IC_{50} (enz)= 19 nM
EC_{50} (cells)= 39 nM

Fig. 15 FTIs from Laboratoires Pierre Fabre and Servier

tolerated and orally bioavailable with a half-life of 15–20 h in healthy volunteers [181]. Development of AZD-3409 has now been discontinued.

Coming from a collaboration between Pfizer and OSI Pharmaceutical, CP-609754 has recently entered phase II, but the structure of this molecule has not been disclosed [182]. Moreover, in recent patent applications [183] Pfizer claimed several compounds bearing a striking resemblance to Johnson & Johnson's tipifarnib. One enantiomer (Fig. 4) was emphasized but no data were presented.

6 Clinical Experience

The early phase I clinical experience with FTIs was encouraging, with evidence of biological activity, good tolerability, and preliminary reports of clinical activity warranting further evaluation of at least tipifarnib and lonafarnib in phase II and phase III clinical trials. Several other FTIs discussed previously have advanced to the point of providing published phase I data, but have been halted or the status of their further development is not known.

The FTIs that have continued in clinical development are oral agents, which is a practical advantage in the chronic cancer treatment setting. The compounds have demonstrated varying degrees of activity and tolerability as oral agents in phase I trials [184, 185]. With inhibition of FTase activity as the principal mechanism of all FTI action, some commonality in FTI-related adverse events has been observed. These include myelosuppression (e.g., neutropenia and thrombocytopenia), fatigue, nausea, and neurosensory symptoms [123, 184–186]. In phase II studies, overall activity of the FTI class against the major solid tumors (prostate, pancreatic, colon, and non-small cell lung cancer) has been disappointing with the exception of early studies with tipifarnib in advanced breast cancer [187]. Some interesting activity has also been noted in glioma with tipifarnib [188]. The most consistent activity has been observed in hematological malignancies with the most promising activity observed in AML and myelodysplastic syndrome [108, 189]. As clinical development of FTIs in hematological malignancies and solid tumors is the subject of several recent in-depth reviews [190–194], it will not be further discussed here.

7 Further Developments

Several groups have shown that protein farnesylation also occurs in trypanosomatid parasites which cause diseases such as African sleeping sick-

ness, Chagas disease, and leismaniasis or in the malaria parasite, and that FTIs which are well tolerated in man are toxic for these parasites [195–201]. New drugs of low cost are desperately needed to replace existing treatments that show either toxicity, limited efficacy, or face drug resistance problems for the millions of people concerned, mainly in Africa and Latin America. It seemed then clever to capitalize on low toxicity pharmacological data and SAR knowledge of FTI anticancer agents and use them as starting points to accelerate progress in the development of therapeutics for protozoan parasitic diseases.

Some of the FTIs in clinical development (lonafarnib, tipifarnib, BMS-214662) were tested for their ability to inhibit the growth of *Plasmodium falciparum* (the causal parasite of malignant tertian and pernicious malaria) [202]. Although lonafarnib and tipifarnib were not effective against *P. falciparum* cell proliferation, BMS-214662 showed an ED_{50} of 180 nM and this triggered further testing of tetrahydroquinoline (THQ) related compounds. Encouraging results were obtained, with BMS-388891 inhibiting *P. falciparum* FTase (Fig. 16) with subnanomolar IC_{50} and an ED_{50} of 5 nM.

Several groups are also applying their knowledge of FT inhibition to specifically design novel lead structures for antimalarial FTIs, emphasizing simple molecular architecture and straightforward chemical synthesis to aim at low-cost treatments [203–207]. *Trypasonoma brucei* (*Tb*, causal agent of sleeping sickness) and *Trypasonoma cruzi* (Chagas disease) have also recently been the focus of attention. It has been shown that catalytic sites of rat FTase

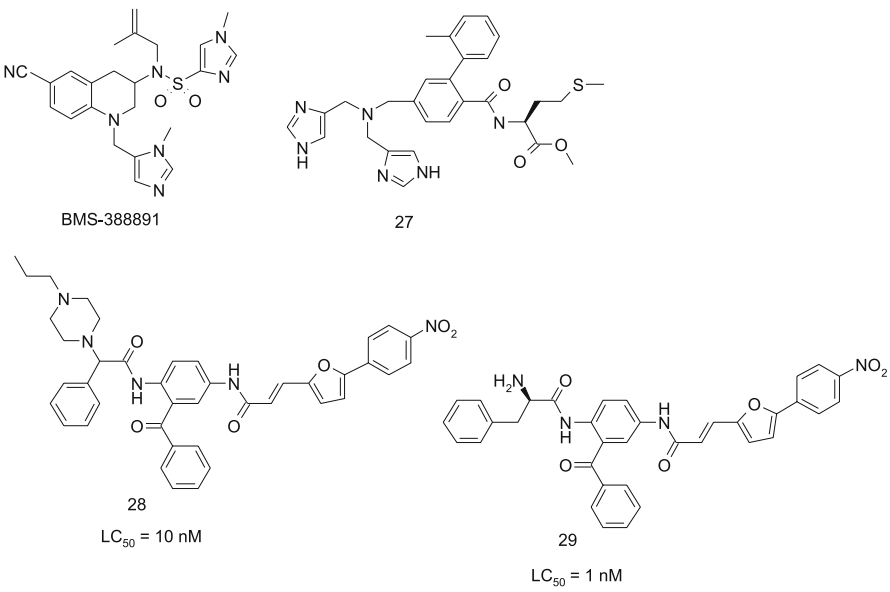


Fig. 16 Further developments of FTIs

and *Tb*FTase share a high homology [208] allowing transfer of knowledge. Thus, compounds based on the 4-aminomethylbenzoic acid scaffold [209] were found to be highly potent inhibitors against *T. brucei*, especially compound **27** (Fig. 16) (ED_{50} = 1.5 nM against bloodstream parasite growth).

Inhibition of *T. cruzi* is still in an early phase but significant results have been obtained using a homology model and benzophenone-based FTIs providing compounds **28** and **29** (Fig. 16), which display in vitro activity against *T. cruzi* in the nanomolar range and induce enhanced survival rates when tested in vivo [210]. It is noteworthy that the concentrations of tipifarnib necessary to inhibit 50% growth of *T. cruzi* amastigotes in culture (4 nM) or its IC_{50} against the *T. cruzi* FTase enzyme (75 nM) are also in the nanomolar range [211]. Hope resides in the fact that optimization of the oral bioavailability and pharmacokinetic properties of these lead compounds could rapidly provide a drug candidate for human clinical trials.

8 Conclusion and Perspectives

Identification of new cancer targets provides challenges to both discovery teams and clinical investigators. Several companies and institutions have already fine-tuned their candidates in clinical trials, capitalizing on further interactions at the binding site to improve their drugability, and are preparing second- or third-generation FTIs. As clinical trials progress, investigations into the molecular mechanisms downstream of Ftase inhibition responsible for antitumor action are advancing in parallel. Over the next several years, cross-fertilization of data between these dual research tracks may generate important clues for optimal clinical use of these new anticancer agents. New insights into modulation of genetic and protein expression profiles via microarray and proteomic techniques may help answer key questions about patient selection, the best dose and schedule for FTI therapy, and the possible benefits of combination regimens. It is also likely, of course, that findings in the clinic will inform and enhance laboratory efforts to pinpoint the diverse molecular actions of FTIs within the cancer cell. Clearly, the synergy between clinical and laboratory FTI research is increasing the momentum of progress in both arenas. As such, FTIs represent an important new therapeutic approach, with significant potential across a range of solid tumors and hematologic malignancies. In addition, FTIs may in the future represent an opportunity for people suffering from parasitic diseases such as malaria or Chagas disease, thus illustrating how pioneer discovery work and an opportunistic approach could ultimately provide benefit to patients in new therapeutic areas.

Acknowledgements The authors would like to thank everyone at Johnson & Johnson who has worked on FTIs over the years.

References

1. Quesney-Huneeus V, Wiley MH, Siperstein MD (1979) Proc Natl Acad Sci USA 76:5056
2. Habenicht AJ, Glomset JA, Ross R (1980) J Biol Chem 255:5134
3. Schmidt RA, Schneider CJ, Glomset JA (1984) J Biol Chem 259:10175
4. Beck LA, Hosick TJ, Sinensky M (1988) J Cell Biol 107:1307
5. Casey PJ, Solski PA, Der CJ, Buss J (1989) Proc Natl Acad Sci USA 86:832
6. Leonard S, Beck L, Sinensky M (1990) J Biol Chem 265:5157
7. Barbacid M (1987) Ann Rev Biochem 56:779
8. Kato K, Cox AD, Hisaka MM, Graham SM, Buss JE, Der CJ (1992) Proc Natl Acad Sci USA 89:6403
9. Jackson JH, Cochrane CG, Bourne JR, Solski PA, Buss JE, Der CJ (1990) Proc Natl Acad Sci USA 87:3042
10. Sinensky M, Beck LA, Leonard S, Evans R (1990) J Biol Chem 265:19937
11. Gibbs JB (1991) Cell 65:1
12. Reiss Y, Goldstein JL, Seabra MC, Casey PJ, Brown MS (1990) Cell 62:81
13. Cox AD, Der CJ (1997) Biochim Biophys Acta 1333:F51
14. Zujewski J, Horak ID, Woestenborghs R, Chiao J, Cusack G, Kohler D, Kremer AB, Cowan KH (1998) Proc Am Assoc Cancer Res 39:270
15. Hudes GR, Schol J, Baab J, Rogatko A, Bol C, Horak I, Langer C, Goldstein LJ, Szarka C, Meropol NJ, Weiner L (1999) Proc Am Soc Clin Oncol 18:601
16. Adjei AA, Erlichman C, Davis JN, Reid J, Sloan J, Statkevich P, Zhu Y, Marks RS, Pitot HC, Goldberg R, Hanson L, Alberts S, Cutler D, Kaufman SH (1999) Proc Am Soc Clin Oncol 18:598
17. Schellens JHM, De Klerk G, Swart M, Palmer PA, Bol CJ, Van't Veer LJ, Tan H, ten Bokkel Huinink WW, Beijnen JH (1999) Proc Am Assoc Cancer Res 40:724
18. Nakagawa K, Yamamoto N, Nishio K, Ohashi Y, End D, Bol Kees, Ito H, Fukuoka M (2001) Proc Am Soc Clin Oncol 20:317
19. Raponi M, Belly RT, Karp JE, Lancet JE, Atkind D, Wang Y (2004) BMC Cancer 4:56
20. Raponi M, Zhang Y, Jatkoe T, Yu J, Lee G, Lancet JE, Karp JE, Thibault A, Wang X (2005) Blood 106:Abstract 2785
21. Maltese WA, Erdman RA (1989) J Biol Chem 264:18168
22. Pompliano DL, Schaber MD, Mosser SD, Omer CA, Shafer JA, Gibbs JB (1993) Biochemistry 32:8341
23. Furfine ES, Leban JJ, Landavazo A, Moomaw JF, Casey PJ (1995) Biochemistry 34:6857
24. Strickland CL, Windsor WT, Syto R, Wang L, Bond R, Wu Z, Schwartz J, Le HV, Beese LS, Weber PC (1998) Biochemistry 37:16601
25. Yokoyama K, McGeady P, Gelb MH (1995) Biochemistry 34:1344
26. Park HW, Boduluri SR, Moomaw JF, Casey PJ, Beese LS (1997) Science 275:1800
27. Long SB, Casey PJ, Beese LS (1998) Biochemistry 37:9612
28. Dunten P, Kammlott U, Crowther R, Weber D, Palermo R, Birktoft J (1998) Biochemistry 37:7907
29. Huang CC, Casey PJ, Fierke CA (1997) J Biol Chem 272:20
30. Hightower KE, Huang CC, Casey PJ, Fierke CA (1998) Biochemistry 37:15555
31. Reiss Y, Seabra MC, Armstrong SA, Slaughter CA, Goldstein JL, Brown MS (1991) J Biol Chem 266:10672
32. Seabra MC, Reiss Y, Casey PJ, Brown MS, Goldstein JL (1991) Cell 65:429
33. Otto JC, Kim E, Young SG, Casey PJ (1999) J Biol Chem 274:8379
34. Gutierrez L, Magee AI, Marshall CJ, Hancock JF (1989) EMBO J 8:1093

35. Seabra MC, Goldstein JL, Sudhof TC, Brown MS (1992) J Biol Chem 267:14497
36. Seabra MC, Brown MS, Slaughter CA, Sudhof TC, Goldstein JL (1992) Cell 70:1049
37. Egan SE, Giddings BW, Brooks MW, Buday L, Sizeland AM, Weinberg RA (1993) Nature 363:45
38. Stokoe D, Macdonald SG, Cadwallader K, Symons M, Hancock JF (1994) Science 264:1463
39. Moodie SA, Willumsen BM, Weber MJ, Wolfman A (1993) Science 260:1658
40. Kohl NE, Mosser SD, deSolms SJ, Giuliani EA, Pompliano DL, Graham SL, Smith RL, Scolnick EM, Oliff A, Gibbs JB (1993) Science 260:1934
41. James GL, Goldstein JL, Brown MS, Rawson TE, Somers TC, McDowell RS, Crowley CW, Lucas BK, Levinson AD, Marsters JC (1993) Science 260:1937
42. Sepp-Lorenzino L, Ma Z, Rands E, Kohl NE, Gibbs JB, Oliff A, Rosen N (1995) Cancer Res 55:5302
43. End DW, Smets G, Todd AV, Applegate TL, Fuery CJ, Angibaud P, Venet M, Sanz G, Poignet H, Skrzat S, Devine A, Wouters W, Bowden C (2001) Cancer Res 61:131
44. James G, Goldstein JL, Brown MS (1996) Proc Natl Acad Sci USA 93:4454
45. Rowell CA, Kowalczyk JJ, Lewis MD, Garcia AM (1997) J Biol Chem 272:14093
46. Whyte DB, Kirschmeier P, Hockenberry TN, Nunez-Oliva I, James L, Catino JJ, Bishop WR, Pai JK (1997) J Biol Chem 272:14459
47. Grunewald K, Lyons J, Frohlich A, Feichtinger H, Weger RA, Schwab G, Janssen JW, Bartram CR (1989) Int J Cancer 43:1037
48. Forrester K, Almoguera C, Han K, Grizzle WE, Perucho M (1987) Nature 327:298
49. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL (1988) N Engl J Med 319:525
50. Reynolds SH, Anna CK, Brown KC, Wiest JS, Beattie EJ, Pero RW, Iglehart JD, Anderson MW (1991) Proc Natl Acad Sci USA 88:1085
51. Knowles MA, Williamson M (1993) Cancer Res 53:133
52. Nagasu T, Yoshimatsu K, Rowell C, Lewis MD, Garcia AM (1995) Cancer Res 55:5310
53. Maltese WA (1990) FASEB J 4:3319
54. Sun J, Qian Y, Hamilton AD, Sebti SM (1998) Oncogene 16:1467
55. Lobell R, Anthony N, Bell I, Buser C, Desolms J, Dinsmore C, Gibbs J, Graham S, Hartman G, Heimbrook D, Huber H, Lumma W, William T, Kohl N (2001) Proc Am Assoc Cancer Res 42:259
56. Prendergast GC (2001) Histol Histopathol 16:269
57. Hall A (1998) Science 279:509
58. Prendergast GC, Khosravi-Far R, Solski PA, Kurzawa H, Lebowitz PF, Der CJ (1995) Oncogene 10:2289
59. Adamson P, Marshall CJ, Hall A, Tilbrook PA (1992) J Biol Chem 267:20330
60. Baron R, Fourcade E, Lajoie-Mazenc I, Allal C, Couderc B, Barbaras R, Favre G, Faye JC, Pradines A (2000) Proc Natl Acad Sci USA 97:11626
61. Lebowitz PF, Casey PJ, Prendergast GC, Thissen JA (1997) J Biol Chem 272:15591
62. Du W, Lebowitz PF, Prendergast GC (1999) Mol Cell Biol 19:1831
63. Du W, Prendergast GC (1999) Cancer Res 59:5492
64. Liu AX, Du W, Liu JP, Jessell TM, Prendergast GC (2000) Mol Cell Biol 20:6105
65. Liu AX, Cerniglia GJ, Bernhard EJ, Prendergast GC (2001) Proc Natl Acad Sci USA 98:6192
66. Teyssier F, Dalenc F, Pradines A, Couderc B, Ader I, Bonnet J, Cohen-Jonathan E, Toulas C, Favre G (2001) Proc Am Assoc Cancer Res 42:489
67. Van Golen KL, Bao LW, DiVito MM, Wu ZF, Prendergast GC, Merajver SD (2002) Mol Cancer Ther 1:575

68. Chen Z, Sun J, Pradines A, Favre G, Adnane J, Sebti SM (2000) J Biol Chem 275:17974
69. DuHadaway JB, Du W, Donover S, Baker J, Liu A, Sharp DM, Muller AJ, Prendergast GC (2003) Oncogene 22:3578
70. Kamasani U, Huang M, DuHadaway JB, Prochownik EV, Donover PS, Prendergast GC (2004) Cancer Res 64:8389
71. Wolda SL, Glomset JA (1988) J Biol Chem 263:5997
72. Holtz D, Tanaka RA, Hartwig J, McKeon F (1989) Cell 59:969
73. Beck LA, Hosick TJ, Sinensky M (1990) J Cell Biol 110:1489
74. Sinensky M, Fantle K, Dalton M (1994) Cancer Res 54:3229
75. Dalton MB, Fantle KS, Bechtold HA, DeMaio L, Evans RM, Krystosek A, Sinensky M (1995) Cancer Res 55:3295
76. Toth JI, Yang SH, Qiao X, Beigneux AP, Gelb MH, Moulson CL, Miner JH, Young SG, Fong LG (2005) Proc Natl Acad Sci USA 102:12873
77. Capell BC, Erdos MR, Madigan JP, Fiordalisi JJ, Varga R, Conneely KN, Gordon LB, Der CJ, Cox AD, Collins FS (2005) Proc Natl Acad Sci USA 102:12879
78. Glynn MW, Glover TW (2005) Hum Mol Genet 14:2959
79. Mallampalli MP, Huyer G, Bendale P, Gelb MH, Michaelis S (2005) Proc Natl Acad Sci USA 102:14416
80. Crespo NC, Ohkanda J, Yen TJ, Hamilton AD, Sebti SM (2001) J Biol Chem 276:16161
81. Ashar HR, James L, Gray D, Carr D, Black S, Armstrong L, Bishop WR, Kirschmeier P (2000) J Biol Chem 275:30451
82. Li Q, Ke Y, Kapp JA, Fertig N, Medsger TA, Joshi HC (1995) Biochem Biophys Res Commun 212:220
83. Liao H, Winkfein RJ, Mack G, Rattner JB, Yen TJ (1995) J Cell Biol 130:507
84. Schaar BT, Chan GKT, Maddox P, Salmon ED, Yen TJ (1997) J Cell Biol 139:1373
85. Chan GK, Schaar BT, Yen TJ (1998) J Cell Biol 143:49
86. Moasser MM, Sepp-Lorenzino L, Kohl NE, Oliff A, Balog A, Su DS, Danishefsky SJ, Rosen N (1998) Proc Natl Acad Sci USA 95:1369
87. Shi B, Yaremko B, Hajian G, Terracina G, Bishop WR, Liu M, Nielsen LL (2000) Cancer Chemother Pharmacol 46:387
88. de Caestecker MP, Piek E, Roberts AB (2000) J Natl Cancer Inst 92:1388
89. Venkatasubbarao K, Ahmed MM, Mohiuddin M, Swiderski C, Lee E, Gower WR, Salhab KF, McGrath P, Strodel W, Freeman JW (2000) Anticancer Res 20:43
90. Muro-Cacho CA, Rosario-Ortiz K, Livingston S, Munoz-Antonia T (2001) Clin Cancer Res 7:1618
91. Matsushita M, Matsuzaki K, Date M, Watanabe T, Shibano K, Nakagawa T, Yanagitani S, Amoh Y, Takemoto H, Ogata N, Yamamoto C, Kubota Y, Seki T, Inokuchi H, Nishizawa M, Takada H, Sawamura T, Okamura A, Inoue K (1999) Br J Cancer 80:194
92. Brattain MG, Ko Y, Banerji SS, Wu G, Willson JK (1996) J Mammary Gland Biol Neoplasia 4:365
93. Hejlik DP, Kottickal LV, Liang H, Fairman J, Davis T, Janecki T, Sexton D, Peery W, Tavtigian SV, Teng DH, Nagarajan L (1997) Cancer Res 57:3779
94. Adnane J, Bizouarn FA, Chen Z, Ohkanda J, Hamilton AD, Munoz-Antonia T, Sebti SM (2000) Oncogene 19:5525
95. Fralix KD, Jeske N, Freeman JW (2000) Proc Am Assoc Cancer Res 41:440
96. Alcock RA, Dey S, Mohiuddin M, Gallicchio VS, Chatfield LK, Freeman JW (2001) Proc Am Assoc Cancer Res 42:489
97. Basso AD, Mirza A, Liu G, Long BJ, Bishop WR, Kirshmeier P (2005) J Biol Chem 280:31101
98. Lebowitz PF, Sakamuro D, Prendergast GC (1997) Cancer Res 57:708

99. Kohl NE, Omer CA, Conner MW, Anthony NJ, Davide JP, deSolms SJ, Giuliani EA, Gomez RP, Graham SL, Hamilton K, Handt LK, Hartman GD, Kobaln KS, Kral AM, Miller PJ, Mosser SD, O'Neill TJ, Rands E, Schaber MD, Gibbs JB, Oliff A (1995) Nat Med 1:792
100. Liu M, Bryant MS, Chen J, Lee S, Yaremko B, Lipari P, Malkowski M, Ferrari E, Nielsen L, Prioli N, Dell J, Sinha J, Syed J, Korfmacher WA, Nomeir AA, Lin CC, Wang L, Taveras AG, Doll RJ, Njoroge FG, Mallams AK, Remiszewski S, Catino JJ, Girijavallabahan VM, Kirschmeier P, Bishop WR (1998) Cancer Res 58:4947
101. Jiang K, Coppola D, Crespo NC, Nicosia SV, Hamilton AD, Sebti SM, Cheng JQ (2000) Mol Cell Biol 20:139
102. Du W, Liu A, Prendergast GC (1999) Cancer Res 59:4208
103. McFall A, Ulku A, Lambert QT, Kusa A, Rogers-Graham K, Der CJ (2001) Mol Cell Biol 21:5488
104. Ochiai N, Uchida R, Fuchida S, Okano A, Okamoto M, Ashihara E, Inaba T, Fujita N, Matsubara H, Shimazaki C (2003) Blood 102:3349
105. Birkencamp KU, Geugien M, Schepers H, Westra J, Lemmink HH, Vellenga E (2004) Leukemia 18:103
106. Takada Y, Khuri FR, Aggarawal BB (2004) J Biol Chem 279:26287
107. Mijimolle N, Velasco J, Dubus P, Guerra C, Weinbaum CA, Casey PJ, Campuzano V, Barbacid M (2005) Cancer Cell 7:313
108. Karp JE, Lancet JE, Kaufman SH, End DW, Wright JJ, Bol K, Horak I, Tidwell ML, Liesveld J, Kottke TJ, Ange DA, Buddharaju L, Gojo I, Highsmith E, Belly RT, Hohl RJ, Rybak ME, Thibault A, Rosenblatt J (2001) Blood 97:3361
109. Adjei AA, Erlichman C, David JN, Cutler DL, Sloan JA, Marks RS, Hanson LJ, Svinegn PA, Atherton P, Bishop WR, Kirschmeier P, Kaufman SH (2000) Cancer Res 60:1871
110. Raponi M, Lowenberg B, Lancet JE, Harousseau JL, Stone R, Rackoff W, Thibault A, Zhang Y, Atkins D, Wang Y (2004) Blood 104:246A
111. Garcia AM, Rowell C, Ackermann K, Kowalczyk JJ, Lewis MD (1993) J Biol Chem 268:18415
112. Kohl NE, Wilson FR, Mosser SD, Giuliani E, deSolms SJ, Conner MW, Anthony NJ, Holtz WJ, Gomez RP, Lee TJ, Smith RL, Graham SL, Hartman GD, Gibbs JB, Oliff A (1994) Proc Natl Acad Sci USA 91:9141
113. Sun J, Qian Y, Hamilton AD, Sebti SM (1995) Cancer Res 55:4243
114. Nagasu T, Yoshimatsu K, Rowell C, Lewis MD, Garcia AM (1995) Cancer Res 55:5310
115. Vrignaud P, Bissery MC, Lavelle F (1999) Ann NY Acad Sci 886:249
116. Liu A, Prendergast GC (2000) FEBS Lett 481:205
117. Mazet JL, Padieu M, Osman H, Maume G, Mailliet P, Dereu N, Hamilton AD, Lavelle F, Sebti SM, Maume BF (1999) FEBS Lett 460:235
118. Yonemoto M, Satoh T, Arakawa H, Suzuki-Takahashi I, Monden Y, Kodera T, Tanaka K, Aoyama T, Iwasawa Y, Kamei T, Nishimura S, Tomimoto K (1998) Mol Pharmacol 54:1
119. Aoyama T, Satoh T, Yonemoto M, Shibata J, Nonoshtia K, Arai S, Kawakami K, Iwasawa Y, Sano H, Tanaka K, Monden Y, Kodera T, Arakawa H, Suzuki-Takahashi I, Kamei T, Tomimoto K (1998) J Med Chem 41:143
120. Tahir SK, Gu WZ, Zang HC, Leal J, Lee JY, Kovar P, Saeed B, Cherian SP, Devine E, Cohen J, Warner R, Wang YC, Stout D, Arendsen DL, Rosenberg S, Ng SC (2000) Eur J Cancer 36:1161
121. Bottex-Gauthier C, Vidal D, Picot F, Potier P, Menichini F, Appendino G (1993) Biotechnol Ther 4:77

122. Shaikenov TE, Adekenov SM, Williams RM, Prashad N, Baker FL, Madden TL, Newman R (2001) Oncol Rep 8:173
123. Zujewski J, Horak ID, Bol CJ, Woestenborghs R, Bowden C, End DW, Piotrovsky VK, Chiao J, Belly RT, Todd A, Kopp WC, Kohler DR, Chow C, Noone M, Hakim FT, Larkin G, Gress RE, Nussenblatt RB, Kremer AB, Cowan KH (2000) J Clin Oncol 18:927
124. Mallams AK, Rossman RR, Doll RJ, Girijavallabhan VM, Ganguly AK, Petrin J, Wang L, Patton R, Bishop WR, Carr DM, Kirschmeier P, Catino JJ, Bryant MS, Chen KJ, Korfmacher WA, Nardo C, Wang S, Nomeir AA, Lin CC, Li Z, Chen J, Lee S, Dell J, Lipari P, Malkowski M, Yaremko B, King I, Liu M (1998) J Med Chem 41:877
125. Strickland CL, Weber PC, Windsor WT, Wu Z, Le HV, Albanese MM, Alvarez CS, Cesarz D, del Rosario J, Deskus J, Mallams AK, Njoroge FG, Piwinski JJ, Remiszewski S, Rossman R, Taveras AG, Vibulbhan B, Doll RJ, Girijavallabhan VM, Ganguly AK (1999) J Med Chem 42:2125
126. Mallams AK, Njoroge FG, Doll RJ, Snow ME, Kaminski JJ, Rossman RR, Vibulbhan B, Bishop WR, Kirschmeier P, Liu M, Bryant MS, Alvarez C, Carr D, James L, King I, Li Z, Lin CC, Nardo C, Petrin J, Remiszewski SW, Taveras AG, Wang S, Wong J, Catino J, Girijavallabhan V, Ganguly AK (1997) Bioorg Med Chem 5:93
127. Njoroge FG, Taveras AG, Kelly J, Remiszewski S, Mallams AK, Wolin R, Afonso A, Cooper AB, Rane DF, Liu YT, Wong J, Vibulbhan B, Pinto P, Deskus J, Alvarez CS, Del Rosario J, Connolly M, Wang J, Desai J, Rossman RR, Bishop WR, Patton R, Wang L, Kirschmeier P, Bryant MS, Nomeir AA, Lin CC, Liu M, McPhail AT, Doll RJ, Girijavallabhan VM, Ganguly AK (1998) J Med Chem 41:4890
128. Njoroge FG, Vibulbhan B, Rane DF, Bishop WR, Petrin J, Patton R, Bryant MS, Chen KJ, Nomeir AA, Lin CC, Liu M, King I, Chen J, Lee S, Yaremko B, Dell J, Lipari P, Malkowski M, Li Z, Catino J, Doll RJ, Girijavallabhan V, Ganguly AK (1997) J Med Chem 40:4290
129. Kuo SC, Chen F, Hou D, Kim-Meade A, Bernard C, Liu J, Levy S, Wu GG (2003) J Org Chem 68:4984
130. Rokosz LL, Huang CY, Reader JC, Stauffer TM, Chelsky D, Sigal NH, Ganguly AK, Baldwin JJ (2005) Bioorg Med Chem Lett 15:5537
131. Huang CY, Stauffer TM, Strickland CL, Reader JC, Huang H, Li G, Cooper AB, Doll RJ, Ganguly AK, Baldwin JJ, Rokosz LL (2006) Bioorg Med Chem Lett 16:507
132. Njoroge FG, Vibulbhan B, Pinto P, Strickland CL, Bishop WR, Kirschmeier P, Girijavallabhan V, Ganguly AK (2003) Bioorg Med Chem 11:139
133. Njoroge FG, Vibulbhan B, Shi X, Strickland C, Kirschmeier P, Bishop WR, Nomeir A, Girijavallabhan V (2004) Bioorg Med Chem Lett 14:5899
134. Taveras AG, Aki C, Chao J, Doll RJ, Lalwani T, Girijavallabhan V, Strickland CL, Windsor WT, Weber P, Hollinger F, Snow M, Patton R, Kirschmeier P, James L, Liu M, Nomeir A (2002) J Med Chem 45:3854
135. Njoroge FG, Vibulbhan B, Pinto P, Strickland CL, Kirschmeier P, Bishop WR, Girijavallabhan V (2004) Bioorg Med Chem 14:5877
136. Njoroge FG, Vibulbhan B, Pinto P, Strickland C, Bishop WR, Nomeir A, Girijavallabhan V (2006) Bioorg Med Chem Lett 16:984
137. Venet M, End D, Angibaud P (2003) Curr Top Med Chem 3:1095
138. Reid TS, Beese LS (2004) Biochemistry 43:6877
139. Angibaud P, Bourdrez X, End DW, Freyne E, Janicot M, Lezouret P, Ligny Y, Mannens G, Damsch S, Mevellec L, Meyer C, Muller P, Pilatte I, Poncelet V, Roux B, Smets G, Van Dun J, Van Remoortere P, Venet M, Wouters W (2003) Bioorg Med Chem Lett 13:4365

140. Angibaud P, Bourdrez X, Devine A, End DW, Freyne E, Ligny Y, Muller P, Mannens G, Pilatte I, Poncelet V, Skrzat S, Smets G, Van Dun J, Van Remoortere P, Venet M, Wouters W (2003) Bioorg Med Chem Lett 13:1543
141. Skotnicki JS, Zask A, Nelson FC, Albright JD, Levin JI (1999) Ann NY Acad Sci 878:61
142. Hunt JT, Ding CZ, Batorsky R, Bednarz M, Bhide R, Cho Y, Chong S, Chao S, Gullo-Brown J, Guo P, Kim SH, Lee FY, Leftheris K, Miller A, Mitt T, Patel M, Penhallow BA, Ricca C, Rose WC, Schmidt R, Slusarchyk WA, Vite G, Manne W (2000) J Med Chem 43:3587
143. Johnston SRD (2003) IDrugs 6:72
144. Lackner MR, Kindt RM, Carroll PM, Brown K, Cancilla MR, Chen C, de Silva H, Franke Y, Guan B, Heuer T, Hung T, Keegan K, Lee JM, Manne V, O'Brien C, Parry D, Perez-Villar JJ, Reddy RK, Xiao H, Zhan H, Cockett M, Plowman G, Fitzgerald K, Costa M, Ross-Macdonald P (2005) Cancer Cell 7:325
145. Camacho LH, Soignet SL, Pezzulli S, Canales C, Aghajanian C, Spriggs DS, Damle B, Sonnichsen D (2001) Proc Am Soc Clin Oncol 20:Abstract 311
146. Lombardo LJ, Camuso A, Clark J, Fager K, Gullo-Brown J, Hunt JT, Inigo I, Kan D, Koplowitz B, Lee F, McGlinchey K, Qian L, Ricca C, Rovnyak G, Traeger S, Tokarski J, Williams DK, Wu LI, Zhao Y, Manne V, Bhide RS (2005) Bioorg Med Chem Lett 15:1895
147. Anthony NJ, Gomez RP, Schaber MD, Mosser SD, Hamilton KA, O'Neil TJ, Koblan KS, Graham SL, Hartman GD, Shah D, Rands E, Kohl NE, Gibbs JB, Oliff A (1999) J Med Chem 42:3356
148. Williams TM, Bergman JM, Brashear K, Breslin MJ, Dinsmore CJ, Hutchinson JH, MacTough SC, Stump CA, Wei DD, Zartman CB, Bogusky MJ, Culberson JC, Buser-Doepner C, Davide J, Greenberg IB, Hamilton KA, Koblan KS, Kohl NE, Liu D, Lobell RB, Mosser SD, O'Neill TJ, Rands E, Schaber MD, Wilson F, Senderak E, Motzel SL, Gibbs JB, Graham SL, Heimbrook DC, Hartman GD, Oliff AL, Huff JR (1999) J Med Chem 42:3779
149. Breslin MJ, deSolms SJ, Giuliani EA, Stokker GE, Graham SL, Pompliano DL, Mosser SD, Hamilton KA, Hutchinson JH (1998) Bioorg Med Chem 8:3311
150. Bell IM, Gallichio SN, Abrams M, Beshore DC, Buser CA, Culberson JC, Davide J, Ellis-Hutchings M, Fernandes C, Gibbs JB, Graham SL, Hartman GD, Heimbrook DC, Homnick CF, Huff JR, Kassahun K, Koblan KS, Kohl NE, Lobell RB, Lynch JJ, Miller PA, Omer CA, Rodrigues AD, Walsh ES, Williams TM (2001) J Med Chem 44:2933
151. Soignet S, Yao SL, Britten C, Spriggs D, Pezzulli S, McCreery H, Mazina K, Deutsch P, Lee Y, Lobell R, Rosen N, Rowinsky E (1998) Proc Am Assoc Cancer Res 40:517
152. Britten CD, Rowinsky E, Yao SL, Soignet S, Rosen N, Eckhardt SG, Drengler R, Hammond L, Siu LL, Smith L, McCreery H, Pezzulli S, Lee Y, Lobell R, Deutsch P, Von Hoff D, Spriggs D (1999) Proc Am Soc Clin Oncol 18:597
153. Reid TS, Long SB, Beese LS (2004) Biochemistry 43:9000
154. Lackner MR, Kindt RM, Carroll PM, Brown K, Cancilla MR, Chen C, de Silva H, Franke Y, Guan B, Heuer T, Hung T, Keegan K, Lee JM, Manne V, O'Brien C, Parry D, Perez-Villar JJ, Reddy RK, Xiao H, Zhan H, Cockett M, Plowman G, Fitzgerald K, Costa M, Ross-MacDonald P (2005) Cancer Cell 7:325
155. Bell IM, Gallicchio SN, Abrams M, Beese LS, Beshore DC, Bhimnathwala H, Bogusky MJ, Buser CA, Culberson JC, Davide J, Ellis-Hutchings M, Fernandes C, Gibbs JB, Graham SL, Hamilon KA, Hartman GD, Heimbrook DC, Homnick CF, Huber HE, Huff JR, Kassahun K, Koblan KS, Kohl NE, Lobell RB, Lynch JJ, Robinson R, Rodrigues AD, Taylor JS, Walsh ES, Williams TM, Zartman CB (2002) J Med Chem 45:2388

156. Dinsmore CJ, Zartman CB, Bergman JM, Abrams MT, Buser CA, Culberson JC, Davide JP, Ellis-Hutchings M, Fernandes C, Graham SL, Hartman GD, Huber HE, Lobell RB, Mosser SD, Robinson RG, Williams TM (2004) Bioorg Med Chem Lett 14:639
157. deSolms SJ, Ciccarone TM, MacTough SC, Shaw AW, Buser CA, Ellis-Hutchings M, Fernandes C, Hamilton KA, Huber HE, Kohl NE, Lobell RB, Robinson RG, Tsou NN, Walsh ES, Graham SL, Beese LS, Taylor JS (2003) J Med Chem 46:2973
158. Bergman JM, Abrams MT, Davide JP, Greenberg IB, Robinson RG, Buser CA, Huber HE, Koblan KS, Kohl NE, Lobell RB, Graham SL, Hartman GD, Williams TM, Dinsmore CJ (2001) Bioorg Med Chem Lett 11:1411
159. Henry KJ, Wasicak J, Tasker AS, Cohen J, Ewing P, Mitten M, Larsen JJ, Kalvin DM, Swenson R, Ng SC, Saeed B, Cherian S, Sham H, Rosenberg SH (1999) J Med Chem 42:4844
160. Curtin ML, Florjancic AS, Cohen J, Gu WZ, Frost DJ, Muchmore SW, Sham HL (2003) Bioorg Med Chem Lett 13:1367
161. Lin NH, Wang L, Cohen J, Gu WZ, Frost D, Zhang H, Rosenberg S, Sham H (2003) Bioorg Med Chem Lett 13:1293
162. Gwaltney SL, O'Connor SJ, Nelson LTJ, Sullivan GM, Imade H, Wang W, Hasvold L, Li Q, Cohen J, Gu WZ, Tahir SK, Bauch J, Marsh K, Ng SC, Frost DJ, Zhang H, Muchmore S, Jakob CG, Stoll V, Hutchins C, Rosenberg SH, Sham HL (2003) Bioorg Med Chem Lett 13:1363,1363
163. Tong Y, Lin NH, Wang L, Hasvold L, Wang W, Leonard N, Li T, Li Q, Cohen J, Gu WZ, Zhang H, Stoll V, Bauch J, Marsh K, Rosenberg SH, Sham HL (2003) Bioorg Med Chem Lett 13:1571
164. Hasvold LA, Wang W, Gwaltney SL, Rockway TW, Nelson LTJ, Mantei RA, Fakhoury SA, Sullivan GM, Li Q, Lin NH, Wang L, Zhang H, Cohen J, Gu WZ, Marsh K, Bauch J, Rosenberg SH, Sham HL (2003) Bioorg Med Chem Lett 13:4001
165. Wang L, Wang GT, Wang X, Tong Y, Sullivan G, Park D, Leonard NM, Li Q, Cohen J, Gu WZ, Zhang H, Bauch JL, Jakob CG, Hutchins CW, Stoll VS, Marsh K, Rosenberg SH, Sham HL, Lin NH (2004) J Med Chem 47:612
166. Wang L, Lin NH, Li Q, Henry RF, Zhang H, Cohen J, Gu WZ, Marsh KC, Bauch JL, Rosenberg SH, Sham HL (2004) Bioorg Med Chem Lett 14:4603
167. Li Q, Claiborne A, Li T, Hasvold L, Stoll VS, Muchmore S, Jakob CG, Gu W, Cohen J, Hutchins C, Frost D, Rosenberg SH, Sham HL (2004) Bioorg Med Chem Lett 14:5367
168. Li Q, Wang GT, Li T, Gwaltney SL, Woods KW, Claiborne A, Wang X, Gu W, Cohen J, Stoll VS, Hutchins C, Frost D, Rosenberg SH, Sham HL (2004) Bioorg Med Chem Lett 14:5371
169. Wang GT, Wang X, Wang W, Hasvold LA, Sullivan G, Hutchins CW, O'Conner S, Gentiles R, Sowin T, Cohen J, Gu WZ, Zhang H, Rosenberg SH, Sham HL (2005) Bioorg Med Chem Lett 15:153
170. Li Q, Woods KW, Wang W, Lin NH, Claiborne A, Gu WZ, Cohen J, Stoll VS, Hutchin C, Frost D, Rosenberg SH, Sham HL (2005) Bioorg Med Chem Lett 15:2033
171. Gu WZ, Joseph I, Wang YC, Frost D, Sullivan GM, Wang L, Lin NH, Cohen J, Stoll VS, Jakob CG, Muchmore SW, Harlan JE, Holzman T, Walten KA, Ladror US, Anderson MG, Kroeger P, Rodriguez LE, Jarvis KP, Ferguson D, Marsh K, Ng S, Rosenberg SH, Sham HL, Zhang H (2005) Anticancer Drugs 16:1069
172. Rozema MJ, Kruger AW, Rohde BD, Shelat B, Bhagavatula L, Tien JJ, Zhang W, Henry RF (2005) Tetrahedron 61:4419
173. Ferguson D, Rodriguez LE, Palma JP, Refici M, Jarvis K, O'Connor J, Sullivan GM, Frost D, Marsh K, Bauch J, Zhang H, Lin NH, Rosenberg S, Sham HL, Joseph IBJK (2005) Clin Cancer Res 11:3045

174. Angibaud P, Saha AK, Bourdrez X, End DW, Freyne E, Lezouret P, Mannens G, Mevellec L, Meyer C, Pilatte I, Poncelet V, Roux B, Smet G, Van Dun J, Venet M, Wouters W (2003) Bioorg Med Chem Lett 13:4361
175. Prevost GP, Pradines A, Brezak MC, Lonchampt MO, Viossat I, Ader I, Toulas C, Kasprzyk P, Gordon T, Favre G, Morgan B (2001) Int J Cancer 91:718
176. Perez M, Maraval C, Dumont S, Lamothe M, Schambel P, Etievant C, Hill B (2003) Bioorg Med Chem Lett 13:1455
177. Le Diguarher T, Ortuno JC, Dorey G, Shanks D, Guilbaud N, Pierre A, Fauchere JL, Hickman JA, Tucker GC, Casara PJ (2003) Bioorg Med Chem 11:3193
178. Le Diguarher T, Ortuno JC, Shanks D, Guilbaud N, Pierre A, Raimbaud E, Fauchere JL, Hickman JA, Tucker GC, Casara PJ (2004) Bioorg Med Chem Lett 14:767
179. Smith AC, Tosca PJ, Prezioso J, Grossi I, Ryan M, Zutshi A, Lee S, Son WC, Juhn SH, Tomaszewski JE, Turner N (2001) Proc Am Assoc Cancer Res 42:489
180. Sorbera LA, Fernandez R, Castaner JR (2001) Drugs Future 26:453
181. McCormack P, Macpherson M, Wilson D, Lindsay A, Parry T, Holt A, Hughes AJ (2004) Clin Oncol 22:3172
182. Press release from Ninth Annual JP Morgan H&Q Investing in Biotechnology Conference, July 2001, London
183. Lyssikatos JP, La Greca SD, Yang BV (2000) Quinolin-2-one derivitives useful as anticancer agents. WO 00/12498
184. Patnaik A, Rowinsky EK (2001) Early clinical experience with farnesyl protein transferase inhibitors. In: Sebti SM, Hamilton AD (eds) Farnesyltransferase inhibitors in cancer therapy. Humana, Totowa, NJ, pp 233–249
185. Hahn SM, Bernhard E, McKenna WG (2001) Semin Oncol 28:86
186. Schellens JHM, de Klerk G, Swart M, Palmer PA, Bol CJ, van't Veer LJ, Tan S, de Gast GC, Beijnen JH, ten Bokkel Huinink W (2000) Proc Am Soc Clin Oncol 19:715
187. Johnson SR, Hickish T, Houston S, Ellis PA, Howes A, Thibualt A (2002) Proc Am Soc Clin Oncol 21:138
188. Cloughesy TF, Kuhn J, Wen P, Chang SM, Schiff D, Greenberg H, Junck L, Robins I, DeAngelis LM, Yung A, Groves M, Fink K, Abrey LE, Lieberman F, Mehta MP, Raizer JJ, Hess K, Prados M (2002) Proc Am Soc Clin Oncol 21:317
189. Kurzrock R, Albitar M, Cortes JE, Estey EH, Faderl SH, Garcia-Manero G, Thomas DA, Giles FJ, Ryback ME, Thibault A, De Porre P, Kantarjian HM (2004) J Clin Oncol 22:1287
190. Lancet JE, Karp JE (2003) Blood 102:3880
191. Karp JE, Lancet JE (2005) Curr Mol Med 5:643
192. Caponigro F, Casale M, Bryce J (2003) Expert Opin Investig Drugs 12:943
193. Jabbour E, Kantarjian H, Cortes J (2004) Leuk Lymphoma 45:2187
194. Appels NMGM, Beijnen JH, Schellens JHM (2005) Oncologist 10:565
195. Yokoyama K, Lin Y, Stuart KD, Gelb MH (1997) Mol Biochem Parasitol 87:61
196. Field H, Blench I, Croft S, Field MC (1996) Mol Biochem Parasitol 82:67
197. Yokoyama K, Trobridge P, Buckner FS, Scholten J, Stuart KD, Van Voorhis WC, Gelb MH (1998) Mol Biochem Parasitol 94:87
198. Chakrabarti D, Azam T, DelVecchio C, Qiu L, Park Y, Allen CM (1998) Mol Biochem Parasitol 94:175
199. Yokoyama K, Trobridge P, Buckner FS, Van Voorhis WC, Stuart KD, Gelb MH (1998) J Biol Chem 273:26497
200. Chakrabarti D, Da Silva T, Barger J, Paquette S, Patel H, Patterson S, Allen CM (2002) J Biol Chem 277:42066

201. Gelb MH, Van Voorhis WC, Buckner FS, Yokoyama K, Eastman R, Carpenter EP, Panethymitaki C, Brown KA, Smith DF (2003) Mol Biochem Parasitol 126:155
202. Nallan L, Bauer KD, Bendale P, Rivas K, Yokoyama K, Horney CP, Pendyala PR, Floyd D, Lombardo LJ, Williams DK, Hamilton A, Sebti S, Windsor WT, Weber PC, Buckner FS, Chakrabarti D, Gelb MH, Van Voorhis WC (2005) J Med Chem 48:3704
203. Schlitzer M (2005) Curr Med Chem Anti-Infective Agents 4:277
204. Glenn MP, Chang SY, Hucke O, Verlinde CLMJ, Rivas K, Horney C, Yokoyama K, Buckner FS, Pendyala PR, Chakrabarti D, Gelb M, Van Voorhis WC, Sebti SM, Hamilton AD (2005) Angew Chem Int Ed Engl 44:4903
205. Kettler K, Sakowski J, Wiesner J, Ortmann R, Jomaa H, Schlitzer M (2005) Pharmazie 60:323
206. Kettler K, Wiesner J, Fucik K, Sakowski J, Ortmann R, Dahse HM, Jomaa H, Schlitzer M (2005) Pharmazie 60:677
207. Ryckebusch A, Gilleron P, Millet R, Houssin R, Lemoine A, Pommery N, Grellier P, Sergheraert C, Henichart JP (2005) Chem Pharm Bull 53:1324
208. Buckner FS, Yokoyama K, Nguyen L, Grewal A, Erdjument-Bromage H, Tempst P, Strickland CL, Xiao L, Van Voorhis WC, Gelb MH (2000) J Biol Chem 275:21870
209. Ohkanda J, Buckner FS, Lockman JW, Yokoyama K, Carrico D, Eastman R, De Lucas-Fradley K, Davies W, Croft SL, Van Voorhis WC, Gelb MH, Sebti SM, Hamilton AD (2004) J Med Chem 47:432
210. Esteva MI, Kettler K, Maidana C, Fichera L, Ruiz AM, Bontempi EJ, Andersson B, Dashe HM, Haebel P, Ortmann R, Klebe G, Schlitzer M (2005) J Med Chem 48:7186
211. Hucke O, Gelb MH, Verlinde CLMJ, Buckner FS (2005) J Med Chem 48:5415

Top Med Chem (2007) 1: 169–206
DOI 10.1007/7355_2006_005

Published online: 18 January 2007

Survival Signaling

Carlos Garcia-Echeverria

Novartis Institutes for BioMedical Research, Oncology Research, 4002 Basel, Switzerland
carlos.garcia-echeverria@novartis.com

1 Introduction . . . 171

2 Insulin-like Growth Factor I Receptor . . . 172
2.1 Targeting the Extracellular Domain of IGF-IR with Humanized Monoclonal Antibodies . . . 173
2.2 Modulating IGF-IR Function with Kinase Inhibitors . . . 174

3 Phosphatidylinositol 3-Kinases . . . 178
3.1 Kinase Inhibitors of PI3K . . . 179

4 3-Phosphoinositide-dependent Protein Kinase-1 . . . 183
4.1 Kinase Inhibitors of PDK1 . . . 184

5 Protein Kinase B . . . 186
5.1 Kinase Inhibitors of PKB . . . 187

6 Mammalian Target of Rapamycin . . . 190
6.1 Inhibitors of mTOR . . . 190

7 Other Medicinal Chemistry Approaches to Block the Survival Pathway . . . 192
7.1 Phosphatidylinositol Analogues . . . 192
7.2 Inositol Polyphosphates . . . 194
7.3 Phospholipids . . . 194
7.4 Heat-Shock Protein Inhibitors . . . 195

8 PI3K/PKB Pathway Modulators with Unknown Mechanism of Action . . . 197

9 Conclusions and Outlook . . . 199

References . . . 200

Abstract The phosphatidylinositol-3-kinase/protein kinase (PI3K/PKB) signaling pathway—also known as the survival or anti-apoptotic pathway—plays an important role in controlling cell growth, proliferation and survival. Whatever the mechanism, the prevalence of PI3K/PKB signaling abnormalities in human cancer cells has suggested the potential use of PI3K/PKB pathway modulators as novel targeted therapeutic agents. Although often non-selective for the intended target, early PI3K/PKB inhibitors have been extensively used as molecular probes for improving our understanding of the biological processes associated with this pathway. A few of these early compounds, or closely related analogues, have provided proof-of-concept in preclinical settings and have entered clinical trials. This work covers recent salient achievements in the identification and development of PI3K/PKB pathway modulators, updating recent reports on this class of potential targeted anticancer drugs.

Keywords Hsp90 · IGF-IR · Inositol polyphosphate · mTor · PDK1 · PI3K · Phosphatidylinositol · Phospholipids · PKB

Abbreviations

Akt	protein kinase B
bid	bis in die, twice a day
COX-2	cyclooxygenase-2
EGFR	erbB-1, HER1, epidermal growth factor receptor
	erbB-2, Her2, Neu, epidermal growth factor related receptor 2
DNA-PK	DNA-dependent protein kinase
DPIEL	D-3-deoxy-phosphatidyl-*myo*-inositol ether lipid
FACS	fluorescence activated cell sorting
GLUT4	glucose transporter 4
GPCRs	G-protein-coupled receptors
GSK3	glycogen synthase kinase-3
HBE	human bronchial epithelial
Hsp	heat-shock protein
17-HWT	17-hydroxywortmannin
IGF	insulin-like growth factor
IGF-IR	insulin-like growth factor-I receptor
InsR	insulin receptor
i.p.	intraperitoneal
IRS-1	insulin receptor substrate-1
IRS-2	insulin receptor substrate-2
i.v.	intravenous administration
FKBP	FK-506-binding protein
MM	multiple myeloma
mTOR	mammalian target of rapamycin
NADH	nicotinamide adenine dinucleotide
NCI	National Cancer Institute
NSCLC	non-small cell lung cancer
PDK1	3-phosphoinositide-dependent protein kinase-1
PDK2	3-phosphoinositide-dependent protein kinase-2
PEG	poly(ethylene glycol)
PIAs	phosphatidylinositol analogues
PI3K	phosphatidylinositol-3-kinase
PKA	protein kinase A
PKB	protein kinase B
PKC	protein kinase C
PPP	picropodophyllin
po	per os (oral administration)
PtdIns(3,4,5)P3	phosphatidylinositol-3,4,5-triphosphate
PTEN	phosphatase and tensin homologue deleted on chromosome 10
s.c.	sub-cutaneous
SCID	mice, severe combined immunodeficient mice
TSC	tuberous sclerosis complex

1 Introduction

The phosphatidylinositol-3-kinase/protein kinase (PI3K/PKB) signaling pathway—also known as the survival or anti-apoptotic pathway—plays an important role in controlling cell growth, proliferation and survival. Whatever the mechanism, the prevalence of PI3K/PKB signaling abnormalities in human cancers and its potential biological effects (e.g., competitive growth advantage, evasion from apoptosis and therapy resistance) has suggested the potential use of PI3K/PKB pathway modulators as novel targeted therapeutic agents. Following this strategy, a number of compounds have demonstrated antitumor activity in preclinical and clinical settings by targeting directly or indirectly the different components of this pathway. This work covers recent salient medicinal chemistry achievements in the identification of these pathway modulators, updating recent reports on this class of potential cancer drugs [1–4]. To help the reader, each section of this piece of work starts with a brief background to understand the target rationale and then moves on to the lead discovery, drug optimization, and clinical results stages as appropriate. A schematic representation of signaling through this pathway and the pathway components covered herein are shown in Fig. 1.

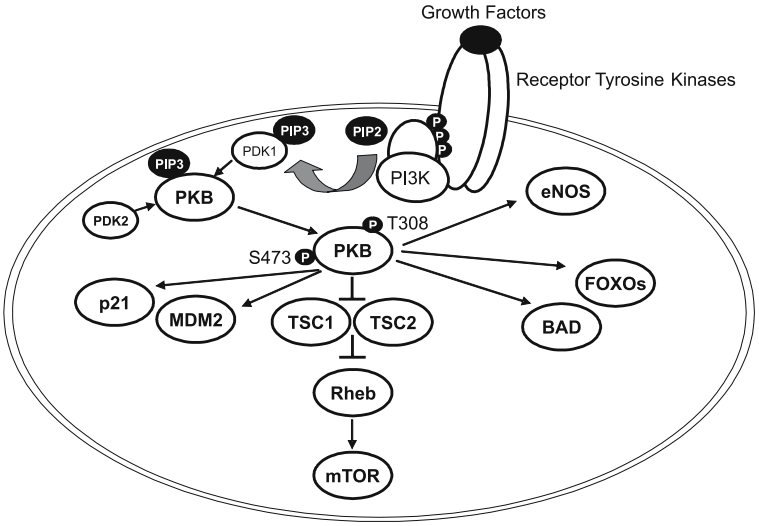


Fig. 1 Schematic representation of signaling through the PI3K/PKB pathway

2
Insulin-like Growth Factor I Receptor

The Insulin-like Growth Factor-I Receptor (IGF-IR) is a member of the insulin receptor family of tyrosine kinases. This transmembrane-spanning protein is composed of two α- and two β-subunits linked by disulfide bonds. While the α-subunits are extracellular, the β-subunits span the plasma membrane and encompass an intracellular tyrosine kinase domain devoted to the initiation of several signal transduction cascades. Signaling through IGF-IR is initiated upon binding of the cognate ligand—Insulin-like growth factor-I (IGF-I) or II (IGF-II) —to the extracellular domain of the receptor. It is thought that this peptide-protein interaction induces a conformational change that results in auto-transphosphorylation of each β-subunit at specific tyrosine residues within the intracellular kinase domain and outside the catalytic domain. Activation of the receptor triggers, through docking and/or phosphorylation of several transduction molecules (e.g., IRS-1, IRS-2 or Shc), results in activation of downstream signaling pathways, of which the Ras/Raf/MAPK pathway is primarily responsible for mitogenesis, and the survival PI3K/PKB pathway appears to play a major role in mediating the IGF-IR biological functions.

A growing body of evidence links IGF-IR activation and downstream signaling to human tumor biology. From an epidemiological point-of-view, up-regulated levels of IGF-IR, IGF-I or both have been documented in carcinomas of the lung, breast, thyroid, colon, and prostate [5], and, although contradictory among some studies, it is generally accepted that increased risk of solid tumors is associated with high levels of IGF-I in plasma [6–8].

Parallel to the preceding epidemiological findings, a broad range of experimental studies have revealed that IGF-IR function is implicated in most of the hallmarks of cancer, i.e., self sufficiency in growth signals, evasion from apoptosis, tissue invasion and metastasis, as well as angiogenesis [9–11]. From the preceding biological functions, it is probably the anti-apoptotic activity of the IGF-I/IGF-II/IGF-IR axis [12, 13] that makes IGF-IR an attractive therapeutic target in anticancer drug discovery. Activation of IGF-IR signaling has been shown to protect cancer cells from apoptosis induced by DNA damaging agents, targeted anticancer drugs, and radiation [14–16]. Conversely, inhibition of IGF-IR signaling by various approaches was reported to enhance the in vivo or in vitro sensitivity of selected cancer cells to radiation and antitumor agents [17–19]. Thus, the use of an IGF-IR modulator could be envisioned as a single agent for the treatment of IGF-IR dependent malignancies or in combination with established therapeutic modalities.

Among the different drug discovery approaches explored in the past few years to modulate IGF-IR function, two of them—antagonistic antibodies and small molecular mass kinase inhibitors—represent, at this point in time, the

most likely clinically viable options. These two approaches will hereafter be described and discussed in more detail.

2.1 Targeting the Extracellular Domain of IGF-IR with Humanized Monoclonal Antibodies

As in the case of other receptor tyrosine kinases like erbB1 and erbB2, efforts have been directed to identify and develop humanized antibodies that block the physical interaction between IGF-IR and its cognate ligands. Although obtained by applying different approaches, these antibodies have been shown in a variety of preclinical systems to specifically bind to the extracellular domain of IGF-IR, preventing downstream signaling.

On the basis of the current available information, the most advanced IGF-IR antibody seems to be CP-751,871, which is in Phase I clinical trials. This fully human IgG2 anti-IGF-IR antibody displays a high binding affinity for its target (K_d = 1.5 nM), potently blocks receptor autophosphorylation (IC_{50} = 0.42 nM), and shows selectivity toward human and monkeys, as opposed to rat, dog, rabbit, and marmoset. As observed also by other IGF-IR antibodies in cellular and in vivo settings, binding of CP-751,871 to its target induces internalization of IGF-IR and downregulation of the receptor at the plasma membrane. Upon intraperitoneal administration, the in vivo antitumor efficacy of CP-751,871 was demonstrated, as single or in combination studies with doxorubicin, 5-fluorouracil, or tamoxifen, in xenograft tumor models derived from cells transformed by the overexpression of human IGF-IR (3T3-IGF-IR), colorectal cancer cells (Colo205), or breast cancer cells (MCF-7). CP-751,871 is currently undergoing Phase I clinical trials in patients with multiple myeloma (relapse, refractory, or stable phase). Although no data have been disclosed yet concerning safety or responses, fluorescence activated cell sorting (FACS) analyses have shown that the antibody downregulates IGF-IR expression on multiple myeloma cell and granulocytes in samples collected from treated multiple myeloma patients.

Other representative antagonistic antibodies that have been described over the past few years are EM164 [20], IMC-A14 [21], and h7C10 [22].

In addition to the preceding biopharmaceuticals, a novel strategy, which uses a bispecific antibody to target simultaneously IGF-IR and Epidermal Growth Factor Receptor (EGFR), merits special attention. BsAb-IGF-IR-EGFR combines in a single antibody two previously identified neutralizing antibodies [23]. It binds to the extracellular domains of IGF-IR and EGFR blocking their activation and downstream signaling. Although in vivo activity has not been reported yet for this bispecific recombinant human antibody, this approach may expand the therapeutic application of engineered antibodies and represents an intriguing new twist in exploiting biopharmaceuticals for targeted anticancer therapy.

2.2 Modulating IGF-IR Function with Kinase Inhibitors

Parallel to the efforts directed to block the extracellular domain of IGF-IR with antibodies, drug discovery activities have also been aimed at modulating IGF-IR function by targeting its intracellular kinase domain. As shown in this section, several series of drug-like small molecules have been discovered and are currently being optimized.

A disproportional number of low-molecular mass inhibitors currently in preclinical studies and clinical trials are directed against the ATP-binding cleft of the targeted protein kinase. Initially, inhibition of protein kinases by ATP-site directed inhibitors was considered unlikely to result in selectivity due to the assumption that the ATP binding pocket of purine-binding enzymes is highly conserved. In this context, the identification of specific low-molecular mass inhibitors of IGF-IR kinase activity has proven to be a major challenge for medicinal chemistry due to the high sequence identity at the kinase domains of IGF-IR and InsR (around 84%) and, in particular, at the ATP-binding pocket [24]. Notably, the amino acids that line the ATP binding cleft of these two kinases are strictly conserved, and only two residues (Ala^{85} and His^{87} in InsR *versus* Thr and Arg in IGF-IR) that are close, but do not have a direct interaction with ATP are different. Additional confirmation for a high structural similarity was obtained when the X-ray structures of the recombinant kinase domains of IGF-IR and InsR in different activation forms were available [25–29]. On the basis of the high sequence identity and structural similarity, it would be reasonable to predict that the identification and development of selective ATP-site directed inhibitors for IGF-IR is an unachievable objective, but, for some compound classes, this assumption has proven to be wrong.

Potent and cellular selective IGF-IR kinase inhibitors have been reported for a new class of pyrrolo[2,3-*d*]pyrimidine derivatives. A representative example of this chemotype is NVP-ADW742 (compound **1**, Fig. 2) [30]. As expected from the high sequence and structural kinase identity of these two proteins, the compound is equipotent against IGF-IR and InsR enzyme activity in biochemical assays using monomeric recombinant kinase domains (IC_{50} = 0.14 μM and 0.12 μM, respectively), but, under similar experimental conditions, it shows around 16-fold selectivity for the IGF-IR (IC_{50} of 0.17 μM) *versus* InsR (IC_{50} of 2.8 μM) in model cellular autophosphorylation assays. The selectivity achieved at the cellular level with NVP-ADW742 and derivatives thereof suggest conformation differences between the native forms of IGF-IR and InsR—from the unactivated to the fully activated form—that can effectively be exploited for drug discovery. Although the resolution of the X-ray structures of the unactivated and activated IGF-IR and InsR full-length proteins has not been accomplished yet, we can imagine that the activation of these kinases must require complex conformation rearrange-

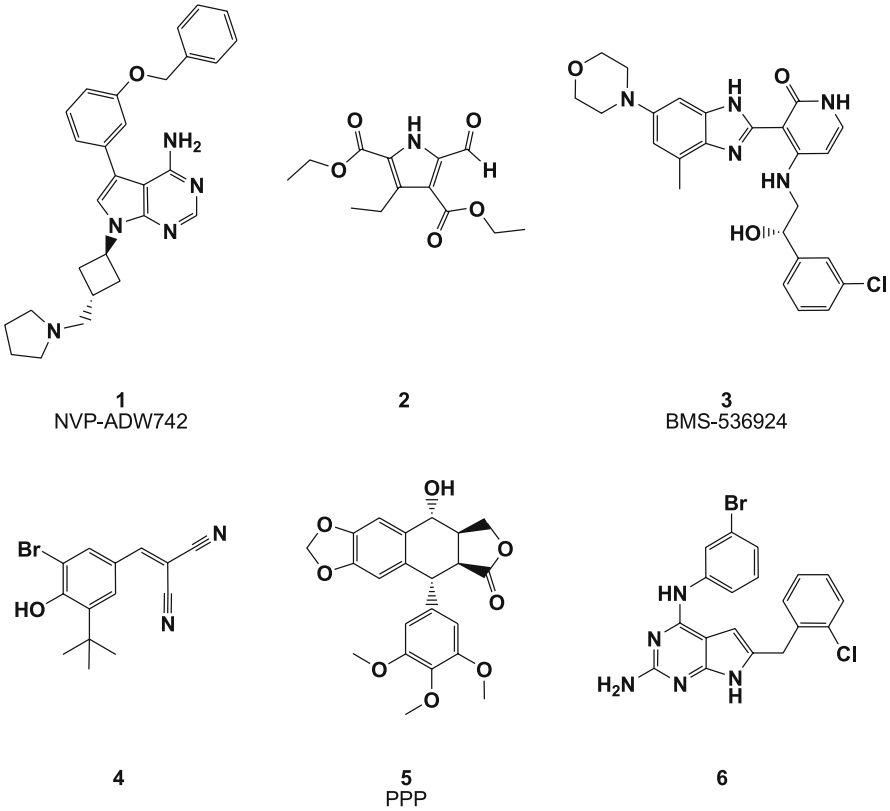


Fig. 2 Representative examples of kinase inhibitors of IGF-IR

ments that may result in relative dissimilarities in the conformational states and topography of their ATP-binding pockets. Along this line of thought, the X-ray structures of the unphosphorylated kinase domains of IGF-IR and InsR have revealed some differences in the ATP-binding pocket, particularly, in the nucleotide and activation loops. Interestingly, recent biochemical and structural studies have revealed that pyrrolo-5-carboxaldehyde derivatives (e.g., compound 2, Fig. 2) exhibited a greater, albeit modest, IGF-IR selectivity against InsR ($\sim$ 6 fold) when targeting the unphosphorylated form of the targeted enzyme [31].

Inhibition of IGF-IR autophosphorylation by NVP-ADW742 results in a plethora of pro-apoptotic molecular events that may account for its effectiveness as a single agent and in enhancing the antitumor activity of a broad spectrum of chemotherapeutic and anticancer targeted agents. Initial in vivo proof-of-concept of the potential therapeutic benefit of blocking IGF-IR kinase activity in tumor cells was obtained in an orthotopic multiple myeloma (MM) model of bone and bone marrow disease. In this mice model, MM

lesions are established after i.v. injection of luciferase-expressing human MM-1S cells, and tumor burden and its response to therapy is quantified by whole-body non-invasive bioluminescence imaging. The observed anatomic distribution of bone injuries (e.g., the axial skeleton and long bones) is consistent with the presentation of disease in human multiple myeloma patients. When used alone or in combination with cytotoxic agents (e.g., melphalan), NVP-ADW742 suppresses tumor growth and prolongs survival of mice without significant toxicity [30]. Additional preclinical experiments support the potential application of these inhibitors in combination with established antitumor modalities for the treatment of small cell lung cancers [32] or musculoskeletal tumors (e.g., Ewing's sarcoma) [33].

Recently, benzoimidazole derivatives have also been recently disclosed as ATP-site directed inhibitors of IGF-IR [34]. BMS-536924 (compound **3**, Fig. 2) is equipotent against IGF-IR and InsR (IC_{50} = 100 and 73 nM, respectively) in biochemical assay, but, as far as cellular mode of action is concerned, no cellular IGF-IR or InsR autophosphorylation inhibition has been disclosed yet. As expected from its mechanism of action, BMS-536924 blocks with good potency the proliferation of a series of tumor cell lines known to be dependent on IGF-IR mediated signaling (e.g., IC_{50} = 202 nM, RD1). The antiproliferative potency of BMS-536924 on tumor cell lines could be clearly delineated on the basis of down-regulation of the anti-apoptotic PI3K/PKB pathway. Treatment of mice (100/300 mpk qd or 50/100 mpk bid, po) bearing established tumors (e.g., IGF-IR Sal or Colo205) resulted in strong inhibition of tumor growth relative to a vehicle-treated control group. The animals treated at the efficacious doses (50/100 mpk bid, po) did not show a significant elevation in glucose levels at the end of the in vivo antitumor efficacy experiment, but upon glucose challenge (oral glucose tolerance test) a significant elevation in glucose levels was observed at the highest active dose (100 mpk bid, po).

Although less explored, drug discovery efforts have also been directed to modulate IGF-IR kinase activity by compounds that do not necessarily interact with the ATP-binding cleft. Initial attempts to inhibit IGF-IR enzymatic activity with non-ATP competitive inhibitors resulted in the identification of several tyrphostin-type compounds (e.g., compound **4**, Fig. 2) that showed weak activity in blocking IGF-IR autophosphorylation ($IC_{50} \approx 7-13$ μM), but some selectivity over InsR (4- to 8-fold) [35]. Confirmation of the possibility to identify potent and selective non-ATP competitive inhibitors of IGF-IR was obtained recently with a cyclolignan derivative, termed picropodophyllin (PPP; compound **5**, Fig. 2). In an initial report, it was demonstrated that PPP potently inhibited IGF-IR autophosphorylation (IC_{50} = 0.04 μM) in intact cells and was selective against a panel of other receptor tyrosine kinases, including InsR [36]. ATP-kinetic studies confirmed that the compound did not interfere with the IGF-IR tyrosine kinase activity at the level of the ATP binding site, suggesting an alternative mechanism of action. Additional efforts to

elucidate its kinase inhibitory mechanism showed that PPP interferes with the phosphorylation of Tyr-1136 in the activation loop of the kinase, while sparing the other two tyrosines (Tyr-1131 and Tyr-1135) [37]. As shown by X-ray crystallography, P-Tyr-1136 stabilizes the conformation of the activation loop, while P-Tyr-1131 and P-Tyr-1135 destabilize the autoinhibitory conformation of the activation loop. Without structural information it is difficult to understand how PPP blocks preferentially Tyr-1136 phosphorylation, but this effect has an interesting cellular outcome. Thus, PPP treatment of IGF-IR overexpressing cells results in a preferential inhibition on the PI3K/PKB pathway, as opposed to the MAPK pathway. We do not know how PPP's unusual mechanism of action may affect its antitumor activity and/or toxicity, but independent of this, the identification of PPP and the elucidation of its mechanism of action confirm that other therapeutic approaches besides ATP mimetics can be exploited for this challenging kinase.

As in the case of the pyrrolo[2,3-*d*]pyrimidines reported before, PPP has shown potent antiproliferative activity against a panel of multiple myeloma cell lines and freshly purified primary MM tumor cells when used alone or in combination with cytotoxic agents. This cellular activity was later confirmed in vivo using the 5T33MM mouse model [38]. Thus, PPP (ip, 20 mg/kg, bid) reduced the bone marrow burden and serum paraprotein concentration in the 5T33MM mice by 77% and 90%, respectively, compared to the control group. These effects resulted also in a significant increase in survival (10 days). Overall, the compound was well tolerated in the in vivo experiments and no increase in serum and glucose levels were observed.

As in the case of non-ATP competitive kinase inhibitors, the possibility to target the unphosphorylated form of a kinase and take advantage of the conformational differences that may occur in this state has been poorly investigated (vide supra, compound **2**, Fig. 2) [31]. In this context, it is of interest to note that the possibility to target the unphosphorylated form of IGF-IR has been explored using a continuous coupled spectrophotometric assay [39]. In this biochemical assay, production of ADP is coupled to the oxidation of NADH, which is measured as a decrease in absorbance at 340 nm. This screening approach led to the identification of a group of 6–5 ring-fused compounds (pyrrolo[2,3-*d*]pyrimidine and 2,4-diaminofuro[2,3-*d*]pyrimidines derivatives; e.g., compound **6**, Fig. 2) that showed some selectivity over InsR ($\approx$ 3-fold). Competition experiments with 5′-(β,γ-imido)triphosphate, which is a non-hydrolyzable ATP analog, showed that compound **6** is not a pure competitive inhibitor with respect to ATP, suggesting a complex mode of inhibition. No additional studies with this class of compounds have been reported yet.

In addition to preceding inhibitors, a number of patent specifications claim protein kinase inhibitors active against IGF-IR (e.g., 4-(pyrazol-3-ylamino)pyrimidines, imidazopyrazines, 2,4-pyrimidinediamines, pyrazolyl-pyrimidinyl-amino derivatives and indolesulfonamides). We can expect that

the diverse chemical scaffolds covered in these specifications may represent promising new lead compounds for the future generation of ATP-site directed IGF-IR kinase-selective inhibitors.

In terms of potential clinical proof-of-concept with this approach, INSM 18 (structure and biological activity not disclosed; Insmed Inc.) seems to be the only low-molecular mass IGF-IR kinase inhibitor to have entered clinical trials (http://www.insmed.com, press releases). INSM 18, which is also active against the human erbB2 receptor, has demonstrated antitumor activity in mice bearing breast, lung, pancreatic and prostate human xenografts. Insmed Inc. announced in October 2004 that it had initiated a Phase I clinical trial in patients with relapsed prostate cancer (unspecified route). No data from this study are available yet.

3 Phosphatidylinositol 3-Kinases

The phosphatidylinositol 3-kinases (PI3Ks) are widely expressed lipid kinases that phosphorylate phosphoinosites at the D-3 position of the inositol ring. These enzymes function as key signal transducers downstream of cell-surface receptors. The eight members of the PI3K family are grouped into three classes based on their primary amino acid sequence, in vitro substrate specificity, structure and mode of regulation [40]. The subject of this section is class I PI3Ks, which is composed of two subgroups, IA and IB.

Class I PI3Ks catalyzed the formation of phosphatidylinositol-3,4,5-triphosphate, PtdIns(3,4,5)P_3—also referred to as PIP3—a process that is reverted by the action of a phosphatase, phosphatase and tensin homologue deleted on chromosome 10 (PTEN).

Genetic aberrations within class I PI3Ks are common in human cancer. Thus, PI3KCA, which encodes the catalytic p110α, is amplified and overexpressed in some cancers of the uterine cervix and the ovary [41]. Somatic missense mutations have also been recently identified in a substantial fraction of solid tumors, in particular, colorectal cancers (74 of 199), glioblastomas (4 of 15), gastric cancer (4 of 12), breast cancer (1 of 12), and lung cancers (1 of 24) [42]. The mutations primarily result in single amino acid substitutions and most of them (> 85%) map to a few hot spots within the catalytic and helical domains. Functional analyses revealed that the three most frequently observed mutants of p110α—E542K, E545K and H1047R—increase lipid kinase activity and induce oncogenic transformation [43]. Parallel to these findings, a high frequency of coexistent PI3KCA/PTEN mutations ($\sim$ 26%, 66 samples) has been reported in endometrial carcinoma [44].

Other biological alterations can also affect the correct regulation of PIP3 signal transducers. Loss of the PTEN protein or function has been found

in a large fraction of advanced human cancer, including glioblastomas, endometrial, breast, thyroid, prostate cancer and melanoma [45–47]. In addition, germline mutation of PTEN also results in autosomal dominant cancer syndromes, and several related conditions (e.g., Bannayan–Riley–Ruvalcaba syndrome or Proteus syndrome) [45].

Overall, the preceding genetic aberrations suggests that they may play an important role in cancer pathogenesis, and provide strong support for the potential targeted therapeutic anticancer application of agents that inhibit the catalytic activity of wild-type or mutated class I PI3Ks [1, 2, 4, 48].

3.1 Kinase Inhibitors of PI3K

Two well known and isoform unselective PI3K inhibitors are the fungal metabolite wortmannin (compound **7**, Fig. 3) and LY294002 (compound **8**, Fig. 3). These two compounds have served as powerful research tools for more than a decade to elucidate the role of PI3Ks in human tumorigenesis and evaluate the potential utility of PI3K inhibitors as cancer therapeutics.

Wortmannin, which is a metabolite antibiotic originally isolated from *Penicillium wortmanni* [49], is an irreversible inhibitor that forms a covalent bond with a conserved lysine residue (Lys-802 of p110α and Lys-883 of p110γ) in the ATP-binding cleft of the lipid kinase [50]. Because of its mechanism of action, wortmannin inhibits PI3K enzymatic activity in the low nanomolar range ($IC_{50} \approx 2$ nM) [51, 52], and it is somewhat non-specific against other lipid and protein kinases (e.g., myosin light-chain kinase, $IC_{50} = 260$ nM; and polo-like kinase, $IC_{50} = 24$ nM) [53, 54].

LY294002 is an ATP-competitive PI3K inhibitor ($IC_{50} = 1.40\ \mu M$) that was identified from a medicinal chemistry optimization process using quercetin (compound **9**, Fig. 3) —a previously described PI3K inhibitor, $IC_{50} = 3.8\ \mu M$—as a model [55–58]. Additional studies have shown that LY294002 has a broad activity profile, inhibiting class I PI3K, PI3KC2β and 2γ, mammalian target of rapamycin (mTOR, casein kinase 2, and DNA-PK, all with IC_{50} values in the μM range) [59].

In addition to the crossover inhibition of other lipid and protein kinases, wortmannin and LY294002 suffer from unfavorable pharmaceutical properties and severe toxicity. For instance, the clinical use of wortmannin has been precluded due to its instability under physiological conditions—half-life of 8 min to 13 min—and acute liver and hematologic toxicity [60]. Despite the preceding drawbacks, these two compounds have been used broadly in the past few years and have provided proof-of-concept for the anticancer activity—at least for pan-PI3K inhibitors—in preclinical experimental studies [61]. Of special interest is the observation that wortmannin and LY294002 are cytostatic agents. Thus, a strong G1 arrest is observed upon incubation of these PI3K inhibitors with a variety of tumor cell lines, and induction of

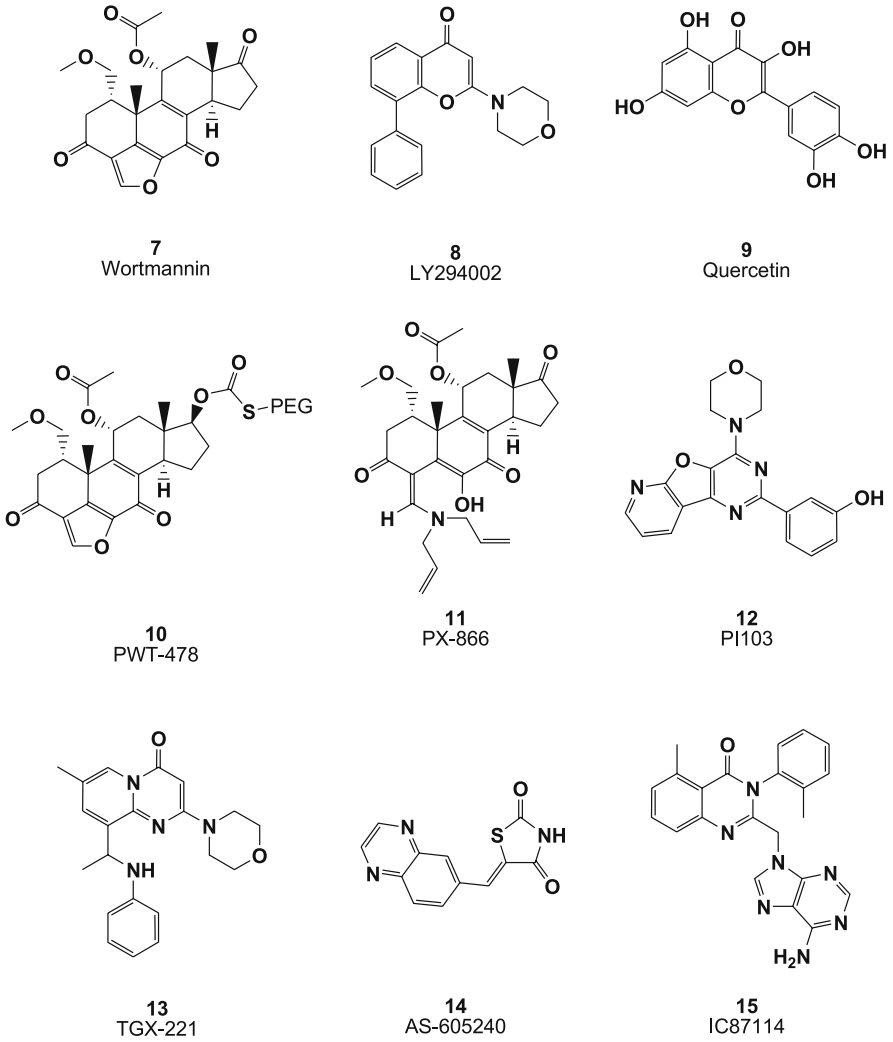


Fig. 3 Representative examples of kinase inhibitors of PI3K

apoptosis is only observed by combination with chemotherapeutic agents or radiation [62]. On the basis of these limited preclinical results, it seems that PI3K inhibitors may exhibit the most therapeutic anticancer value as components of combination regimens.

Using wortmannin as a model, broad-spectrum PI3K inhibitors with improved pharmaceutical properties and therapeutic indexes have been reported in recent publications. PWT-458 (compound **10**, Fig. 3), which is a pegylated derivative of wortmannin, was shown to inhibit PI3K signaling and hold a higher therapeutic index over its parent compound when used in sev-

eral human xenograft tumors (e.g., U87MG, A549 and A498) grown in nude mice [63]. Upon in vivo cleavage of its poly(ethyleneglycol) moiety, PWT-458 releases 17-hydroxywortmannin (17-HWT); efficacious i.v. doses of PWT-458 ranged from 0.5 mg/kg to 10 mg/Kg, achieving a superior therapeutic index over 17-HWT, its parent compound.

Another recent example of wortmannin-based optimization is PX-866 (compound **11**, Fig. 3), which was identified from a compound library of over 100 viridins. The selection criteria was based on its activity in the National Cancer Institute (NCI) human tumor cell line cytotoxicity assay ($IC_{50} \leq 2.2$ nM), and lack of liver toxicity. PX-866 potently inhibits PI3Kα, γ and δ (IC_{50} = 5.5, 9.0 and 2.7 nM, respectively), but unlike wortmannin, shows a certain level of selectivity over PI3Kβ ($IC_{50} > 300$ nM) and higher biological stability [60, 64]. The compound blocks activation of PKB in cellular settings (IC_{50} = 25 nM and 16.8 nM against A549 and HT-20 cells, respectively), and when given i.p. or i.v. to nude mice (6, 8, 9, or 12 mg/kg, qd), exhibited in vivo antitumor activity against s.c. OvCar-3, HT-29, or A-549 human tumor xenografts (T/C values in the 30 to 62 range). Moreover, PX-866 increases the in vivo antitumor effects of chemotherapeutic drugs (e.g., cisplatin), targeted anticancer agents (e.g., gefitinib) and radiation treatment. A major toxicity of PX-866 administration to SCID mice was hyperglycemia with decreased glucose tolerance. This effect, which can be ascribed to blocking insulin signaling through PI3K, was insensitive to the antihyperglycemic drug metformin but was reversed by insulin and the hypoglycemic drug pioglitazone, which is a peroxisome proliferator-activated receptor-γ activator. In addition to the effect on glucose, there was also a decreased gain in body weight and a significant increase in white blood cells. All of these changes were reversed upon cessation of treatment.

In addition to the efforts made to overcome the limitations encountered with wortmannin, high-throughput screening and medicinal chemistry activities have been directed to develop isoform—particularly PI3Kα—or pan-PI3K inhibitors, but the paucity of public information about isoform selective PI3K inhibitors is a clear indicator that this approach has proven to be a major challenge for medicinal chemists. Although often the isoform-activity data are not provided, a number of patent specifications describing pan-PI3K inhibitors including compounds that exhibit some selectivity for individual isoforms have been published in the past few years. Overall, the new generation of PI3K inhibitors have better pharmacological characteristics than the early inhibitors as well as improved PI3K selectivity profile. Some of these compounds are briefly reviewed in the following paragraphs.

Imidazopyrimidines and pyridofuropyrimidines with class I PI3K selectivity have been disclosed in several patent cases [65–67], and some biological data have been presented at scientific meetings [68–70]. For example, PI103 (compound **12**; Fig. 3) exhibited IC_{50} values of 1.5 nM, 3.0 nM, 3.0 nM, and 15 nM against p110α, p110β, p110δ, and p110γ, respectively, in biochemi-

cal assays [69]. Medicinal chemistry efforts to improve its specificity profile and pharmaceutical properties resulted in the identification of PI509 (structure not disclosed; IC_{50} = 4.5 nM, 37 nM, 19 nM, and 112 nM for p110α, β, δ, and γ, respectively) and PI540 (structure not disclosed; IC_{50} = 13 nM, 44 nM, 9 nM, and 321 nM for p110α, β, δ, and γ, respectively). These two compounds, which showed an excellent selectivity profile against a panel of 72 protein kinases, inhibit in a dose-dependent manner the phosphorylation of PKB in a cellular setting (IC_{50} = 12 nM and 10 nM for PI-509 and PI-540, respectively). In vivo antitumor activity (80% reduction in tumor growth) was observed when PI540 was administered i.p. (200 mg/kg qd or 100 mg/kg bid) to nude mice bearing U87MG subcutaneous xenografts [70].

Pyridopyrimidines and benzopyranones have been described as potent and selective PI3Kβ inhibitors [71, 72]. A representative example of this type of inhibitor is TGX-221 (compound **13**; Fig. 3), which showed an IC_{50} value of 5 nM against PI3Kβ, and exhibited good selectivity over the other PI3K isoforms: 20-fold against PI3Kδ and > 1000-fold over PI3Kα and γ. The main therapeutic utility for these PI3Kβ isoform selective inhibitors seems to be as antithrombotic agents [73, 74], and a compound, KN309 (structure not disclosed), was scheduled to enter Phase I clinical trials in 2005. No anticancer activity for these inhibitors has been reported yet.

In contrast to class IA PI3K members, PI3Kγ is mainly activated by seven-transmembrane G-protein-coupled receptors (GPCRs), through its regulatory subunit p101 and G-protein $\beta\gamma$ subunits. Potent and selective PI3Kγ inhibitors containing the thiazolidine-2,4-dione scaffold have been described in a recent publication [75]. AS-605240 (compound **14**, Fig. 3) is an ATP-competitive inhibitor that blocks PI3Kγ enzymatic activity (K_i = 7.8 nM) as well as PI3Kγ-mediated signaling and chemotaxis in vitro and in vivo. The compound is isoform selective with over 30-fold selectivity for PI3Kγ and β, and 7.5-fold selectivity over PI3Kα. This selectivity profile can be ascribed to key interactions with the ATP-binding pocket of the target enzyme. Analysis of the crystallographic structure of AS-605240 bound to the catalytic domain of PI3Kγ revealed that the negatively charged nitrogen of the thiazolidine-2,4-dione template forms a salt-bridge interaction with the side chain of Lys833, whereas the nitrogen peptide backbone of Val882 forms a hydrogen bond with the nitrogen of the quinoxyline ring of the inhibitor. In accordance with its biological profile, AS-605240 suppresses upon oral administration the progression of joint inflammation and damage in both lymphocyte-independent and dependent mouse models of rheumatoid arthritis. These results show the potential pharmacological application of PI3Kγ inhibition in the treatment of chronic inflammatory disorders, but no data are available yet on the potential use of AS-605240 or derivatives thereof in the treatment of cancers.

Novel quinazoline derivatives like IC87114 (compound **15**, Fig. 3) have been disclosed as inhibitors of p110δ with good selectivity over the other

three isoforms (IC_{50} = 0.5 μM, PI3Kδ, > 100 μM, PI3Kα, 75 μM, PI3Kβ, and 29 μM, PI3Kγ) [76]. In this context, it has been recently demonstrated that the p110δ isoform of PI3K is consistently expressed at a high level in blast cells from acute myeloid leukemia (AML), and the p110δ-selective inhibitor IC87114 is able to inhibit the proliferation of these malignant cells without affecting the proliferation of normal hematopoietic progenitor cells [77]. In addition to their potential therapeutic application in the treatment of cancers of hematopoietic origin, p110δ inhibitors may also be of interest in controlling breast cancer cell chemotaxis [78].

In addition to the preceding chemotypes, thiazoles, quinolin-2-ones and cycloalkanothieno-pyrimido-thiazoles [79], azolepyrimidines derivatives, trisubstituted pyrimidines, benzo[*b*]thiophenecarboxamides and benzofurancarboxamides [80–83], and substituted benzopyranones have also been claimed in different patents as PI3K inhibitors [1].

The identification and optimization of the preceding PI3K inhibitors has certainly benefited by having early access to structural information [84, 85]. X-ray crystal structures of the p110 subunit of PI3Kγ [86] have provided a detailed molecular map of the ATP-binding cleft of this special family of kinases. Sequence alignment and homology models have revealed that the residues forming the ATP-binding pocket are strictly conserved within the class I PI3K [59]; by contrast, some amino acid differences between class IA PI3K members and PI3Kγ exist at the entrance of the ATP cleft. Modulating and fine-tuning the interactions of the modified molecular scaffold with the amino acids that line the entrance to the ATP binding pocket of the different PI3K isoforms may provide an opportunity to obtain spectrum-selective PI3K inhibitors [75, 76].

Parallel to the current synthetic efforts to identify and develop isoform selective PI3K inhibitors or compounds that target PI3K mutants, studies with small interfering RNAs (siRNAs) are expected to improve our knowledge of the degree of selectivity that may be needed to have an antitumor response and therapeutic index, as well as the degree of knockdown of target activity required.

4
3-Phosphoinositide-dependent Protein Kinase-1

The 3-phosphoinositide-dependent protein kinase-1 (PDK1) is a 556-amino acid enzyme composed of three well-differentiated motifs: an *N*-terminal domain, a constitutively activated serine/threonine kinase domain, and a Pleckstrin homology (PH) domain at its C-terminus [87–91]. The attractiveness of PDK1 as a potential anticancer target is linked to its ability to control the activity of a diverse set of AGC kinase members, in particular the three PKB isoforms [92]. Full activation of PKB requires phosphorylation at two sites,

one within the activation loop (e.g., Thr-308 for PKBα), and one within the C-terminus (e.g., Ser-473 for PKBα). Phosphorylation of the critical and conserved threonine residue in the activation loop of the three PKB isoforms is carried out by PDK1 at the plasma membrane [93–97]. In addition to PKB, other members of the AGC kinase subfamily like p70 ribosomal S6 kinase, serum- and glucocorticoid-induced protein kinase and protein kinase C (PKC) are phosphorylated by this promiscuous kinase [92, 98, 99].

4.1 Kinase Inhibitors of PDK1

Contrary to the other major components of the PI3K/PKB survival pathway, the development of PDK1 inhibitors would be, to a certain extent, simpler as only a single PDK1 isoform exists in human cells. Moreover, the observation that PDK1 hypomorphic mice expressing only approximately 10% of the normal level of PDK1 display no obvious harmful phenotype—they are 40–50% smaller than control animals — [100], indicates that a PDK1 inhibitor may provide effective anticancer therapy at an acceptable therapeutic index.

The most potent PDK1 kinase inhibitor reported to date is UCN-01 (compound **16**; Fig. 4; IC_{50} = 6 to 33 nM) [101], a staurosporine analogue isolated from the culture broth of *Streptomyces* sp. Originally developed as an inhibitor of calcium-dependent PKC, UCN-01 has the capacity to inhibit a broad spectrum of kinases [102], including other members of the AGC subfamily of kinases (e.g., IC_{50} = 491 nM for PKB) [103]. UCN-01-induced PDK1 inhibition has also been observed in in vivo murine and human tumor xenografts [101].

UCN-01 is currently being explored in cancer patients in Phase I/II clinical trials, both as a single agent and in combination with conventional chemotherapeutic drugs (e.g., cytarabine, topotecan). In this last scenario, results from a Phase I clinical trial in combination with topotecan showed antitumor activity in 12 patients with advanced solid tumor with one partial response and three cases of stable disease. However, and due to the low selectivity of UCN-01, it is unclear which inhibited kinase(s) are suppressing growth and survival of cancer cells in these clinical studies.

In addition to pulmonary toxicity, nausea/vomiting, lactic acidosis and transaminitis, UCN-01 induced insulin resistance during Phase I clinical trials. As shown recently with rat adipose cells, this effect may be due to UCN-01 inhibition of PKB Thr-308 phosphorylation—no effect on Ser-473 was observed in this study—and subsequent blockade of GLUT4 translocation in response to insulin [104]. If this mode of action is confirmed in the ongoing clinical trials and contrary to what was observed in the PDK1 hypomorphic mice (vide supra) [100], insulin resistance may represent an important hurdle in the development of PDK1 inhibitors and, in general, of any agent that blocks the PI3K/PKB pathway in adipose and muscle cells.

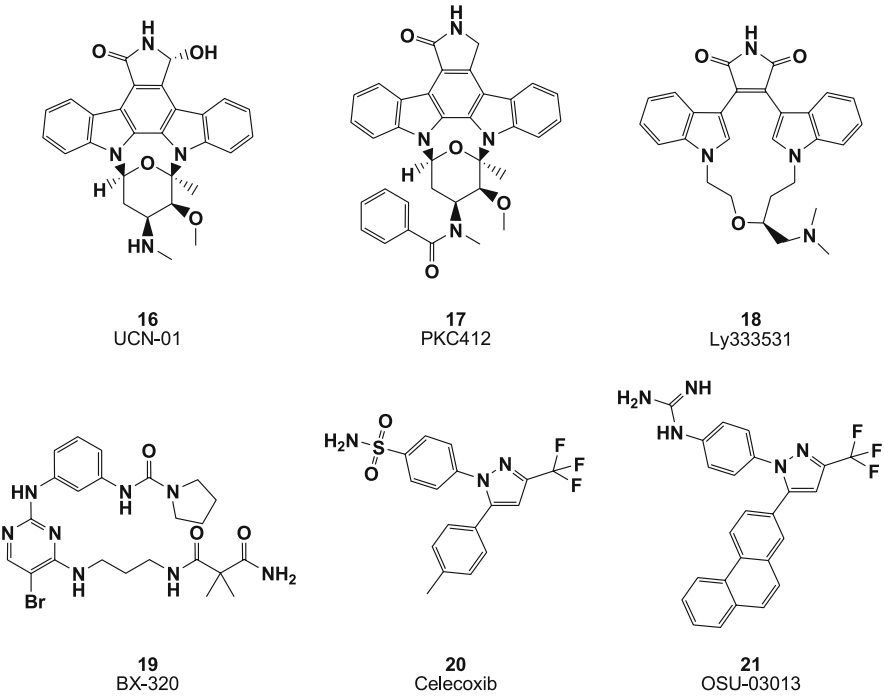


Fig. 4 Representative examples of kinase inhibitors of PDK1

In addition to UCN-01 and other staurosporine or maleimide derivatives (e.g., compounds **17** and **18**; Fig. 4) [102, 103, 105–107], PDK1 kinase activity is inhibited by aminopyrimidines. A representative example of this compound class is BX-320 (compound **19**, Fig. 4), a PDK1 inhibitor (IC_{50} = 30 nM) that displays good selectivity over protein kinase A (PKA, 35-fold) [108]. BX-320 blocks the growth in soft agar of a wide range of tumor cell lines (IC_{50} = 0.093 to 1.32 μM), and shows efficacy in a metastasis mouse model (200 mg/kg bid).

Although other signaling targets cannot be excluded, it has been found that celecoxib (compound **20**, Fig. 4), which is a cyclooxygenase-2 (COX-2) inhibitor, can block the activation of PKB [109] in a variety of cancer cells by inhibiting PDK1 activity [110]. In an immunoprecipitated assay, the compound inhibited in a dose-dependent manner the kinase activity of PDK1 with an IC_{50} value of 3.5 μM. However, weaker inhibitory activity (IC_{50} = 30 μM) has been obtained when celecoxib was tested in another cell-free assay using the recombinant PDK1 protein [111]. Interestingly, celecoxib inhibits PDK1 by competing with ATP for binding, and on the basis of this result and using a structure-based design optimization strategy, celecoxib derivatives (e.g., OSU-03013, compound **21**; Fig. 4) with slightly improved antiproliferative activity (IC_{50} = 3 μM) were identified [111].

Epidemiological studies have suggested an inverse association between non-steroidal anti-inflammatory drugs treatment and risk for certain type of cancers, in particular breast tumors. Moreover, preclinical experiments showed that celecoxib could induce apoptosis in cancer cells in in vitro and in vivo settings [112, 113]. The preceding epidemiological and preclinical findings prompted the clinical evaluation of celecoxib for the treatment of human cancers. Celecoxib is currently being investigated in Phase III clinical studies as a therapy for bladder and colorectal cancers, and in Phase II for prostate cancer. Parallel to these studies, additional Phase II clinical trials are also investigating the use of celecoxib in combination with paclitaxel and carboplatin in the treatment of patients with non-small cell lung cancer, and as a single agent in the prevention of skin cancer in patients with actinic keratoses. The compound has received marketing approval in several countries for the treatment of familial adenomatous polyposis. As for other indications, the potential cardiovascular risks of COX-2 inhibitors may limit the use of celecoxib in cancer patients.

Initial insights into how PDK1 kinase selectivity might be designed into ATP-competitive inhibitors have been provided by the solving of the X-ray co-crystal structures of PKA, PKB, and PDK1 bound to ATP, ATP analogs, or kinase inhibitors [102, 114–116]. As expected from the high sequence identity between the members of the AGC subfamily of kinases, the structural studies have shown that the kinase domain of PDK1 presents a high structural similarity with the one of PKA [116, 117]. As shown in the previous paragraphs, it is therefore not a surprise that, thus far, no sufficiently specific inhibitor of PDK1 has been reported.

In addition to the preceding chemotypes, a limited number of patent specifications include PDK1 as a target for the inhibitors exemplified in the cases. Thus, 5-aminocarbonylindazoles, pyrazolopyrimidines, triazolo[1,5-*a*]-pyrimidines, pyrazolopyrroles and phthalimides have been claimed to be active against PDK1 [1], but further biological data are awaited.

5 Protein Kinase B

Protein kinase B (PKB), which is also known as Akt, is a serine/threonine kinase that also belongs to the AGC kinase subtype. Three mammalian isoforms—PKBα, β, and γ have been identified. These proteins are broadly expressed and, although isoform-specific patterns of expression exists in some tissues, the three kinases have a similar organizational structure: an amino-terminal PH domain, a central serine/threonine catalytic domain, and a short regulatory region at the carboxy terminal end containing the so-called hydrophobic motif [118–120].

The PKB enzymes catalyze the phosphorylation of serine/threonine residues at consensus phosphorylation sites—ArgXxxArgXxxXxxSer/Thr—in molecular targets, including proteins with key roles in the regulation of cell growth and survival. PKB is downstream of PI3K and is a critical node in this signal pathway. To be fully activated, PKB requires translocation to the plasma membrane and its phosphorylation by other kinases at two key regulatory residues, Thr308 and Ser473 (numbering for PKBα) [97, 121–124]. The threonine residue is located in the T-loop, also known as the activation-loop, whilst the serine amino acid is C-terminal to the catalytic domain in the hydrophobic regulatory region of the protein. Following growth factor stimulation of PI3K, PKB is recruited at the plasma membrane, an event accomplished by physical, direct interaction between its PH domain and the generated PtdIns(3,4,5)P_3 molecules (see Sect. 3). Co-localization of PDK1 at the plasma membrane allows the phosphorylation of Thr308 by this enzyme, which is necessary and sufficient for PKB activation [90, 96, 121–123, 125]. However, maximal enzymatic activation requires phosphorylation of Ser473. The molecular identity/ies of the kinase/s, often termed as PDK2, involved in the phosphorylation of Ser473 is still controversial and has been hypothesized, among other proteins, to be DNA-PK [126] or the mTOR/rictor complex [127].

PKB isoforms have been found to be overexpressed in a variety of human tumors, and, at the genomic level, to be amplified in gastric adenocarcinomas (PKBα), ovarian (PKBβ), pancreatic (PKBβ), and breast (PKBβ) cancers [128, 129]. In addition, many tumor cells display elevated levels of PI3K products as a result of deletion/mutations of PTEN, activation of Ras or expression of autocrine growth factors (vide supra). The biological consequences of uncontrolled PKB activation in tumor cells are critical for inducing, among other effects, a survival signal that allows them to withstand apoptotic stimuli. Thus, PKB protects tumor cells from death by phosphorylating and inactivating components of the intrinsic apoptosis pathway [130].

5.1 Kinase Inhibitors of PKB

Non-selective PKB inhibitors have been extensively used as tool compounds to elucidate the role of this kinase in the biology of human cancers. Thus, PKB isoforms are potently inhibited by promiscuous kinase inhibitors like staurosporine (compound **22**, Fig. 5; IC_{50} = 48 to 11 nM for PKBα) [131–133] and derivatives thereof (e.g., compound **23**, Fig. 5) [134]. Another example of a potent and non-selective pan-PKB inhibitor is Ro-31-8220 (compound **24**, Fig. 5), a PKC inhibitor (IC_{50} = 10 nM) [135] that also interferes with PKB kinase activity (IC_{50} = 240 nM for PKBα).

Initial medicinal chemistry attempts to improve PKB kinase inhibitory selective exploited a previously described PKA inhibitor (compound **25**, Fig. 5;

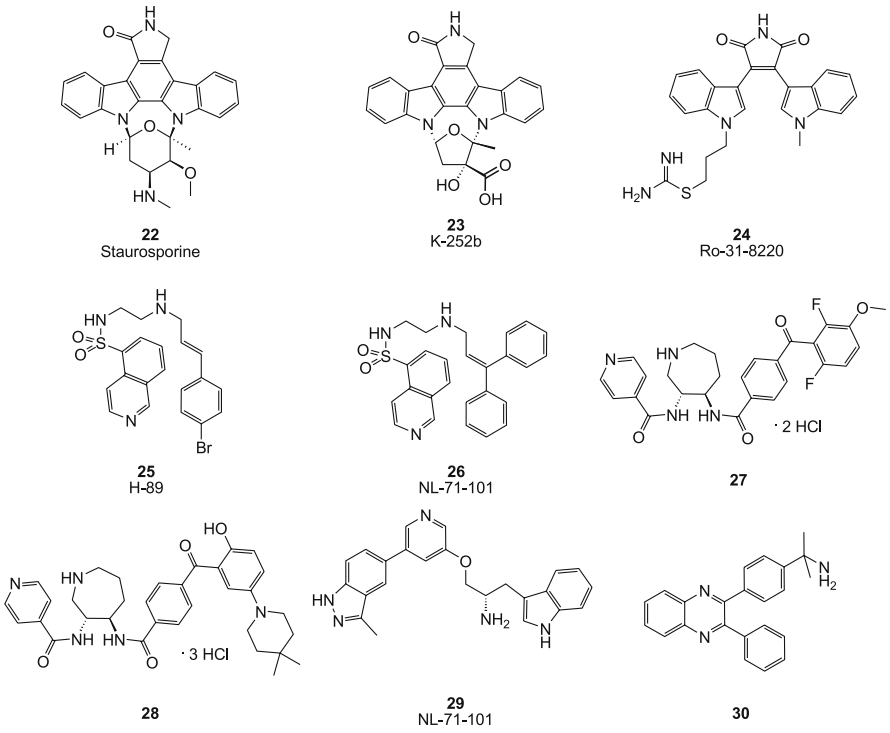


Fig. 5 Representative examples of kinase inhibitors of PKB

K_i = 48 nM for PKA) [136], as a starting point in the optimization process [137]. Obtained by parallel synthesis, NL-71-101 (compound **26**, Fig. 5) inhibits PKB enzymatic activity in vitro (IC_{50} = 3.7 μM), blocks GSK3β phosphorylation in intact cells ($IC_{50} \approx 25$ μM), and causes programmed cell death in tumor cells (e.g., OVCAR-3), albeit at high concentrations ($ED_{50} \leq$ 100 μM). In terms of its in vitro selectivity profile, NL-71-101 is 2-fold less active against PKA and PKC (IC_{50} = 9–11 μM) and inactive against PKC and p38 ($IC_{50} \geq 100$ μM).

Exploiting the X-ray crystal structure of (–)-balanol, which is a potent inhibitor of AGC-kinases, in complex with PKA, medicinal chemistry efforts have been directed to impart PKB selectivity and improve its pharmaceutical properties [138–141]. Starting with compound **27** (Fig. 5) as a lead compound (IC_{50} = 5 nM for PKBα and PKA), a new series of potent PKB inhibitors (e.g., compound **28**, Fig. 5; IC_{50} = 20 nM for PKB versus IC_{50} = 1900 nM for PKA) were obtained by exploiting a binding site in PKB that is narrower in PKA. The X-ray structure of compound **28** bound to PKA shows that the piperidine moiety of the inhibitor adopts an energetically unfavorable envelop conformation to avoid a steric clash with the side chain of phenylalanine-187; these negative interactions do not occur in the modeled complex of the inhibitor

bound to PKB; in this protein, the amino acid at position 187 is leucine and not phenylalanine. The activity of these azepane derivatives in cellular settings or in vivo models has not been reported yet.

Promising PKB inhibitors have also been obtained from indazole-pyridine-based derivatives [142]. These new inhibitors, exemplified by A-443654 (compound **29**, Fig. 5; K_i = 160 pM), are reversible, ATP competitive inhibitors able to decrease the phosphorylation of PKB downstream targets in cells (e.g., GSK3α/β, FOXO3, TSC2, and mTOR) and in vivo in a dose-dependent manner. Interestingly, and as reported for other PKB-inhibitors, the preceding biological effect is associated with a concomitant increase in the Thr-308 and Ser-473 phosphorylation of PKB. This effect is blocked if PI3K inhibitors are co-administered.

Synergy was observed when A-443654 was combined with doxorubicin or camptothecin [143]. In animal experiments, the compounds showed antitumor activity as single agents and in combination regimens in a number of tumor xenografts, but the dosing period is limited due to malaise and weight loss. Although further studies with other chemotypes will be required to assess the generality of these in vivo findings, the side effects observed in animal models raise concerns that the therapeutic application of PKB inhibitors will be limited by mechanism-based metabolic toxicities.

In addition to the preceding kinase inhibitors, a number of patents describe compounds that are claimed to be active against PKB (e.g., diamino triazines, diaminopyrimidines or 5-aminocarbonylindazoles) [1, 144]. Although in general no kinase inhibitory data are provided in these patents, the diverse chemical scaffolds covered in these specifications may represent promising new chemotypes for the future generation of PKB kinase-selective inhibitors.

If the identification of potent and selective PKB kinase inhibitors has proven to be a difficult task, the design of PKB isoform selective modulators represents a higher medicinal chemistry challenge. The kinase domain of PKBα has a sequence identity of 90% and 87% with PKBβ and PKBγ, respectively. Notably, the amino acids that line the ATP binding cleft of PKBα are strictly conserved in PKBβ, and only one amino acid is different in PKBγ alanine in PKBα/β *versus* valine in PKBγ). A detailed molecular map of the ATP-binding pocket of these kinases has been obtained recently by solving the X-ray crystal structure of ΔPH-PKBβ with adenyl-imidodiphosphate tetralithium salt (AMP-PNP) and a GSK3β-derived peptide [114, 144, 145], but the structure-based design of PKB isoform selective inhibitors will probably require access to the structures of the full PKB isoforms. Amino acids away from the ATP binding pocket or the interaction of the kinase domain with other parts of the protein may alter the size and shape of the ATP-binding cleft and hence the binding affinity and selectivity of an ATP-competitive or allosteric inhibitor.

Eventually, other pockets besides the ATP-binding cleft can be exploited for the identification and development of PKB kinase modulators. Thus,

allosteric inhibitors of PKB containing the 2,3-diphenylquinoxaline or 5,6-diphenyl-pyrazin-2(1H)-one scaffolds have been described in recent publications [134, 146–149]. A high-throughput homogenous time-resolved fluorescence kinase assay was used to screen around 270 000 compounds for their ability to inhibit PKB enzymatic activity. The inhibitors identified exhibit a linear mixed-type inhibition against ATP and peptide substrate, show isozyme selectivity, and are only active against the full length protein (e.g., compound **30**, Fig. 5; IC_{50} = 2.7 μM for PKBα versus IC_{50} > 250 μM for ΔPH-PKBα). Although the mechanism of inhibition by these compounds has not been fully elucidated and there is not structural information yet, it seems that these molecules may bind outside the ATP-binding pocket, interacting with the PH domain and/or hinge region and probably promoting the formation of an inactive conformation. Interestingly, incubation of tumor cells with these allosteric inhibitors results in significant reduction of the phosphorylation of both Thr-308 and Ser473, which is something unexpected for a "classical" PKB kinase inhibitor. This interesting work not only illustrates alternative ways to block kinase activity, but shows that this is probably the way to go when targeting an isoenzyme or a member of a closely related kinase family.

6
Mammalian Target of Rapamycin

The mammalian target of rapamycin (mTOR) is a 290-KDa serine-threonine kinase that regulates both cell growth and cell-cycle progression. This protein is downstream of the PI3K/PKB pathway and recent studies have established a biological route from PKB to mTOR. In its unphosphorylated form, tuberous sclerosis complex (TSC) 2 is bound to TSC1 in a complex that blocks mTOR activation. Phosphorylation of TSC2 by PKB disrupts the TSC1/TSC2 complex, allowing the activation of mTOR by Ras homolog enriched in brain (Rheb). mTOR activation leads to the phosphorylation of several downstream signaling effectors and transcription factors that influence cell proliferation, angiogenesis and survival [150].

6.1
Inhibitors of mTOR

Extensive preclinical studies have shown that sensitivity to mTOR inhibition may correlate with aberrant activation of the PI3K pathway or loss of functional tuberous sclerosis complex (TSC), as occurred in patients with tuberous sclerosis syndrome [151, 152]. Rapamycin (compound **31**, Fig. 6), which was the first compound shown to inhibit mTOR kinase activity, is an approved drug for prevention of allograft rejection. Rapamycin is a macrocyclic

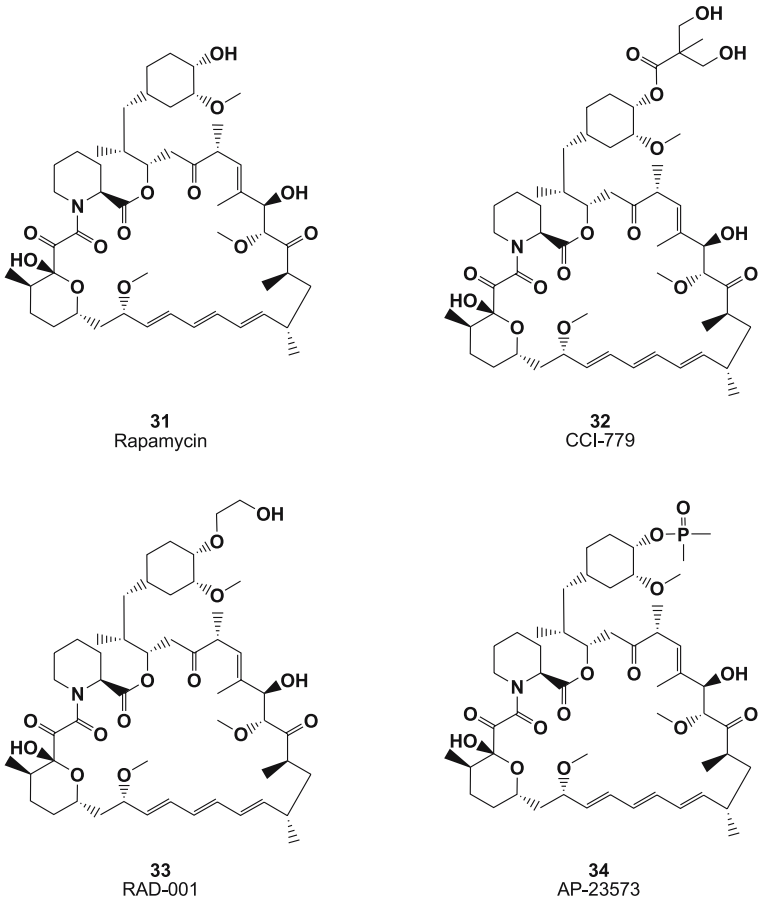


Fig. 6 Representative examples of mTOR inhibitors

triene antibiotic produced by *Streptomyces hygroscopicus*, a streptomycete that was isolated from a soil sample collected from Easter Island (Rapa Nui). The mechanism of action of rapamycin differs from that of other kinase inhibitors. The natural product initially forms a complex with the FKBP-25 cellular receptor. This complex, in turn, binds to a 133 amino acid hydrophobic FKBP:RAPA binding domain located immediately upstream of the kinase sequence. The formation of this complex interferes with the kinase activity of mTOR, but it does not inhibit all the functions of mTOR, nor do they inhibit the mTOR/rictor complex [153, 154]. In fact, mTOR inhibition by rapamycin and derivatives thereof can result in activation of PKB [155]. This intriguing effect suggests that targeting upstream of mTOR in the survival pathway might be required to interrupt feedback loops and achieve optimal therapeutic activity in cancer cells.

Currently, three rapamycin derivatives—CCI-779 (compound **32**, Fig. 6), RAD-001 (compound **33**, Fig. 6), and AP-23573 (compound **34**, Fig. 6)—are being evaluated in cancer clinical trials. All these mTOR inhibitors have shown potent cytostatic activity in cellular settings and in vivo antitumor activity in a variety of hematological and solid tumor preclinical models as single agents and in combination with standard cancer therapeutics, targeted anticancer agents, and radiation [2, 151, 156]. It is important to mention that CCI-779 and RAD-001 are pro-drugs of rapamycin, while stability and in vitro studies along with in vitro metabolism studies have shown that AP-23573 is not.

Recent review papers have covered in detail the available clinical results with the mTOR inhibitors [151, 156]. Overall, the compounds are well tolerated and may induce prolonged stable disease and increase time to progression in a subset of cancer patients. In particular, promising activity has been reported for CCI-779 in patients with mantle cell non-Hodgkin's lymphoma [151].

7
Other Medicinal Chemistry Approaches to Block the Survival Pathway

Although much of the drug discovery efforts have been directed to modulate the enzymatic activity of the different components of the survival pathway, other therapeutic modes have been successfully explored for pathway interruption. Some of these alternative medicinal chemistry approaches are briefly reviewed in this section.

7.1
Phosphatidylinositol Analogues

Phosphatidylinositol lipid analogues, which are structurally similar to the products of PI3Ks, have been designed and synthesized to interact with PH domains and disrupt the activation of the PI3K/PKB pathway in tumor cells. A representative example of this class of inhibitors is D-3-deoxyphosphatidyl-*myo*-inositol ether lipid (DPIEL, compound **35**, Fig. 7) [157]. This compound specifically binds to the PH domain of PKB, blocking the translocation of this protein from the cytoplasm to the plasma membrane and thus preventing PKB phosphorylation and activation ($IC_{50} = 1.5 \pm 0.3\ \mu M$). DPIEL inhibits the proliferation of MCF-7 and HT29 tumor cell lines with IC_{50} values of 7.2 and 2.1 μM, respectively. Replacement of the phosphate linkage of DPIEL with a carbamate group or varying the nature of the lipid groups on the diacylglycerol-like side chain of DPIEL resulted in derivatives that were less potent than the parent compound at inhibiting PKB in cells (18- to 8-fold) and also less specific for the PH domain of PKB. In addition

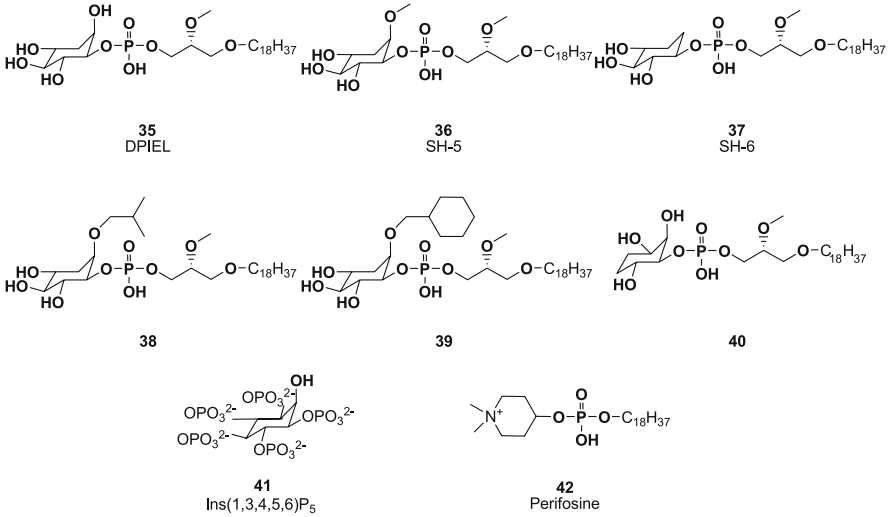


Fig. 7 Other modulators of the PI3K/PKB pathway

to its modest cellular activity, further development of DPIEL was hampered by its poor in vivo profile. Thus, oral administration of DPIEL resulted in low bioavailability due to acid lability, and i.v. administration resulted in massive hemolysis and animal death [158].

In addition to DPIEL, other phosphatidylinositol analogues (PIAs) (e.g., SH-5 and SH-6, compounds **36** and **37**, respectively, Fig. 7) have been recently reported [159, 160]. When used at 5 or 10 μM, SH-5 and SH-6, which are supposed to be more resistant to phosphatidylinositol-specific phospholipase C mediated degradation [161], effectively block the phosphorylation and activation of PKB in HL60AR tumor cells, and sensitize this and other leukemic tumor cell lines to the effects of etoposide and cytarabine. No effect on the survival rate of hematopoetic precursor cells was observed if the compounds are used at 5 μM.

To improve metabolic stability and inhibitory potency, the inositol ring of the phosphatidyl-*myo*-inositol scaffold has also been the subject of extensive chemical modifications [162]. Exploiting a model of the interaction of PtdIns(3,4,5)P_3 with the PH domain of PKB, selected substituents were introduced at individual sites of the inositol ring to maximize hydrogen-bonding or polar interactions between the modified PIA and the target protein. These modifications resulted in the identification of new PIAs (e.g., compounds **38–40**, Fig. 7) that inhibited the activation of PKB in H1703 cells with IC_{50} values around 2 to 4 μM. As for other PIAs, the cellular effect on PKB phosphorylation was not due to inhibition of upstream kinases. Interestingly, induced programmed cell death was observed when tumor cell lines with high PKB activity were incubated with these compounds ($c = 10$ μM) for

24 h. No information about the in vivo activities of these PIAs is available yet.

7.2 Inositol Polyphosphates

Inositol and its phosphorylated forms have been exploited to antagonize the activation of the PI3K-pathway by competing with the binding of $PtdIns(3,4,5)_3$ to PH domains [163]. A representative example of this compound class is $Ins(1,3,4,5,6)P_5$ (compound **41**, Fig. 7) [164] (for other examples see also [165]). This highly negatively charged molecule is able to inhibit PKB kinase activity in vitro (43% at 50 μM), and to induce apoptosis in ovarian, lung, and breast cancer cell lines (e.g., 40% at 100 μM using SCL-H69 cells). In a cellular setting, $Ins(1,3,4,5,6)P_5$ ($c = 50$ μM) sensitizes cancer cells to the apoptotic effect of a variety of chemotherapeutic agents (e.g., paclitaxel). Furthermore, the compound (50 mg/kg/day; ip) blocks the growth of SKOV-3 xenograft implanted s.c. in nude mice without signs of toxicity, as judged by parallel monitoring body weight [166]. *Ex-vivo* analyses of tumor tissue showed that PKB phosphorylation at Ser473 and Thr308 was inhibited after 12 days of treatment. Contrary to what one could expect, $Ins(1,3,4,5,6)P_5$ is rapidly and efficiently internalized by cells and is only minimally converted into different metabolites.

Interestingly, other inositol polyphosphates (e.g., $Ins(1,2,3,4,5,6)P_6$) have little or no effect in the preceding biological assays. This specificity can be ascribed to key interactions of $Ins(1,3,4,5,6)P_5$ with the PH domain of PKB; in particular, the presence of the phosphate group at position 1 and the free OH group at position 2 [167, 168].

7.3 Phospholipids

This compound class has been exploited in recent years to modulate membrane function and signaling targets that use naturally occurring lipid moieties as substrates or co-factors. Several publications have shown that perifosine (NSC 639966, compound **42**, Fig. 7), which is an orally active analogue of alkylphosphocholine, blocks the activation and phosphorylation of PKB in cellular settings [169, 170]. Although the mechanism of action of perifosine is not fully understood, one hypothesis is that, following insertion into the cellular membrane, perifosine interferes with PKB membrane localization by inhibiting the association of its PH domain with $PtIns(3,4,5)P_3$. Interference with PKB plasma membrane localization has been demonstrated [171] using immunofluorescence imaging [169]. Perifosine has exhibited broad antineoplastic activity in preclinical studies. Of special interest is the synergistic antiproliferative effect observed when perifosine was used in combination

with UCN-01 in cell culture assays with PC3 and A459 tumor cells [172], and with the anti-EGF receptor antibody cetuximab in PTEN-deficient cancer cells [173]. Blocking simultaneously distinct components of the PI3K/PKB pathway seems to enhance the inhibition of phosphorylation of PKB along with activation of the apoptotic pathway.

Perifosine is currently undergoing Phase II clinical trials. These studies are conducted by the NCI for prostate, head and neck, breast and pancreatic cancers, as well as melanomas and sarcomas. Encouraging evidence of antitumor activity as a single agent or in combination with radiation was reported in Phase I clinical trials, in particular, in the treatment of non-small cell lung cancer patients. However, no significant clinical activity was observed in Phase II clinical studies in previously untreated patients with metastatic melanoma [174], and in patients with progressive, metastatic androgen-independent prostate cancer [175].

7.4 Heat-Shock Protein Inhibitors

The 90 kDa heat-shock proteins (Hsp90s) are ATP-dependent molecular chaperones involved in the folding and stability of a selected range of substrates, the so-called "client proteins" [176]. The Hsp90 family of chaperones is composed of four isoforms: Hsp90α, Hsp90β, GRP94, and TRAP-1. The Hsp90 chaperone binds to the "client protein" in the presence of other partner proteins to produce a multiprotein complex that folds the target substrate into its biologically active conformation. Binding and release of Hsp90 "client proteins" is regulated by the activity of the N-terminal ATPase domain, which binds and hydrolyzes ATP to mediate a series of association-dissociation cycles between Hsp90 and its substrate.

Heat-shock proteins are believed to be involved in dealing with the cellular stress associated with the hostile cancer environment, as well as being essential for the proper function of key oncogenic proteins. Many of the proteins that interact with Hsp90 are key players in signal transduction pathways that are essential to mediate and sustain tumor cell growth and survival. The group of Hsp90 client proteins includes PKB and PDK1, and a functional chaperone is required for their correct folding and stability [177, 178]. Thus, inhibition of Hsp90 leads to the ubiquination and proteasome mediated degradation of PDK1 and PKB [179].

The chaperoning function of Hsp90 can be "switched-off" by inhibiting its ATP-ase activity [180, 181].

Initial attention in the development of Hsp90 inhibitors as anticancer agents was focused on two natural products, geldanamycin (compound **43**, Fig. 8) and radicicol (also called monorden, compound **44**, Fig. 8). These compounds bind into the ATP-binding cleft of the N-terminal domain of Hsp90 preventing the chaperone from cycling between its ADP and ATP-bound

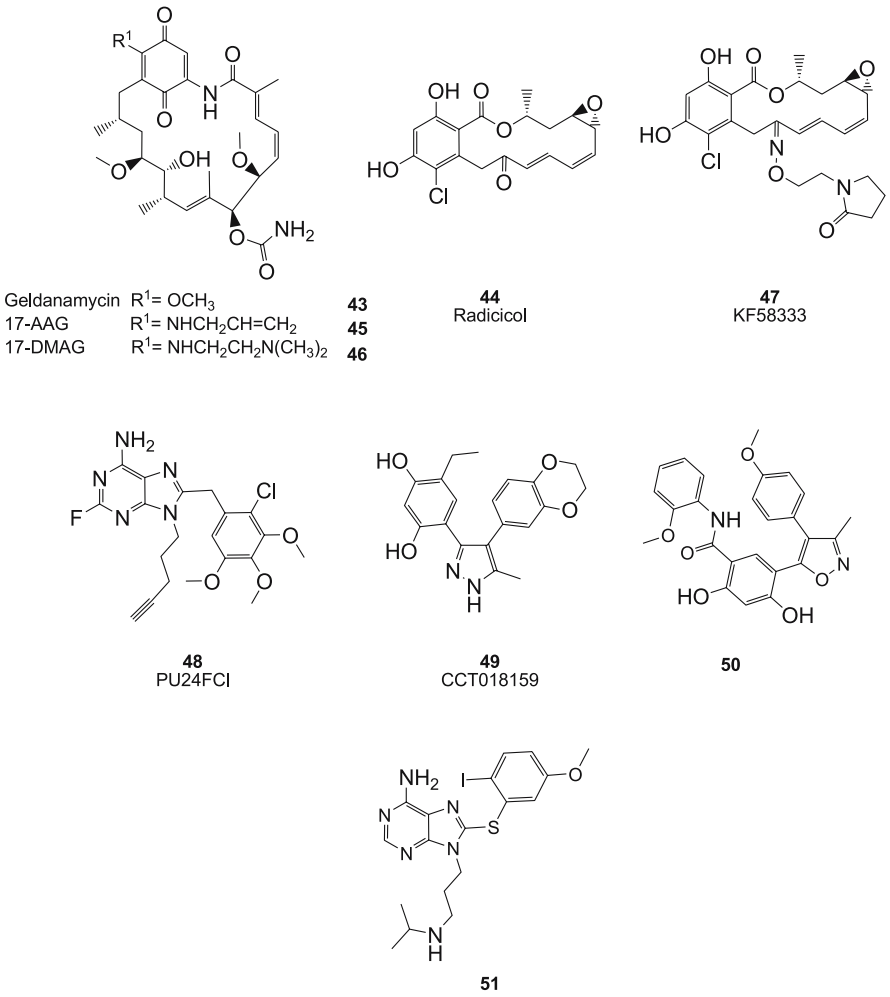


Fig. 8 Representative examples of Hsp90 inhibitors

conformations [182, 183]. These natural products showed potent antitumor activities in preclinical models [184], but, due to several development issues, the clinical evaluation of these compounds has not been pursued. In the case of geldanamycin, extensive medicinal chemistry efforts have been made to generate analogues with improved pharmaceutical properties. One of these derivatives, 17-allyamino-17-demethoxygeldanamycin (17-AAG, compound **45**, Fig. 8) [185], has undergone Phase I clinical trials and Phase II monotherapy trials began last year (malignant melanoma). Although 17-AAG has shown some encouraging clinical responses, it presents important drawbacks (e.g., liver toxicity and cumbersome formulation) that may limit its clinical application. KOS953 and CNF1010, which contain proprietary forms

of 17-AAG in novel, optimized formulations, are also undergoing Phase I clinical trials.

More recently, novel geldanamycin analogues with increased chemical/metabolic stability and formulation options have been reported or claimed. Among these new compounds, 17-(2-dimethylaminoethyl)amino-17-demethoxygeldanamycin (17-DMAG, KOS1022; compound **46**, Fig. 8) [186] and IPI504 (structure not disclosed; a pro-drug of 17-AAG) [187] have entered Phase I clinical trials.

In terms of clinical activity, promising results from a Phase I trial investigating KOS953 in patients with multiple myeloma were presented at the American Society of Hematology meeting in 2005. Among the 22 patients who received KOS953, one patient had a measurable response while two showed partial responses.

As in the case of geldanamycin, synthetic efforts have been directed to identify radicicol derivatives with improved in vivo activity [188, 189]. A representative example of this new class of inhibitors is KF58333 (compound **47**, E-form isomer, Fig. 8), which increased the median survival time of mice inoculated with K562 chronic myelogenous leukemic cells when given i.v. at 50 mg/kg [189].

In addition to the medicinal chemistry activities around the preceding natural products, structure-based design and high-throughput screening approaches have been taken to identify new chemotypes that inhibit Hsp90 ATPase activity [190, 191]. Representative examples of low-molecular mass Hsp90 inhibitors reported to date are purine- (e.g., PU24FCl, compound **48**, Fig. 8) [192, 193], pyrazole- (e.g., CCT018159, compound **49**, Fig. 8) [194], and isoxazole- (e.g., compound **50**, Fig. 8) [180] based compounds. A recent publication has shown that a new series of purine-based Hsp90 inhibitors (e.g., compound **51**, Fig. 8) can slow s.c. tumor growth in nude mice upon oral dosage, albeit high doses are needed (200 mg/kg/day) [195].

8
PI3K/PKB Pathway Modulators with Unknown Mechanism of Action

Effective PI3K/PKB pathway interruption has also been reported with a series of compounds whose mechanism of action is still unknown. The three examples reviewed in this section also illustrate the potential application and challenges that chemical genetics may face in this area of drug discovery.

Triciribine, also known as Akt/protein kinase B signaling inhibitor-2 (API-2); compound **52**, Fig. 9), was identified by screening the National Cancer Institute Diversity Set chemical library (1992 compounds). The screen was performed using a cell-based proliferation assay with PKBβ transformed NIH3T3 cells, and triciribine scored positive in this assay with an IC_{50} value of 50 nM [196]. Although the compound blocks the cellular phosphorylation

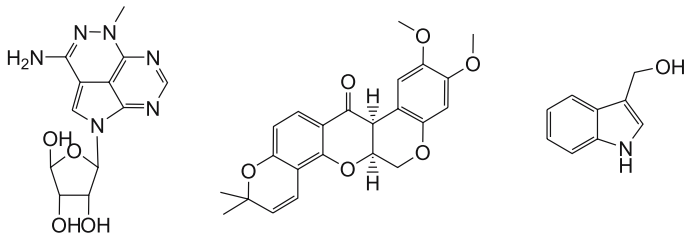


Fig. 9 PI3K/PKB pathway modulators with unknown mechanism of action

of PKB ($IC_{50} < 0.5\,\mu M$) and induces apoptosis in human tumor cells with elevated levels of PKB, it does not inhibit the kinase activity of recombinant myr-PKBβ, PI3K, or PDK1. In vivo antitumor activity (tumor growth inhibition $\geq 80\%$) against a panel of s.c. human tumors xenografts (e.g., OVCAR3, OVAR8, and PANC1) with aberrant PKB activation was observed when triciribine was administered i.p at 1 mg/kg/day. Ex vivo analyses of tumor samples showed that the compound blocks PKB phosphorylation at Thr308 and Ser473 without affecting protein content. At the therapeutic dose, the compound did not affect body weight and glucose levels in plasma. The compound fulfilled the preclinical requirements and entered Phase I clinical trials a few years ago. Hepatotoxicity, hypertriglyceridimia, thrombocytopenia, hypocalcemia, and hyperglycemia have been reported as the most common side effects, and, due to its side effects at high doses, the compound has been limited in the clinic. Phase II clinical trials have been performed in cancer patients with advanced solid tumors, but it appears that further development of triciribine has been discontinued. Independently of this, further understanding of the molecular mechanism of the action of triciribine [e.g., molecular target(s)] would be greatly beneficial for the design of new analogues with improved pharmaceutical properties.

Deguelin (compound **53**, Fig. 9), which is a rotenoid isolated from *Munduleasericea* (Legumonosae) [197] and synthesized from rotenone [198], has been shown to inhibit in a dose- and time-dependent manner the growth of transformed human bronchial epithelial (HBE; $IC_{50} < 10^{-8}$ M) [199] and NSCLC cells [200] by blocking the PI3K/PKB-mediated signaling pathway [199, 201]. The compound inhibited PI3K activity and reduced P-PKB levels at concentrations of 10^{-7} M, but had no discernable effect on MAPK pathway [199]. Although it is unclear how deguelin inhibits PKB activity at this moment, experimental results seem to indicate that the mechanism of action of deguelin involves PI3K-dependent and independent pathways [201]. In addition to its effects on the PI3K/PKB pathway, it has also been shown that deguelin induces inhibition of COX-2 expression in HBE cells [199]. Preclinical findings with carcinogenesis models have pointed to the potential

therapeutic use of deguelin for chemoprevention in early-stage lung carcinogenesis and in the treatment of lung cancer [201, 202]. However, no recent developments for deguelin have been reported.

Indole-3-carbinol (compound **54**, Fig. 9) and derivatives thereof have been shown to inhibit the phosphorylation and activation of PKB in tumor-derived breast and prostate cell lines [203, 204]. As in the other examples in this section, the precise mechanism of compound-mediated inhibition of the PI3K/PKB pathway needs to be elucidated, but it is clear that these molecules are not acting as direct kinase inhibitors. Thus, indole-3-carbinol did not inhibit PKB kinase activity when added to an in vitro kinase assay at concentrations up to 750 μM [203]. Recently, the biological activity of an optimized natural indole derivative (structure not disclosed) has been reported [205]. SR13668 inhibits phosphorylation of PKB in MDA-MB-468 breast cancer cells (c = 75 to 100 μM) and tumor xenografts (50 mg/kg/day, po). Interestingly, assessment of glucose metabolism in mice treated at doses ten times higher than were needed for antitumor activity showed no adverse effect on the fasting glucose concentration after 14 days of oral treatment. The compound also significantly inhibited angiogenesis in the choroallantoic membrane assay, and was effective at inhibiting adriamycin-, cisplatin-, and tamoxifen-resistant breast cancer cell lines. Although this compound was selected for inclusion in the National Cancer Institutes' "Rapid Access to Preventive Intervention Development" (RAPID) program, its development status is unclear at this point in time.

9 Conclusions and Outlook

As shown in the preceding sections, substantial drug discovery efforts have been devoted in the past few years to identify and develop therapeutic agents able to specifically down regulate the PI3K/PKB pathway in tumor cells. Although often non-selective for the intended target, first generation inhibitors have been extensively used as molecular probes for improving our understanding of biological processes associated with this pathway. A few of these early inhibitors, or closely related analogues, have provided proof-of-concept in preclinical settings and entered clinical trials. The differences in therapeutic response and toxicity observed with these compounds suggest that inhibiting the PI3K/PKB pathway at different nodes might yield very different antitumor activities and therapeutic windows. Overall, we can conclude that the introduction of PI3K/PKB inhibitors as potential targeted anticancer agents is still in an early stage, and that additional preclinical and clinical investigations with more selective inhibitors, or at least better characterized compounds, will be required to determine where and how to target the PI3K survival pathway in order to provide the best efficacy and therapeutic window.

References

1. Stauffer F, Holzer P, Garcia-Echeverria C (2005) Curr Med Chem—Anti-Cancer Agents 5:449
2. Hennessy BT, Smith DL, Ram PT, Lu Y, Mills GB (2005) Nature Rev 4:988
3. Pommery N, Hénichart J-P (2005) Mini-Reviews in Medicinal Chemistry 5:1125
4. Chen YL, Law P-Y, Loh HH (2005) Curr Med Chem—Anti-Cancer Agents 5:575
5. Macaulay VM (1992) Br J Cancer 65:311
6. Ma J, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH, Stampfer MJ (1999) J Natl Cancer Inst 91:620
7. Yu H, Spitz MR, Mistry J, Gu J, Hong WK, Wu X (1999) J Natl Cancer Inst 91:151
8. Hankinson SE, Willette WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, Polla M (1998) Lancet 351:1373
9. Mauro L, Salerno M, Morelli C, Boterberg T, Bracke ME, Surmacz E (2003) J Cell Phys 194:108
10. Valentinis B, Baserga R (2001) Mol Pathol 54:133
11. Baserga R (2000) Oncogene 19:5574
12. O'Connor R (1998) Adv Biochem Eng—Biotechnol 62:137
13. Werner H, Le Roith D (1997) Crit Rev Oncogenesis 8:71
14. Yu D, Watanabe H, Shibuya H, Miura M (2003) J Biol Chem 278:6702
15. Lu Y, Zi X, Mascarenhas D, Pollak M (2001) J Natl Cancer Inst 93:1852
16. Grothey A, Voigt W, Schober C, Muller T, Dempke W, Schmoll HJ (1999) J Cancer Res Clin 125:166
17. Wen B, Deutsch E, Marangoni E, Frascona V, Maggiorella L, Abdulkarim B, Chavaudra N, Bourhis J (2001) Br J Cancer 85:2017
18. Scotlandi K, Avnet S, Benini S, Manara MC, Serra M, Cerisano V, Perdichizzi S, Lollini PL, De Giovanni C, Landuzzi L, Picci P (2002) Int J Cancer 101:11
19. Beech DJ, Parekh N, Pang Y (2001) Oncol Rep 8:325
20. Maloney EK, McLaughin JL, Dagdigian NE, Garrett LM, Connors KM, Zhou X-M, Blättler WA, Chittenden T, Sing R (2003) Cancer Res 63:5073
21. Burtrum D, Zhu Z, Lu D, Anderson DM, Prewett M, Pereira DS, Bassi R, Abdullah R, Hooper AT, Koo H, Jimenez X et al. (2003) Cancer Res 63:8912
22. Goetsch L, Gonzales A, Leger O, Beck A, Pauwles PJ, Haeuw JF, Corvaia N (2005) Int J Cancer 113:316
23. Lu D, Zhang H, Ludwig D, Persaud A, Jimenez X, Burtrum D, Balderes P, Liu M, Bohlen P, Witte L, Zhu Z (2004) J Biol Chem 4:2856
24. Ullrich A, Gray A, Tam AW, Yang-Feng T, Tshobokawa M, Collins C, Henzel W, Le Bon T, Kathuria S, Chen E et al. (1986) EMBO J 5:2503
25. Munshin S, Kormienko M, Hall DL, Reid JC, Waxman L, Stirdivant SM, Darke PL, Kuo LC (2002) J Biol Chem 277:38797
26. Pautsch A, Zoephel A, Ahorn H, Spevak W, Hauptmann R, Nar H (2001) Structure 9:955
27. Wei L, Hubbard SR, Hendrickson WA, Ellis L (1995) J Biol Chem 270:8122
28. Hubbard SR, Wei L, Ellis L, Hendrickson WA (1994) Nature 372:746
29. Hubbard SR, Till JH (2000) Annu Rev Biochem 69:373
30. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Akiyama M, Hideshima T, Chauhan D, Joseph M, Libermann TA, Garcia-Echeverria C, Pearson MA, Hofmann F, Anderson KC (2004) Cancer Cell 5:221
31. Bell IM, Stirdivant SM, Ahern J, Culberson JC, Darke PL, Dinsmore CL, Drakas RA, Gallicchio SN, Graham SL, Heimbrook DC et al. (2005) Biochemistry 44:9430

32. Warshamana-Greene GS, Litz J, Buchdunger E, Garcia-Echeverria C, Hofmann F, Krystal GW (2005) Clin Cancer Res 11:1563
33. Scotlandi K, Manara MC, Nicoletti G, Lollini PL, Lukas S, Benini S, Croci S, Perdichizzi S, Zambelli D, Serra M, Garcia-Echeverria C, Hofmann F, Picci P (2005) Cancer Res 65:3868
34. Wittman M, Carboni J, Attar R, Balasubramanian B, Balimane P, Brassil P, Beaulieu F, Chang Ch, Clarke W et al. (2005) J Med Chem 48:5639
35. Parrizas M, Gazit A, Levitzki A, Wertheimer E, LeRotih D (1997) Endocrinology 138:1427
36. Girnita A, Girnita L, del Prete F, Bartolazzi A, Larsson O, Axelson M (2004) Cancer Res 64:236
37. Vasilcanu D, Girnita A, Girnita L, Vasicanu R, Axelson M (2005) Oncogene 23:7854
38. Menu E, Jernberg-Wiklung JJ, Stromberg T, De Raeve H, Girnita L, Larsson O, Axelson M, Asosingh K, Nilsson K, Camp BV, Vanderkerken K (2006) Blood 107:655
39. Li W, Favelyukis S, Yang J, Zeng Y, Yu J, Gangjee A, Miller WT (2004) Biochem Pharmacol 68:145
40. Berrie CP (2001) Expert Opin Inv Drug 10:1085
41. Shayesteh L, Lu Y, Kuo WL, Baldocchi R, Godfrey T, Collins C et al. (1999) Nat Genet 21:99
42. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ et al. (2004) Science 304:554
43. Kang S, Bader AG, Vogt PK (2005) Proc Natl Acad Sci USA 102:802
44. Oda K, Stokoe D, Taketani Y, McCormick F (2005) Cancer Res 65:10669
45. Eng C (2003) Human Mutat 22:183
46. Cantley LC (2002) Science 296:1655
47. Cantley LC, Neel BG (1999) Proc Natl Acad Sci USA 96:4240
48. Workman P (2004) Transactions 32:393
49. Brian PW, Hemming HG, Norris GLF (1957) Brit Mycol Soc Trans 40:365
50. Wymann MP, Bulgarelli-Leva G, Zvelebil MJ, Pirola L, Vanhaesebroeck B, Waterfield MD, Panayotou G (1996) Mol Cell Biol 16:1722
51. Stoyonova S, Bulgarelli-Leva G, Kirsch C, Hanck T, Klinger R, Wetzler R, Wymann MP (1997) Biochem J 324:489
52. Berrie CP (2001) Expert Opin Inv Drug 10:1085
53. Davies SP, Reddy H, Caivano M, Cohen P (2000) Biochem J 351:95
54. Liu Y, Shreder KR, Gai W, Corral S, Ferris DK, Rosenblum JS (2005) Chem Biol 12:99
55. Matter WF, Brown RF, Vlahos CJ (1992) Biochem Bioph Res Co 186:624
56. Vlahos CJ, Matter WF, Hui KY, Brown RF (1994) J Biol Chem 269:5241
57. Brunn GJ, Williams J, Sabers C, Wiederrecht G, Lawrence JC Jr, Abraham RT (1996) EMBO J 15:5256
58. Davies SP, Reddy H, Caivano M, Cohen P (2000) Biochem J 351:95
59. Knight ZA, Chiang GG, Alaimo PJ, Kenski DM, Ho CB, Coan K, Abraham RT, Shokat KM (2004) Bioorg Med Chem 12:4749
60. Ihle NT, Williams R, Chow S, Chew W, Berggren MI, Paine-Murrieta G, Minion DJ, Halter RJ, Wipf P, Abraham R, Kirkpatrick L, Powis G (2004) Molecular Cancer Ther 3:763
61. Hu L, Hofmann J, Lu Y, Mills GB, Jaffe RB (2002) Cancer Res 62:1087
62. Brognard J, Clark AS, Ni Y, Dennis PA (2001) Cancer Res 61:3986
63. Yu K, Lucas J, Zhu T, Zask A, Gaydos C, Toral-Barza L, Gu J, Li F, Chaudhary I, Cai P, Lotvin J, Petersen R, Ruppen M, Fawzi M, Ayrai-Kaloustain S, Mansour T, Frost P, Gibbons J (2005) Cancer Biol Ther 4:538

64. Ihle NT, Paine-Murrieta G, Berggren MI, Baker A, Tate WR, Wipf P, Abraham RT, Kirkpatrick DL, Powis G (2005) Mol Cancer Ther 4:1349
65. Hayakawa M, Kaizawa H, Kawaguchi KI, Matsuda K, Ishikawa N, Koizumi T, Yamano M, Okada M, Ohta M, Patent WO01083481
66. Hayakawa M, Kaizawa H, Moritomo H, Kawaguchi KI, Koizumi T, Yamano M, Matsuda K, Okada M, Ohta M, WO01083456
67. Parker P, Waterfield M, WO4017950
68. Ahmadi K, Waterfield M (2004) In: Lennarz WT, Lane MD (eds) Encyclopedia Biological Chemistry. Elsevier/Academic Press 3:281
69. Ahmadi K, Alderton W, Chuckowree I, Depledge P, Folkes A, Pergl-Wilson G, Saghir N, Shuttleworth S, Wan N, Raynaud F (2004) European J Cancer Suppl 2:97
70. Ahmadi K, Alderton W, Chuckowree I, Depledge P, Folkes A, Pergl-Wilson G, Saghir N, Shuttleworth S, Wan N, Raynaud F, Saghir NSS, Wan NC, Zhyvoloup A (2004) Proc EORTC-NCI-AACR, abstract 320, Poster
71. Robertson AD, Jackson S, Kenche V, Yaip C, Parbaharan H, Thompson P, WO1053266
72. Jackson SP, Robertson AD, Kenche V, Thompson P, Prabaharan H, Anderon K, Abbott B, Goncalves I, Nesbitt W, Schoenwaelder S, Saylik D, WO04016607
73. Yap CL, Anderson KE, Hughan SC, Dopheide SM, Salem HH, Jackson SP (2002) Blood 99:151
74. Jackson SP, Schoenwaelder SM, Goncalves I, Nesbitt WS, Yap CL, Wright CE, Kenche V, Anderson KE, Dopheide SM et al. (2005) Nature Med 11:507
75. Camps M, Rückle T, Ji H, Ardissone V, Rintelen F, Shaw J, Ferrandi C, Chabert C, Gillieron C et al. (2005) Nature Med 11:936
76. Sadhu C, Dick K, Tino WT, Staunton DE (2003) Biochem Biophys Res Commun 308:764
77. Sujobert P, Bardet V, Cornillet-Lefebvre P, Hayflick JS, Prie N et al. (2005) Blood 106:1063
78. Sawyer C, Sturge J, Bennett DC, O'Hare MJ, Allen WE, Bain J, Jones GE, Vanhaesebroeck B (2003) Cancer Res 63:1667
79. Melese T, Perkins EL, Nguyen ATQ, Sun D, WO03034997
80. Gogliotti RD, Mucciolo KL, Para KS, Visnick M, WO04056820
81. Bruendl MM, Connolly MK, Goodman AP, Gogliotti RD, Lee HT, Plummer MS, Sexton KE, Reichard GA, Visnick M, Wilson MW, WO04108715
82. Gogliotti RD, Lee HT, Sexton KE, Visnick M, WO04108709
83. Gogliotti RD, Lee HT, Sexton KE, Visnick M, US04248953
84. Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP, Williams RL (2001) Molecular Cell 6:909
85. Walker EH, Perisic O, Ried C, Stephens L, Williams RL (2000) Nature 402:313
86. Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP, Williams RL (2001) Molecular Cell 6:909
87. Tian X, Rusanescu G, Hou W, Schaffhausen B, Feig LA (2002) EMBO J 21:1327
88. Stephens L, Anderson K, Stokoe D, Erdjument-Bromage H, Painter GF, Holmes AB, Gaffney PRJ, Reese CB, McCormick F, Tempst P, Coadwell J, Hawkins PT (1998) Science 279:710
89. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PRJ, Reese CB, Cohen P (1997) Curr Biol 7:261
90. Hawkins PT, Welch H, McGregor A, Eguinoa A, Gobert S, Krugmann S, Anderson K, Stokoe D, Stephens L (1997) Biochem Soc Trans 25:1147
91. Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PRJ, Reese CB, Cohen P (1997) Curr Biol 7:261

92. Mora A, Komander D, van Aalten DMF, Alessi DR (2004) Semin Cell Dev Biol 15:161
93. Alessi DR, Deak M, Casamayor A, Caudwell FB, Morrice N, Norman DG, Gaffney P, Reese CB, Macdougall CN, Harbison D, Ashworth A, Bownes M (1997) Curr Biol 7:776
94. Stokoe D, Stephens LR, Copeland T, Gaffney PRJ, Reese CB, Painter GF, Holmes AB, McCormick F, Hawkins PT (1997) Science 277:567
95. Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA (1996) EMBO J 15:6541
96. Thomas CC, Deak M, Alessi DR, van Aalten DM (2002) Curr Biol 12:1256
97. Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA (1996) EMBO J 15:6541
98. Wick MJ, Ramos FJ, Chen H, Quon MJ, Dong LQ, Liu F (2003) J Biol Chem 278:42913
99. Casamayor A, Morrice NA, Alessi DR (1999) Biochem J 342:287
100. Lawlor MA, Mora A, Ashby PR, Williams MR, Murray-Tait V, Malone L, Prescott AR, Lucocq JM, Alessi DR (2002) EMBO J 21:3728
101. Sato S, Fujita N, Tsuruo T (2002) Oncogene 21:1727
102. Komander D, Kular GS, Bain J, Elliott M, Alessi DR, van Aalten DMF (2003) Biochem J 375:255
103. Fujita N, Tsuruo T (2003) Cancer Chemother Pharmacol 52:S24
104. Kondapaka SB, Zarnowski MJ, Yver DR, Sausville EA, Cushman SW (2004) Clin Cancer Res 10:7192
105. Hill MM, Andjelkovic M, Brazil DP, Ferrari S, Fabbro D, Hemmings BA (2001) J Biol Chem 276:25643
106. Tsuruo T, Naito M, Tomida A, Fujita N, Mashima T, Sakamoto H, Haga N (2003) Cancer Sci 94:15
107. Komander D, Kular GS, Bain J, Elliott M, Alessi DR, van Aalten DMF (2003) Biochem J 375:255
108. Feldman RI, Wu JM, Polokoff MA, Kochanny MJ, Dinter H, Zhu D, Biroc SL, Alicke B, Bryant J, Yuan S, Buckman BO, Lentz D, Ferrer M, Whitlow M, Adler M, Finster S, Chang Z, Arnaiz DO (2005) J Biol Chem 280:19867
109. Hsu AL, Chingm TT, Wang DS, Song S, Rangnekar VM, Chen CS (2000) J Biol Chem 275:11397
110. Arico S, Pattingre S, Bauvy C, Gane P, Barbat A, Codogno P, Ogier-Denis E (2002) J Biol Chem 277:27613
111. Zhu J, Huang J-W, Tseng P-H, Yang Y-T, Fowble J, Shiau C-W, Shaw Y-J, Kulp SK, Chen C-S (2004) Cancer Res 64:4309
112. Song X, Lin HP, Johnson AJ, Tseng PH, Yang YT, Kulp SK, Chen C (2002) J Natl Cancer Inst 94:585
113. Yamazaki R, Kusunoki N, Matsuzaki T, Hashimoto S, Kawai S (2002) FEBS Lett 531:278
114. Yang J, Cron P, Good VM, Thompson V, Hemmings BA, Barford D (2002) Nat Struct Biol 9:940
115. Komander D, Kular GS, Schuttelkopf AW, Deak M, Prakash KRC, Bain J, Elliott M, Garrido-Franco M, Kozikowski AP, Alessi DR, van Aalten DMF (2004) Structure 12:215
116. Biondi RM, Komander D, Thomas CC, Lizcano JM, Deak M, Alessi DR, van Aalten DMF (2002) EMBO J 21:4219
117. Biondi RM, Cheung PCF, Casamayor A, Deak M, Currie RA, Alessi DR (2000) EMBO J 19:979
118. Yang J, Cron P, Thompson V, Good VM, Hess D, Hemmings BA, Barford D (2002) Mol Cell 9:1227

119. Jones PF, Jakubowicz T, Pitossi FJ, Maurer F, Hemmings BA (1991) Proc Natl Acad Sci USA 88:4171
120. Jones PF, Jakubowicz T, Hemmings BA (1991) Cell Regul 2:1001
121. Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA (1996) EMBO J 15:6541
122. Andjelkovic M, Alessi DR, Meier R, Fernandez A, Lamb NJ, Frech M, Cron P, Cohen P, Lucocq JM, Hemmings BA (1997) J Biol Chem 272:31515
123. Bellacosa A, Chan TO, Ahmed NN, Datta K, Malstrom S, Stokoe D, McCormick F, Feng J, Tsichlis P (1998) Oncogene 17:313
124. Jahn T, Seipel P, Urschel S, Peschel C, Duyster J (2002) Mol Cell Biol 22:979
125. Alessi DR, Deak M, Casamayor A, Caudwell FB, Morrice N, Norman DG, Gaffney P, Reese CB, Macdougall CN, Harbison D, Ashworth A, Bownes M (1997) Curr Biol 7:776
126. Feng J, Park J, Cron P, Hess D, Hemmings BA (2004) J Biol Chem 279:41189
127. Sarbassov D, Guertin DA, Ali SM, Sabatini DM (2005) Science 307:1098
128. Hill MM, Hemmings BA (2002) Pharmacol Ther 93:243
129. Mitsiades CS, Mitsiades N, Koutsilieris M (2004) Curr Cancer Drug Targets 4:235
130. Downward J (2006) Sem Cell Dev Biol 15:177
131. Li Q, Zhu GD (2002) Curr Top Med Chem 2:939
132. Kumar CC, Diao R, Yin Z, Liu Y, Samatar AA, Madison V, Xiao L (2001) Biochim Biophys Acta 1526:257
133. Masure S, Haefner B, Wesselink JJ, Hoefnagel E, Mortier E, Verhasselt P, Tuytelaars A, Gordon R, Richardson A (1999) Eur J Biochem 265:353
134. Barnett SF, Defeo-Jones D, Fu S, Hancock PJ, Haskell K, Jones RE, Kahana JA, Kral AM, Leander K, Lee LL, Malinowski J, McAvoy EM, Nahas DD, Robinson RG, Huber HE (2004) Biochem J 385:399
135. Davis PD, Elliott LH, Harris W, Hill CH, Hurst SA, Keech E, Kumar MK, Lawton G, Nixon JS, Wilkinson SE (1992) J Med Chem 35:994
136. Chijiwa T, Mishima A, Hagiwara M, Sano M, Hayashi K, Inoue T, Naito K, Toshioka T, Hidaka H (1990) J Biol Chem 265:5267
137. Reuveni H, Livnah N, Geiger T, Klein S, Ohne O, Cohen I, Benhar M, Gellerman G, Levitzki A (2002) Biochemistry 41:10304
138. Breitenlechner CB, Wegge T, Berillon L, Graul K, Marzenell K, Friebe WG, Thomas U, Schumacher R, Huber R, Engh RA, Masjost B (2004) J Med Chem 47:1375
139. Breitenlechner CB, Friebe W-G, Brunet E, Werner G, Graul K, Thomas U, Künkele K-P, Schäfer W, Gassel M, Bossemeyer D, Huber R, Engh RA, Masjost B (2005) J Med Chem 48:163
140. Gassel M, Breitenlechner CB, Ruger P, Juknischke U, Schneider T, Huber R, Bossemeyer D (2003) J Mol Biol 329:1021
141. Friebe WG, Masjost B, Schumacher R, WO03076429
142. Luo Y, Shoemaker AR, Liu X, Woods KW, Thomas SA, de Jong R, Han EK, Li T, Stoll VS, Powlas JA, Oleksijew A, Mitten MJ, Shi Y et al. (2005) Mol Cancer Res 4:977
143. Shi Y, Liu X, Han E, Guan R, Shoemaker AR, Oleksijew A, Woods KW, Fisher JP, Klinghofer V, Lasko L, McGonigal T, Li Q, Rosenberger SH, Giranda VL, Luo Y (2005) Neoplasia 7:992
144. Chen YL, Law P-Y, Loh HH (2005) Curr Med Chem—Anti-Cancer Agents 5:575
145. Yang J, Cron P, Thompson V, Good VM, Hess D, Hemmings BA, Barford D (2002) Molecular Cell 9:1227
146. Zhao Z, Leister WH, Robinson RG, Stanley FB, Defeo-Jones D, Jones RE, Hartman GD, Huff JR, Huber HE, Duggan ME, Lindsley CW (2005) Bioorg Med Chem Lett 15:905

147. Lindsley CW, Zhao Z, Leister WH, Robinson RG, Barnett SF, Defeo-Jones D, Jones RE, Hartman GD, Huff JR, Huber HE, Duggan ME (2005) Bioorg Med Chem Lett 15: 905
148. DeFeo-Jones D, Barnett SF, Fu S, Hancock PJ, Haskell KM, Leander KR, McAvoy E, Robinson RG, Duggan ME, Lindsley CW, Zhao Z, Huber HE, Jones RE (2005) Mol Cancer Ther 4:271
149. Barnett SF, Defeo-Jones D, Fu S, Hancok PJ, Haskell K, Jones RE, Kahana JA, Kral AM, Leander K, Lee LL, Malinowski J, McAvoy EM, Nahas DD, Robinson RG, Huber HE (2005) Biochem J 385:399
150. Richardson CJ, Schalm SS, Blenis J (2004) Sem Cell Dev Biol 15:147
151. Dancey JE (2005) Expert Opin Invest Drug 14:313
152. Sawyers CL (2003) Cancer Cell 4:343
153. Oshiro N, Yoshino K, Hidayat S, et al. (2004) Genes Cells 9:359
154. Yonezawa K, Tokunaga C, Oshiro N, Yoshino K (2004) Biochem Biophys Res Comm 313:437
155. Hay N (2005) Cancer Cell 8:179
156. Rao RV, Buckner JC, Sarkaria JN (2004) Curr Cancer Drug Targets 4:621
157. Meuillet EJ, Mahadevan D, Vankayalapati H, Berggren M, Williams R, Coon A, Kozilowski AP, Powis G (2003) Mol Cancer Ther 2:389
158. Egorin MJ, Parise RA, Joseph E, Hamburger DR, Lan J, Covey JM, Eiseman JL (2002) Proc Am Assoc Cancer Res 43:abstract 2996
159. Tabellini G, Tazzari PL, Bortul R, Billi AM, Conte R, Manzoli L, Cocco L, Martelli AM (2004) Brit J Haematol 126:574
160. Kozikowski AP, Sun H, Brognard J, Dennis PA (2005) J Am Chem Soc 125:1144
161. Honda RJ, Zhao Z, Kravchuk AV, Liao H, Riddle SR, Yue X, Bruzik KS, Tsai MD (1998) Biochemistry 37:4568
162. Castillo SS, Brognard J, Petukhov PA, Zhang C, Tsurutani J, Granville CA, Li M, Jung M, West KA, Gills JG, Kozikowski AP, Dennis PA (2004) Cancer Res 64:2782
163. Berrie CP, Falasca M (2000) FASEB J 14:2618
164. Piccolo E, Vignati S, Maffucci T, Innominato PF, Riley AM, Potter BVL, Pandolfi PP, Broggini M, Iacobelli S, Innocenti P, Falasca M (2004) Oncogene 23:1754
165. Razzini G, Berrie CP, Vignati S, Broggini M, Mascetta G, Brancaccio A, Falasca M (2000) FASEB J 14:1179
166. Maffucci T, Piccolo E, Cumashi A, Lezzi M, Riley A, Saiardi A, Godage YH, Rossi C, Broggini M, Lacobelli S, Potter BVL, Innocenti P, Falasca M (2005) Cancer Res 65:8339
167. Thomas CC, Deak M, Alessi DR, van Aalten DMF (2002) Curr Biol 12:1256
168. Takeuchi H, Kanematsu T, Misumi Y, Sakane F, Konishi H, Kikkawa U, Watanabe Y, Katan M, Hirata M (1997) Biochim Biophys Acta 1359:275
169. Kondapaka SB, Singh SS, Dasmahapatra GP, Sausville EA, Roy KK (2003) Mol Cancer Ther 2:1093
170. Ruiter GA, Zerp SF, Bartelin H, Van Blitterswijk WJ, Verheij M (2003) Anticancer Drugs 14:167
171. Engel J, Hilgard P, Klenner T, Kutscher B, Nossner G, Traiser M, Voss V (2000) Drug Future 25:1257
172. Dasmahapatra GP, Didolkar P, Alley MC, Ghosh S, Sausville EA, Roy KK (2004) Clin Cancer Res 10:5242
173. Li X, Luwor R, Lu Y, Liang K, Fan Z (2006) Oncogene 25:525
174. Ernst DS, Eisenhauer E, Wainman N, Davis M, Lohmann R, Baetz T, Belanger K, Smylic M (2006) Invest New Drugs, online (2005), 23(6):569–575

175. Posadas EM, Gulley J, Arlen PM, Trout A, Parnes HL, Wright J, Lee MJ, Chung EJ, Trepel JB, Sparreboom A, Chen C, Jones E, Steinberg SM, Daniels A, Figg WD, Dahut WL (2005) Cancer Biol Ther 4:1133
176. Whitesell L, Lindquist SL (2005) Nature Rev 5:761
177. Basso AD, Solit DB, Chiosis G, Giri B, Tsichlis P, Rosen N (2002) J Biol Chem 277:39858
178. Fujita N, Sato S, Ishida A, Tsuruo T (2002) J Biol Chem 277:10346
179. Solit DB, Basso AD, Olshen AB, Scher HI, Rosen N (2003) Cancer Res 63:2139
180. Dymock BW, Drysdale MJ, McDonald E, Workman P (2004) Expert Opin Ther Pat 14:837
181. Chiosis G, Vilenchik M, Kim J, Solit D (2004) Drug Discov Today 9:881
182. Roe SM, Podromou C, O'Brien R, Ladbury JE, Piper PW, Pearl LH (1999) J Med Chem 42:260
183. Stebbins CE, Russo AA, Scheneider C, Rosen N, Hartl FU, Pavletich NP (1997) Cell 89:239
184. Neckers L, Neckers K (2002) Expert Opin Emer Drug 7:277
185. Jia W, Yu C, Rahmani M, Krystal G, Sausville EA, Dent P, Grant S (2003) Blood 102:1824
186. Tian ZQ, Liu Y, Zhang D, Wang Z, Dong SD, Carreras CW, Zhou Y, Rastelli G, Santi DV, Myles DC (2004) Bioorg Med Chem 12:5317
187. Mitsiades C, Mitsiades N, Rooney M, Negri J, Geer CC et al. (2004) Blood 104:660
188. Yamamoto K, Garbaccio RM, Stachel SJ, Solit DB, Chiosis G, Rosen N, Danishefsky SJ (2003) Angewandte Chemie 42:1280
189. Soga S, Shiotsu Y, Akinaga S, Sharma SV (2003) Curr Cancer Drug Targets 3:359
190. Blagg BSJ, Kerr TD (2006) Med Res Rev, online (2006), 26(3):310–338
191. Janin YL (2005) J Med Chem 48:7503
192. Vilenchik M, Solit D, Basso A, Huezo H, Lucas B, He H, Rosen N, Spampinato C, Modrich P, Chiosis G (2004) Chem Biol 11:787
193. Chiosis G, Lucas B, Huezo H, Solit D, Basso A, Rosen N (2003) Curr Cancer Drug Targets 3:371
194. Rowlands MG, Newbatt YM, Prodomou C, Pearl LH, Workman P, Aherne W (2004) Analyt Biochem 327:176
195. Biamonte MA, Shi J, Hong K, Hurst DC, Zhang L, Fan J, Busch DJ, Karjian PL, Maldonado AA et al. (2006) J Med Chem 49:817
196. Yang L, Dan HC, Sun M, Liu Q, Sun X, Feldman RI, Hamilton AD, Polokoff M, Nicosia SV, Herlyn M, Sebti SM, Cheng JQ (2004) Cancer Res 64:4394
197. Mehta RG, Pezzuto JM (2002) Curr Oncol Rep 4:478
198. Anzenveno PB (1979) J Org Chem 44:2578
199. Chun K-H, Kosmeder JWII, Sun S, Pezzuto JM, Lotan R, Hong AK, Lee H-Y (2003) J Natl Cancer J 95:291
200. Lee H-Y, Suh Y-A, Kosmeder K, Pezzuto JM, Hong WK, Kurie JM (2004) Clin Cancer Res 10:1074
201. Lee H-Y (2004) Biochem Pharmacol 68:1119
202. Udeani GO, Gerhauser C, Thomas CF, Moo RC, Kosmeder JW, Kinghorn AD et al. (1997) Cancer Res 57:3424
203. Howells LM, Gallacher-Horley B, Houghton CE, Manson MM, Hudson EA (2002) Mol Cancer Ther 1:1161
204. Chinni SR, Sarkar FH (2002) Clin Cancer Res 8:1228
205. Jong L, Chao W-R, Amin K, Laderoute K, Orduna J, Sato B, Rice G (2003) AACR-NCI-EORTC Int Conf, abstract 157, Poster

Top Med Chem (2007) 1: 207–291
DOI 10.1007/7355_2006_006

Published online: 13 January 2007

Progress in the Development of Agents to Control the Cell Cycle

Kevin J. Moriarty · Holly Koblish · Dana L. Johnson · Robert A. Galemmo Jr (✉)

Oncology Research Team, Spring House Research and Early Development Site, Johnson & Johnson Pharmaceutical Research and Development, Welsh and McKean Roads, Springhouse, PA 19477, USA
rgalemmo@neuromed.com

1 Introduction . . . 209

2 Cyclin-Dependent Kinases . . . 209
2.1 Biology of the CDKs . . . 209
2.2 Structural Biology of the CDKs . . . 210
2.3 Structure–Activity Relationships of CDK Inhibitors . . . 212
2.3.1 Purines . . . 213
2.3.2 Pyrrolocarbazoles . . . 216
2.3.3 Paullones . . . 219
2.3.4 1*H*-Pyrazolo[3,4-*b*]pyridine . . . 220
2.3.5 3,5-Substituted-Indazoles . . . 222
2.3.6 Indeno[1,2-*c*]pyrazol-4-ones . . . 222
2.3.7 3-Aminopyrazoles . . . 225
2.3.8 Pyrido[2,3-*d*]pyrimidin-7-one . . . 227
2.3.9 Quinazolines . . . 229
2.3.10 Diaryl Ureas . . . 231
2.3.11 Quinox-2(1*H*)-ones . . . 232
2.3.12 Pyrimidines . . . 233
2.3.13 3-(Pyrimidin-4-yl)imidazo[1,2-*a*]pyridine and 3-(Pyrimidin-4-yl)imidazo[1,2-*b*]pyridazines . . . 237
2.3.14 Aminoimidazo[1,2-*a*]pyridines . . . 240
2.3.15 Imidazo[1,2-*a*]pyrazines, Imidazo[1,2-*a*]pyridines, Pyrazolo[1,5-*a*]pyridines and Pyrazolo[1,5-*a*]pyrimidines . . . 242
2.3.16 Oxindoles . . . 244
2.3.17 Diaminotriazoles . . . 247
2.3.18 *N*-Acyl- and *N*-Aryl-2-aminothiazoles . . . 248
2.3.19 5-Benzoyl-2,4 diaminothiazoles . . . 249

3 Aurora Kinases . . . 251
3.1 Biology of the Aurora Kinases . . . 251
3.2 Structural Biology of the Aurora Kinases . . . 252
3.2.1 Aurora-A . . . 252
3.2.2 Aurora-B . . . 255
3.3 Structure–Activity Relationships of Aurora Kinase Inhibitors . . . 256
3.3.1 Pyrazoles and Pyrazolones . . . 256
3.3.2 Pyrrolopyrazoles . . . 259

3.3.3 Thienopyrazoles, Furopyrazoles, Indazoles 260
3.3.4 Pyrrolopyrimidines, Thiazolopyrimidines, Imidazolopyrimidines 262
3.3.5 Quinazolines 263
3.3.6 Quinoline 266
3.3.7 Indolopyrimidines 267
3.3.8 Oxindole 267
3.3.9 5*H*-pyrimidino[5,4-*d*]benzazepines 268

4 Polo-Like Kinases 269
4.1 Biology of the Polo-Like Kinases 269
4.2 Structural Biology of the Polo-Like Kinases 270
4.3 Structure–Activity Relationships of Polo-Like Kinase Inhibitors 271
4.3.1 Scytonemin, Staurosporine, Purvalanol A and Flavinoids 271
4.3.2 Pyrimidinopyrazines 274
4.3.3 Pyrimidines 274
4.3.4 Benzothiazoles 277
4.3.5 5-(Benzimidazol-1-yl)thiophene and 2-(benzimidazol-1-yl)thiazole 277
4.3.6 Imidazotriazines 279
4.3.7 Phenylureas 279
4.3.8 3-Ethylthiazolidinone 280

5 Conclusion 280

References 281

Abstract Inhibitors of the kinases controlling the cell cycle have emerged as an important therapeutic modality for the treatment of cancer. Drug discovery efforts have focused on inhibitors of the cyclin-dependent kinases, the Aurora kinases, and Polo-like kinases. Agents for each kinase are now advancing in human clinical trials. In this review we will summarize the work in this area with special emphasis on the structural biology and structure–activity relationships developed for the many chemotypes explored.

Keywords Aurora · CDK · Cell cycle · Kinase · PLK

Abbreviations

ADME	Absorption, distribution, metabolism and excretion
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
CDK	Cyclin-dependent kinase
CoMSIA	Comparative molecular similarity indices analysis
NCI	National Cancer Institute
Rb(+)	Retinoblastoma positive
SAR	Structure–activity relationship
PLK	Polo-like kinase

1 Introduction

Therapeutic intervention targeting the cell cycle has emerged as an important strategy for the development of new therapeutic agents for the control of cancer. To realize this approach, three kinase families have been studied extensively by the pharmaceutical industry, academia, and government because of their role in driving the proliferation of tumor cells. The most mature effort has been the development of inhibitors of cyclin-dependent kinases (CDK1, CDK2 and CDK4,6), long recognized as enzymes responsible for control of the entry into and exit from the cell cycle in proliferating cells. In the last few years the focus has shifted to the development of inhibitors of Aurora kinase A and B, kinases involved in the regulation of spindle assembly and chromosome processing. Finally, Polo-like kinase-1 (PLK1), a regulator of spindle formation, chromosome segregation, centrosome maturation, and cytokinesis has become the subject of recent interest.

The universal strategy has been to develop inhibitors of the adenosine triphosphate (ATP) binding site of these kinases. The advantages and challenges of this approach can be understood by considering the nature of nucleotide cofactor binding. ATP, a compact, highly solvated heterocycle, binds with modest affinity to a binding domain composed of a fairly conserved peptide sequence found throughout the 518 protein kinases of the human kinome [1]. The challenge for the medicinal chemist has not been to find inhibitors with high affinity for this binding site, but rather to design in specificity. As daunting as this task may appear, however, considerable progress has been made by taking advantage of the great deal of structural biology information that has been generated for this enzyme class [2]. It is clear from the available data, some reviewed here, that kinase inhibitor profiles range from highly selective to quite promiscuous. In this chapter we review the progress that has been made in the discovery of inhibitors of the CDK, Aurora and PLK kinases through the eyes of the medicinal chemist. Our emphasis will be on the available structural data and the structure–activity relationships developed in the design of potent and selective inhibitors of these targets.

2 Cyclin-Dependent Kinases

2.1 Biology of the CDKs

Progression through the four phases of the cell cycle is tightly regulated by the temporal expression and destruction of CDK complexes. The het-

erodimeric complexes of a CDK catalytic subunit and a cyclin regulatory subunit trigger the phosphorylation of key substrates such as Rb to drive the cell cycle [3]. These complexes are themselves regulated both by phosphorylation and through interactions with endogenous cell-expressed CDK inhibitors. CDK4 and CDK6 partner with the D type cyclins to control early to mid-G1 progression in response to mitogenic signals, CDK2 binds cyclin E to drive the cell through the G1 to S checkpoint, then with cyclin A to control progression through S phase. Subsequently, CDK1 forms a complex with cyclin A during G2, then cyclin B to control entry into mitosis.

Clinical evidence supports a significant role for these complexes in tumor development. The catalytic CDK subunits have been shown to be overexpressed in a variety of cancers: breast [4], lung [5], and pancreatic [6] cancers for CDK4; gastric [7], ovarian [8], and hepatocellular [9] cancers for CDK2; and breast [10] and oral squamous cell [11] cancers for CDK1. Often CDK overexpression is accompanied by overexpression of the regulatory cyclin subunits [6–9]. When enhanced expression of cyclins and CDKs is not seen in cancer samples, decreased expression of the naturally occurring CDK inhibitors is often detected, resulting in a net increase in basal activity in the cell [12]. Components of this network of kinases are therefore attractive drug targets for selective and multi-targeted agents; the discovery of compounds of both of these classes will be discussed.

2.2 Structural Biology of the CDKs

CDKs are serine/threonine kinases displaying the typical bilobal kinase motif found in all kinases (Fig. 1) [13]. Because of the strong sequence homology between CDKs, it is expected that their three-dimensional structures will be similar. The structures of the CDKs consist of an amino-terminal lobe rich in β-sheets and a larger, mostly α-helical, carboxy-terminal lobe. The ATP binding site is located in a deep cleft between the two lobes, which are linked together by a "hinge" region that plays an important role in forming the catalytic active site. Crystallographic studies have shown the important influence cyclin binding has on the CDKs. Cyclin binding remodels the kinase architecture from an inactive to an active conformation with respect to substrate binding, positioning of ATP, and configuration of the active site. First, the activation-loop, which blocks substrate access in the monomeric CDKs, is forced outside the catalytic cleft after the cyclin binds and promotes the activating phosphorylation. The activating phosphorylation of the CDKs is catalyzed by the CDK activating kinase CAK, itself a cyclin-dependent kinase consisting of a CDK7 catalytic subunit in complex with cyclin H and an assembly factor Mat1. CAK phosphorylates a conserved serine or threonine site in the activation-loop of the CDKs. Phosphorylation on threonine (CDK1 Thr161; CDK2 Thr160; CDK4 Thr172; CDK6 Thr177) induces a further con-

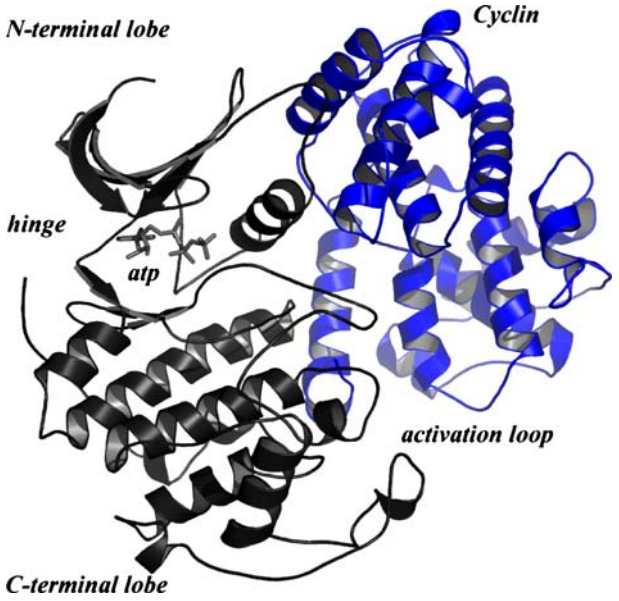


Fig. 1 Ribbon structure of the CDK enzyme complexed with cyclin

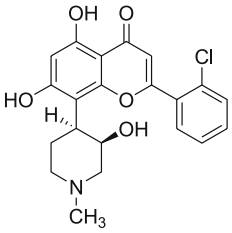
formational change in the CDK, resulting in enhanced interaction between the CDK, the associated cyclin, and the substrate. Both cell cycle CDKs (such as CDK1, CDK2, and CDK4,6) and transcription CDKs (such as CDK7 and Kin28p) have been shown to require CAK phosphorylation to be activated. The second conformational change induced by cyclin binding is found within the ATP binding site where a reorientation of the amino acid side chains induces the alignment of the triphosphate of ATP necessary for phosphate transfer [14–16].

Crystal structures for the kinases CDK2, CDK6 and CDK7 have been reported in the literature. The structures for CDK2 include Apo-CDK2, phosphorylated and unphosphorylated CDK2/cyclin A/ATP complexes, the p27Kip1 inhibitory domain bound to the phospho-CDK2/cyclin A complex, CDK2/cyclin A complex with recruitment peptides, CDK2/cyclin M, CDK2/KAP, CDK2/cyclin E, and with small molecule inhibitors bound to the active site [17–27]. Three structures of CDK6/cyclin V complexed with the inhibitor INK4 have been described as well as an inactive ternary complex between CDK6/viral cyclin D/INK4 and CDK6/cyclin V with a flavone inhibitor bound in the active site [28–32]. One X-ray crystal structure of human CDK7 in complex with ATP has been reported [33]. Although there is no reported structure for wild-type CDK4, crystal structures of a CDK4 mimetic have been described [34]. This approach utilized a CDK2 mutant with the same amino acid sequence as CDK4 in the ATP binding pocket. The mutant enzyme was crystallized both in its free form and with a series of small molecule

inhibitors (vide infra). The structural information gleaned from this CDK4 mimic led to the development of highly specific CDK4 inhibitors. There is no reported crystal structure for CDK1; however, homology models have been constructed based on the CDK2 structure [35, 36].

2.3 Structure–Activity Relationships of CDK Inhibitors

Flavopiridol (alvocidib, **1**) serves as the prototype inhibitor for this class of cell-cycle regulator as the first CDK inhibitor to enter clinical trials [37–39]. Preclinical studies have characterized **1** as a pan-CDK inhibitor active against CDKs – 1, – 2, – 4, – 6 and – 7 with IC_{50} values ranging from 100 to 400 nM. The web page www.clinicaltrials.gov lists 47 human trials of **1** at this time. However, reports from completed clinical studies claim only minor responses for this compound as a single agent or in combination. Dose scheduling in initial trials used long continuous infusions that produced only nanomolar plasma levels of drug and accentuated toxicities. Currently, bolus infusions followed by maintenance infusions are being tried to achieve micromolar levels of drug. A further complication has been the lack of acceptable pharmacodynamic endpoints to gauge effective doses in these early trials. Clinicians are attempting to optimize study design with **1** then follow up with more potent inhibitors of the CDK target [40].



1: Flavopiridol

Structure 1

Extensive efforts by many groups, both in industry and academia, have led to the identification of a number of more potent inhibitors of the CDKs. It is beyond the scope of this chapter to cover them all in detail so representative series have been selected to illustrate how inhibition activity and kinase selectivity have been optimized through the use of X-ray crystallography, modeling, and traditional SAR studies. The reader may turn to many excellent general reviews covering the CDKs for further information [41–47]. A number of more recent compounds with a variety of CDK profiles, including seliciclib (**3**), AG-024322 (**20**), PD0332991 (**32a**), Ro4584820 (**46**) and BMS-387032 (**64**, a.k.a SNS-032), have progressed to clinical trials [48] and data from these studies are awaited.

2.3.1 Purines

Extensive efforts over the last 12 years, carried out in both industrial and academic laboratories, have been focused on purine analogs as CDK inhibitors. Since the discovery of the olomoucine (**2**) [49], the first inhibitor with a reasonable selectivity profile for the CDKs, a number of potent and selective purine analogs have been identified: (R)-roscovitine (CYC202, seliciclib) (**3**), H717 (**4**) and purvalanols A and B (**5a**,**b**). Many of these compounds have undergone preclinical in vivo efficacy studies and clinical evaluation, thus confirming that the CDKs are a proper target for the development of new anticancer drugs [50–52]. This area has been extensively reviewed and these publications offer a more in-depth discussion of the purine chemotype than possible here [53–55]. In this section we will highlight the discovery of NU6102 (**7b**) as a representative example of this chemical class [56–58]. The ATP-competitive purine inhibitor NU2058, **6** (CDK1 $K_i = 5\,\mu M$; CDK2 $K_i = 12\,\mu M$), was the starting point for a structure-based design program carried out by AstraZeneca in collaboration with Newcastle University. A crystal structure of **6** bound to the fully activated CDK2/cyclin A complex was obtained to determine the binding mode of this class of inhibitors. Compound **6** (Fig. 2) formed three hydrogen bonds between the purine and residues Asp81 and Leu83 of the hinge region of CDK2. An edge–face aromatic–aromatic contact was formed between the purine and Phe80 while the O^6-cyclohexylmethyl group is situated in the binding site that accommodates the ribose moiety of ATP. This group forms a hydrophobic interaction with the non-polar glycine-rich loop. Previous SAR studies showed that the O^6-cyclohexylmethyl group was optimal and was retained in subsequent analogs.

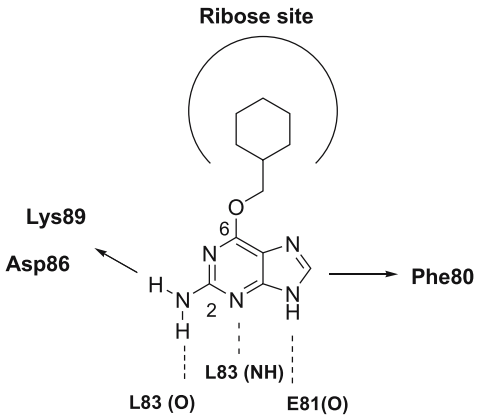
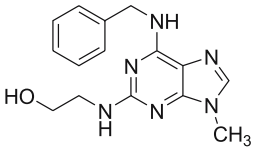
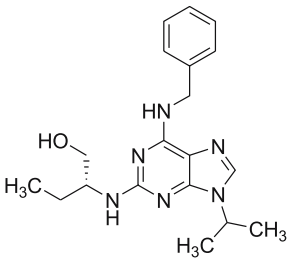


Fig. 2 Binding model for compound **6** in CDK2



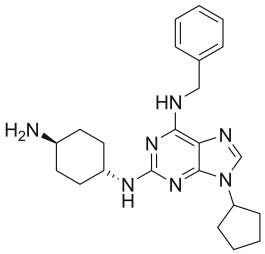
2: Olomoucine

Structure 2



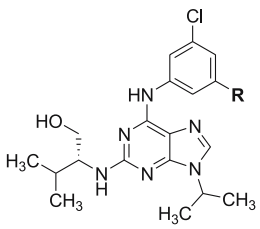
3: Roscovitine

Structure 3



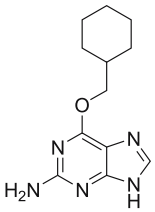
4: H717

Structure 4



5a: R = H, purvalanol A
5b: R = -CO2H, purvalanol B

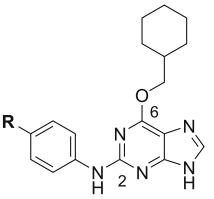
Structure 5



6: NU2058

Structure 6

Analysis of these structural features led to the hypothesis that elaboration at the 2-position of the purine would enhance potency. From the 2-position, groups would contact the "specificity surface" of CDK2 and project out of the ATP binding pocket to solvent. Substitution here offered the potential to increase potency and selectivity, and to improve the physiochemical properties of these inhibitors. Incorporation of an aniline at the 2-position gave **7a**, an inhibitor with a >tenfold increase in potency against CDK2 over **6**. To increase potency further, a sulfonamide group was added to the *para*-position of the aniline ring to form a hydrogen bond to the Lys89. Analog **7b** (NU6102) was a highly potent CDK1 and CDK2 inhibitor (CDK1 K_i = 0.0095 μM; CDK2 K_i = 0.0054 μM), was selective versus CDK4 (> 250-fold compared to CDK2), and inhibited cell growth in MCF-7 tumor cells in vitro. The crystal structure of **7b** bound to the CDK2/cyclin A complex confirmed that the aniline ring was situated within the ATP binding cleft and directed towards the solvent interface. Interestingly, the X-ray structure of **7b** revealed that the sulfonamide group interacts with Asp86, rather than Lys89 as hypothesized. The $-NH_2$ of the sulfonamide donated a hydrogen bond to a side-chain carboxylate oxygen and a sulfonamide oxygen accepted a hydrogen bond from the backbone –NH– of Asp86. The importance of this interaction was highlighted by comparison of *N*-methylsulfonamide **7c** (CDK1



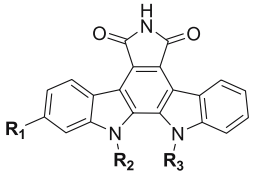
7a: R = -H
7b: R = $-SO_2NH_2$ (NU6102)
7c: R = $-SO_2NHCH_3$
7d: R = $-SO_2N(CH_3)_2$
7e: R = $-SO_2CH_3$

Structure 7

K_i = 0.009 μM; CDK2 K_i = 0.007 μM), that was as active as **7b**, with the *N*,*N*-dimethylsulfonamide **7d** (CDK1 K_i = 0.077 μM; CDK2 K_i = 0.056 μM) that was substantially less potent. These results indicate that at least one sulfonamide H-bond interaction was required for optimal binding to the enzyme. Consistent with this conclusion, the methylsulfone **7e** was equipotent (CDK1 K_i = 0.08 μM; CDK2 K_i = 0.063 μM) to **7d**.

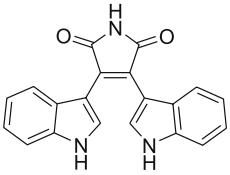
2.3.2 Pyrrolocarbazoles

A screening effort at Lilly identified the natural product arcyriaflavin A (**8a**) as a potent inhibitor of CDK4/cyclin D1 (IC_{50} = 0.16 μM) [59]. SAR development of this lead revealed that substitution at $-R_1$ enhances potency for CDK-4 by about twofold while increasing selectivity against CDK1 and 2, CamKII, and PKA. Addition of an alkyl group at $-R_2$ improved selectivity further. The most potent compound of this series was **8b** (CDK-4/cyclin D1 IC_{50} = 0.042 μM) with good selectivity against CamKII and PKA (IC_{50} > 2.0 μM for both) but with modest inhibition against CDK-2/cyclin E (IC_{50} = 0.121 μM). This compound had submicromolar anti-proliferative activity against HCT-116 and H460 tumor cell lines and induced G1 arrest in both cell lines at 0.5 μM and 2 μM concentrations. Unspecified analogs from this series were claimed to induce tumor growth delay in a HCT-116 tumor xenograft model. A head-to-head comparison of the indolo-[2,3-*a*]pyrrolo[3,4-*c*] carbazoles **8a–e** and their bis-indolylmaleimide synthetic precursors **9** demonstrated that between related analogs the pyrrolocarbazoles were almost tenfold more potent as CDK4 inhibitors than the maleimides [60]. Furthermore, as expected for potent inhibitors of CDK4 and CDK2, **8a–e** caused G1 phase arrest in HCT-116 and H460 tumor cells, while the maleimides (**9**) tested induced G2/M phase cell cycle arrest. These findings are exemplified by **8c–e**. Compound **8c** was a potent inhibitor of CDK4 (IC_{50} = 0.05 μM) and CDK2 (IC_{50} = 0.16 μM) with anti-proliferative activity against HCT-116 (IC_{50} = 0.52 μM) and H460 (IC_{50} = 0.53 μM). Methyl



8a: R_1, R_2, R_3= H (arcyriaflavin A)
8b: R_1= -F; R_2= -H; R_3= $-CH_3$
8c: R_1= -H; R_2=-H; R_3=$-(CH_2)_3NHCH_3$
8d: R_1=-H; R_2=$-CH_3$; R_3=-H
8e: R_1=-H; R_2=$-CH_3$; R_3=$-CH_2CH(OH)CH_2(OH)$

Structure 8

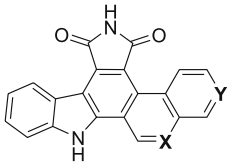


9: (bis-indolylmaleimides)

Structure 9

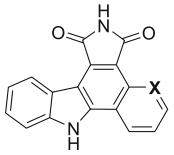
substitution in **8d** gave a compound that was potent against CDK4 (IC_{50} = 0.08 μM) but not against CDK2 (IC_{50} > 1.0 μM). Activity against both HCT-116 (IC_{50} = 0.51 μM) and H460 (IC_{50} = 0.41 μM) cell lines was maintained. Alkylation of both indole nitrogens in **8e** gave a compound with reduced CDK4 inhibition (IC_{50} = 0.26 μM) but potent CDK2 activity (IC_{50} < 0.06 μM). Anti-proliferative activity was submicromolar in both tumor cells lines for **8e**. The proposed binding model for these compounds rationalized much of the observed selectivity by invoking interactions between substituents at – R_2 and – R_3 with the peptide side chains of Asp86, Gln131 (CDK2) or Glu131 (CDK4) and Glu145 in the ATP binding site.

Quinoline and isoquinoline fused pyrrolocabazole analogs were a further elaboration of screening hit **8a** with improved enzyme and cell activity [61]. Compounds **10a** and **10b** represent ring fusions that gave the best CDK4 inhibition activity. The most potent compound was **10a** in both the enzyme and tumor cell anti-proliferation assays (CDK4/cyclin D1 IC_{50} = 0.069 μM, HCT-116 IC_{50} = 0.13 μM, H460 IC_{50} = 0.074 μM) while **10b** was effective in a HCT-116 tumor xenograft model. A broad survey of indole replacements found that the naphthyl[2,1-*a*] analog **10c** was a potent CDK4/cyclin D1 inhibition (IC_{50} = 0.045 μM), with good selectivity against PKA, PKCα, PKCβII, PKCγ, and glycogen synthase kinase-3β (GSK-3β) and modest selectivity versus CDK1 and 2 [62]. Unfortunately, **10c** had poor anti-proliferative activity against the HCT-116 (IC_{50} = 5.05 μM) and H460 (IC_{50} = 2.11 μM) tumor cell lines, possibly due to the low aqueous solubility of this molecule. Other



10a: X = CH; Y = N
10b: X = N; Y = CH
10c: X = CH; Y = CH

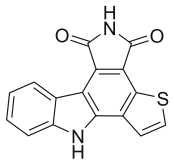
Structure 10



11a: X = N
11b: X = CH

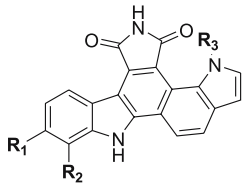
Structure 11

scaffolds, represented by **11a** (IC_{50} = 0.18 μM), **11b** (IC_{50} = 0.26 μM), and **12** (IC_{50} = 0.15 μM), had good activity against CDK4/cyclin D1. The Lilly group published an extensive study of indolo[6,7-*a*]pyrrolo[3,4-*c*]carbazoles (**13a–c**) [63]. Compound **13a** was potent as both a CDK4 (IC_{50} = 0.017 μM) and CDK2 (IC_{50} = 0.006 μM) inhibitor while having anti-proliferative activity in the HCT-116 (IC_{50} = 0.17 μM) and H460 (IC_{50} = 0.23 μM) cell lines. Selectivity against CDK2 was obtained with substitution at R_2. Introduction of a solubilizing group at this position gave **13b** (CDK4 IC_{50} = 0.008 μM; CDK2 IC_{50} = 0.978 μM) and **13c** (CDK4 IC_{50} = 0.019 μM; CDK2 IC_{50} = 1.03 μM). Between the two more soluble analogs, **13b** is superior as an anti-proliferative agent (HCT-116 IC_{50} = 0.20 μM; H460 IC_{50} = 0.14 μM). Compound **13c** was selective when screened against 39 kinases (IC_{50} > 5 μM).



12

Structure 12

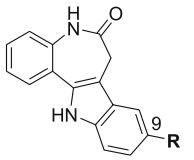


13a: R_1= -OH, R_2, R_3 = H;
13b: R_1=H, R_2=-CH_2CH_2-N-(4-hydroxypiperidine); R_3=-CH_3
13c: R_1= H, R_2=-$CH_2CH_2CH_2$NH-(4-hydroxycyclohexane); R_3=-CH_3

Structure 13

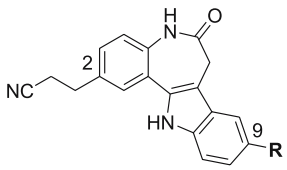
2.3.3 Paullones

With flavopridol (**1**) as a reference, a COMPARE search of compounds evaluated by the NCI as part of the Anti-tumor Drug Screen program (ADS) uncovered kenpaullone (**14a**) as a potential CDK1/cyclin B inhibitor (IC_{50} = 0.4 μM). Subsequent testing confirmed this activity [64]. From a model of **14a** docked in the ATP binding site of CDK2, it was suggested that substitution at the 9-position with a hydrogen bond acceptor might increase affinity by forming a H-bond with a conserved water molecule found in the crystal structure of CDK2 [65]. This hypothesis was confirmed by comparing the unsubstituted paullone scaffold **14b,** with poor CDK1 activity (IC_{50} = 7.0 μM), to 9-CN paullone **14c**, a 300-fold improvement (IC_{50} = 0.024 μM). Substitution with a nitro group at the 9-position gave alsterpaullone (**14d**, IC_{50} = 0.035 μM), a compound with submicromolar antiproliferative activity against the HCT-116 tumor cell line (GI_{50} = 0.83 μM). Further analysis of the model of **14a** docked in CDK2, suggested that substitution on the 2-position of the paullone scaffold with an alkyl chain terminated by a polar group should afford favorable interactions between the polar group and solvent or protein side chains at the solvent interface of the binding pocket. Synthesis and testing of a series of 2-alkyl-9-trifluoromethyl paullones identified the 2-cyanoethyl analog **15a** as a potent inhibitor of CDK1/cyclin B (IC_{50} = 0.047 μM) and tumor cell proliferation (HCT-116 GI_{50} = 0.79 μM) [66]. A further improvement was observed for the 2-cyanoethyl version of alsterpaullone **15b**. This compound was reported to be a remarkably potent inhibitor of CDK1/cyclin B (IC_{50} = 0.00023 μM), CDK5/p25 (IC_{50} = 0.030 μM), and GSK-3β (IC_{50} = 0.0008 μM) [67]. The se-



14a: (kenpaullone) R = Br
14b: (paullone) R = H
14c: R = CN
14d: (alsterpaullone) R = NO_2

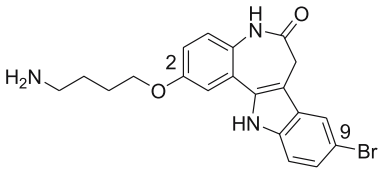
Structure 14



15a: R = CF_3
15b: R = NO_2

Structure 15

lectivity of the paullones was investigated by affinity chromatography. The 4-aminobutoxy group substituted at the 2-position of the kenpaullone scaffold (**16**, nee *gwennpaullone*) was found to be a suitable side chain analog for immobilization to an agarose matrix. Following elution of porcine brain extracts from the modified agarose with increasing concentrations of ATP, analysis of the bound proteins by SDS-PAGE confirmed GSK-3α and GSK-3β as additional kinase targets inhibited by the paullones [68]. The CDK2/cyclin A crystal structure was used as a starting point for a homology model of **14a** bound to the ATP site of CDK1/cyclin B. This model was the basis of a 3D-QSAR study that resulted in the accurate prediction of the CDK1 pIC_{50} values for 9-cyanopaullone **14c** and the 9-nitro alsterpaullone **14d** [69, 70]. Using a CoMSIA analysis, the comparison of several equations with different combinations of electronic and steric parameters, showed that a simple relationship between the polar effect (σ_m) of the 9-substituent gave the best prediction of CDK1 pIC_{50} values. Because derivatives of the paullone scaffold were known to have good activity for GSK-3 and CDK5, targets of interest for diabetes and Alzheimer's disease, the CoMSIA approach was applied to both GSK-3 and CDK5 to provide a rational basis for the design of selective inhibitors of these enzymes [71].

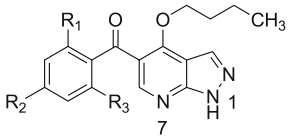


16: gwennpaullone

Structure 16

2.3.4 1*H*-Pyrazolo[3,4-*b*]pyridine

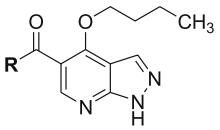
Bristol Myers Squibb has reported a series of 1*H*-pyrazolo[3,4-*b*]pyridines to be CDK1/CDK2 selective inhibitors [72–74]. Initial screening efforts identified SQ-67563 (**17a**), as an inhibitor with moderately potent enzyme activity (CDK1/cyclin B IC_{50} = 0.15 μM, CDK2/cyclin E IC_{50} = 0.11 μM) and selectivity for the CDKs. Optimization efforts led to the discovery of BMS-265246 **17b**, a potent and selective inhibitor of CDK1 and CDK2 (CDK1/cyclin B IC_{50} = 0.006 μM, CDK2/cycline E IC_{50} = 0.009 μM and CDK4 IC_{50} = 0.23 μM). Critical to the activity of these inhibitors was the 2,6-difluorobenzoyl substituent. Benzoyl group substitution analogs revealed a *para* methyl group (**17c**, CDK2/cycline E IC_{50} = 0.21 μM) was preferred over the *ortho* or *meta* substitutions. 2,6-Difluoro-substitution (**17d**, CDK1/cyclin B IC_{50} = 0.032 μM, CDK2/cycline E IC_{50} = 0.064 μM and CDK4 IC_{50} = 21 μM) conferred im-



17a: (SQ-67563) R_1,R_2,R_3 = H
17b: (BMS265246) R_1=F, R_2=CH_3,R_3=F
17c: R_1=H, R_2=CH_3, R_3=H
17d: R_1=F, R_2=H, R_3=F

Structure 17

proved CDK1 and CDK2 activity with selectivity against CDK4. Structure–activity studies showed that replacing the benzoyl group of **17a** with small alkyl groups such as methyl (**18a**, CDK2/cyclin E IC_{50} > 1.0 μM) resulted in a >tenfold loss in activity while a 3-pyridyl ring (**18b**, CDK2/cyclin E IC_{50} = 2.4 μM) caused about a 200-fold loss in potency. An amide (**18c**, R = PhNH-, CDK2/cyclin E IC_{50} = 19 μM) and a methyl ester (**18d**, CDK2/cyclin E IC_{50} > 1.0 μM) at this position were not tolerated. On the other hand, neutral heterocycles, such as the 2-furanyl analog (**18e**, CDK1/cyclin B IC_{50} = 0.28 μM, CDK2/cyclin E IC_{50} = 0.18 μM) had activity comparable to the initial hit **17a.** Compound **17b** was the most potent CDK1/CDK2-selective inhibitor of this chemotype. It was 25- and 11-fold more potent than **17a** versus CDK1 and CDK2, respectively. Furthermore, **17b** has anti-proliferative activity against the A2780 ovarian cancer cell line (IC_{50} = 0.78 μM). X-ray crystal structures of a 4-bromo-2,6-difluorobenzoyl analog and **17a** bound in the ATP binding site of unactivated CDK2 have been reported. These structures reveal that both compounds form a hydrogen bond between the pyrazolopyridine 1-NH and the 7-position ring nitrogen with the backbone amide carbonyl oxygen and NH of Leu83, while the 4-butoxyl side chain spans the region occupied by the ribose of ATP without forming specific contacts with the protein. The 4-bromo-2,6-difluorobenzoyl and benzoyl group of **17b** lie buried within the protein, contacting Phe80 through a lipophilic stacking interaction.

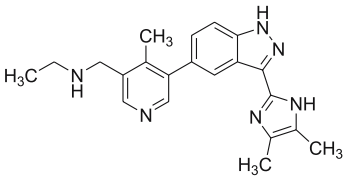


18a: R=CH3
18b: R=3-pyridyl
18c: R=PhNH-
18d: R=MeO-
18e: R=2-furanyl

Structure 18

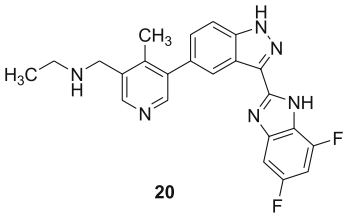
2.3.5
3,5-Substituted-Indazoles

A novel series of 3,5-substituted indazoles were reported by Agouron (now Pfizer) to be CDK2/cyclin A inhibitors [75, 76]. Compound **19** was a potent inhibitor of CDK2/cyclin A (**19** K_i = 0.05 μM), while **20** (AG-024322) was a pan-CDK inhibitor with K_i values in the range of 0.001 to 0.003 μM for CDKs 1, 2, and 4. Compound **20** had potent in vitro anti-proliferative activity against the HCT-116 colon tumor cell line (IC_{50} = 0.030 μM), the lung tumor cell line A549 (IC_{50} = 0.090 μM), breast MDA-MB-468 (IC_{50} = 0.20 μM), and ovarian A2780 (IC_{50} = 0.080 μM). In vivo **20** inhibited tumor cell growth in HCT-116, A2780, and HL60 (leukemia) xenograft models [77]. Compound **20** (AG-024322) is in early human clinical trials.



19

Structure 19

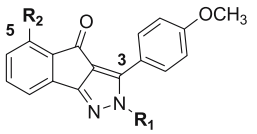


20

Structure 20

2.3.6
Indeno[1,2-*c*]pyrazol-4-ones

High throughput screening of the DuPont Pharmaceuticals (now Bristol-Myers Squibb) compound collection against CDK4/cyclin D led to the identification of **21a** (CDK4/cyclin D IC_{50} = 26 μM, CDK2/cyclin E IC_{50} = 45 μM) [78, 79]. Screening against other CDKs and relevant serine/threonine kinases showed **21a** to be selective for the CDK family. SAR studies with this series demonstrated that the pyrazole NH provided a key interaction with the enzyme since the *N*-methyl analog **21b** was inactive (CDK4/cyclin D IC_{50} > 340 μM, CDK2/cyclin E IC_{50} > 340 μM). Modifications of the aromatic

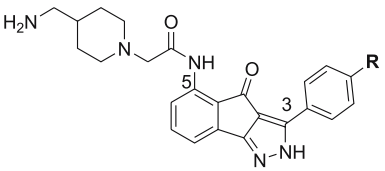


21a: R_1= H, R_2 = H
21b: R_1 = CH_3, R_2 = H
21c: R_1 = H, R_2 = NHAc
21d: R_1 = H, R_2 = NH(CO)CH_2(N-methylpiperazin-1-yl)
21e: R_1 = H, R_2 = NH(CO)CH_2(morpholin-1-yl)
21f: R_1 = H, R_2 = NH(CO)NH_2
21g: R_1 = H, R_2 = NH(CO)N(C_2H_5)$_2$
21h: R_1 = H, R_2 = NH(CO)NH(4-methylpiperazin-1-yl)
21i: R_1 = H, R_2 = NH(CO)NH(morpholin-1-yl)

Structure 21

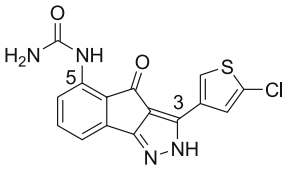
portion of the indenopyrazole to introduce a hydrogen bond donating substituent gave the 5-acetamido analog **21c** (CDK4/cyclin D IC_{50} = 0.46 μM, CDK2/cyclin E IC_{50} = 0.51 μM). Extensive variation at – R_2 suggested the presence of a binding pocket that was rather large and promiscuous [80]. According to modeling studies – R_2 was situated within the ATP binding pocket, not just protruding out to solvent; this hypothesis was confirmed by X-ray crystallography. A variety of substituted *N*-glycyl analogs were prepared with high affinity for CDK2 and CDK4. The most active analogs were *N*-methylpiperazinyl **21d** (CDK4/cyclin D IC_{50} = 0.125 μM, CDK2/cyclin E IC_{50} = 0.012 μM), and the morpholine **21e** (CDK4/cyclin D IC_{50} = 0.195 μM, CDK2/cyclin E IC_{50} = 0.021 μM). The addition of the highly polar substituents to these molecules dramatically improved their physical properties [81]. Replacing the *N*-alkylglycinamide group at – R_2 with a urea also produced potent analogs. The most active was unsubstituted **21f** (CDK4/cyclin D IC_{50} = 0.066 μM, CDK2/cyclin E IC_{50} = 0.007 μM) while disubstitution of the urea, as with the *N*′,*N*′-diethyl analog **21g** (CDK4/cyclin D IC_{50} > 1.3 μM, CDK2/cyclin E IC_{50} > 1.3 μM), rendered the series inactive. This loss in activity was postulated to arise from a combination of intolerance of the binding pocket to branching at this position and the loss of a NH hydrogen bond. Although the urea modification produced potent compounds they were poorly soluble compared to the glycinamides [81]. Introducing a semicarbazide moiety at – R_2 improved the CDK4 activity, giving compounds that were equipotent for both CDK2 and CDK4. Two analogs, **21h** (CDK4/cyclin D IC_{50} = 0.009 μM, CDK2/cyclin E IC_{50} = 0.012 μM) and **21i** (CDK4/cyclin D IC_{50} = 0.012 μM, CDK2/cyclin E IC_{50} = 0.018 μM), were tenfold more potent against CDK4 than the corresponding glycinamides. Molecular modeling studies could account for the improved activity of the ureas and semicarbazides over the glycinamides: the added NH of the semicarbazide or urea functionality formed an additional H-bond with the side chain carbonyl of Asp156.

A variety of groups substituted on the 3-position of the pyrazole were accommodated including phenyl, heterocycles, and alkyl. In the case of the 3-phenyl analogs, small alkyl, aminoalkyl, and alkoxide substituents were tolerated in the *para* position of the phenyl ring. The most potent analogs were a series of glycinamides with either the *para*-dimethyl-aminophenyl (**22a**, CDK4/cyclin D IC_{50} = 0.007 μM, CDK2/cyclin E IC_{50} = 0.015 μM) or *para*-morpholinophenyl (**22b**, CDK4/cyclin D IC_{50} = 0.018 μM, CDK2/cyclin E IC_{50} = 0.026 μM) at the 3-position. In both cases the *N*-4-((aminomethyl)piperidine)glycinamide substituent at the 5-position gave very potent CDK4/cyclin D and CDK2/cyclin E activity. Substitution of the 3-position with a thiophene or thiazole yielded potent analogs in the 5-semicarbazide series [81]. While these compounds were more potent than the 3-phenyl and 3-alkyl analogs, 3-thiophenes and 3-thiazoles usually displayed high plasma protein binding. However, compounds **23** and **24** from this series were shown to have anti-proliferative activity and selectivity for HCT116 (**23** IC_{50} = 0.13 μM; **24** IC_{50} = 0.014 μM) cancer cells over a "normal" human fibroblast cell line AG1523 (**23** IC_{50} > 30 μM; **24** IC_{50} > 21 μM), suggesting the potential for a therapeutic window between spontaneously proliferating cancer cells and quiescent normal cells. They displayed excellent activity against CDK1/cyclin B (**23** IC_{50} = 0.004 μM; **24** IC_{50} = 0.006 μM), CDK2/cyclin E (**23** IC_{50} = 0.013 μM; **24** IC_{50} = 0.014 μM), and CDK4/cyclin D1 (**23** IC_{50} = 0.057 μM; **24** IC_{50} = 0.007 μM) as well as good selectivity against other kinases. Both analogs demonstrated activity against a broad panel of human and murine tumor cell lines.



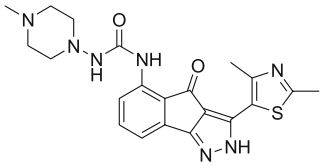
22a: R = $N(CH_3)_2$
22b: R = morpholin-4-yl

Structure 22



23

Structure 23

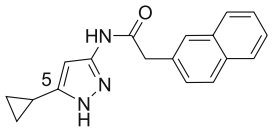


24

Structure 24

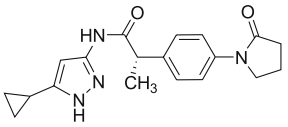
2.3.7
3-Aminopyrazoles

Pharmacia (now Pfizer) reported on a series of *N*-acyl-3-aminopyrazoles as potent CDK2 inhibitors [82–84]. Compounds **25** and **26** inhibited CDK2/cyclin A (**25** and **26** $IC_{50} = 0.037\ \mu M$) and had modest selectivity against CDK1/cyclin B (**25** $IC_{50} = 0.27\ \mu M$; **26** $IC_{50} = 0.208\ \mu M$) and CDK5/p25 (**25** $IC_{50} = 0.114\ \mu M$; **26** $IC_{50} = 0.065\ \mu M$). However, these compounds were > 500-fold selective against CDK4 and a panel of 31 unrelated kinases. Both inhibited A2780 and HT-29 tumor cell proliferation in vitro and exhibited anti-tumor activity in-vivo at 7.5 mg/kg (p.o., bid) in the human ovarian A2780 xenograft model with > 50% inhibition of tumor growth for **25** and > 70% inhibition for **26**. Compounds **25** and **26** had good ADME properties; however **25** had higher plasma protein binding (99% bound) than **26** (74% bound). In the crystal structure of **25** bound to CDK2 (Fig. 3), the 5-position of the pyrazole ring was oriented toward the Phe80 residue, thus the addition of large groups at this site was predicted to cause an unfavorable steric interaction and result in a loss of binding affinity. Consistent with this analysis, only small cycloalkyl groups were accommodated at the 5-position.



25

Structure 25



26

Structure 26

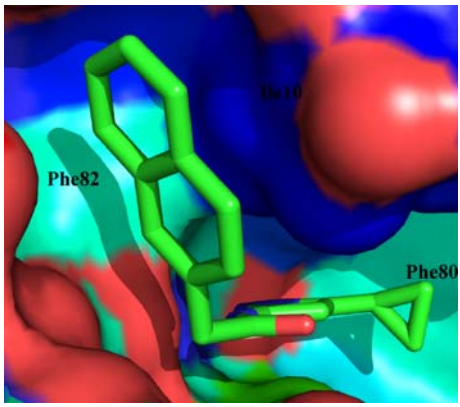
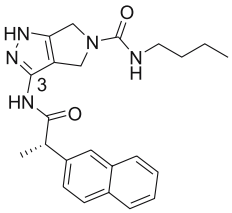


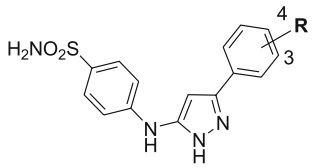
Fig. 3 Compound **25** bound to CDK2

A second series of pyrrolo[3,4-*c*]pyrazoles exemplified by **27** was reported by Pfizer [85]. Compound **27** was a potent CDK2/cyclin A inhibitor (IC_{50} = 0.036 μM) with moderate selectivity against CDK1/cyclin B (20-fold) and CDK5/p25 (fourfold), and good selectivity versus CDK4/cyclin D (> 278 fold) and Aurora-A (> 278-fold). Compound **27** did not significantly inhibit other protein kinases (IC_{50} > 10 μM) in a panel of 22 enzymes; however, it was a potent inhibitor of GSK-3β. This compound was an effective anti-proliferative agent against the A2780 ovarian tumor cell line (IC_{50} = 0.130 μM), as well as the HCT116 (IC_{50} = 0.120 μM) and HT-29 (IC_{50} = 0.070 μM) colon tumor cell lines. Analysis of the cell cycle profile and the CDK2 substrate phosphorylation status demonstrated that the anti-proliferative effect was mediated by CDK2 inhibition. SAR studies found the combination of a 3-arylacetamide substituent with the pyrrolo[3,4-*c*]pyrazole scaffold, as with **27**, produced inhibitors that preferentially inhibited CDK2/cyclin A, whereas, substitution with a 3-benzamide group produced inhibitors of Aurora-A. GlaxoSmith Kline disclosed a series of *N*-aryl 3-aminopyrazoles as inhibitors of CDK2 [86]. Compound **28a** was a potent inhibitor of CDK2 (IC_{50} = 0.00034 μM) with 1000-fold selectivity versus CDK1 and six other ki-



27

Structure 27



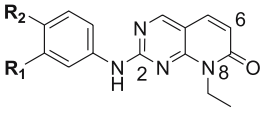
28a: 4-NH_2
28b: 3-Br

Structure 28

nases. In an X-ray crystal structure of **28b** with activated CDK2, this inhibitor was bound in the ATP binding pocket of the kinase with the sulfonamide NH forming a hydrogen bond to the Asp86 residue located at the opening to the ATP binding cleft.

2.3.8 Pyrido[2,3-*d*]pyrimidin-7-one

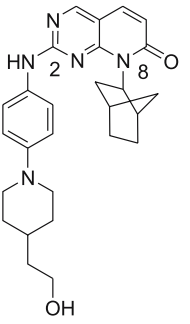
Pfizer/Warner Lambert disclosed in patents and papers the CDK inhibition activity of pyrido[2-3-*d*]pyrimidin-7-ones **29a–e** [87–92]. The 6-aryl substituted pyrido[2-3-*d*]pyrimidin-7-one template was the starting point for the program, though it was known to be active against EGFr, FGFr, PDGFr, and c-Src. Homology binding models suggested that if the 6-aryl group of the pyrido[2-3-*d*]pyrimidin-7-one core was removed, the resulting molecule would be accommodated by the CDK ATP binding site. This was confirmed with compound **29a** (CDK4/cyclin D $IC_{50} = 0.62\ \mu M$). SAR studies of the 2-aniline substitution pattern showed a strong preference for electron releasing substituents in the *para* position. For example, the *m*-methoxyaniline analog **29b** (CDK4/cyclin D $IC_{50} > 40\ \mu M$) was inactive versus CDK4 while the *p*-methoxyaniline **29c** (CDK4/cyclin D $IC_{50} = 0.60\ \mu M$) was equipotent to **29a**. The most effective *para*-substituents in this study were the 2-(dimethylamino)ethoxy analog **29d** (CDK4/cyclin D $IC_{50} = 0.16\ \mu M$) and the *N*-methylpiperizine **29e** (CDK4/cyclin D $IC_{50} = 0.085\ \mu M$). Tertiary amine substituents, as exemplified by **29d** and **29e**, enhanced potency through



29a: R_1, R_2=H
29b: R_1=OCH_3, R_2=H
29c: R_1=H, R_2=OCH_3
29d: R_1=H, R_2=$OCH_2CH_2N(CH_2CH_3)_2$
29e: R_1=H, R_2=N-methypiperizine

Structure 29

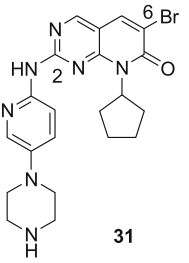
a beneficial electrostatic interaction with the carboxylate side chain of Asp98. SAR studies focused on the 8-position of the pyrido[2-3-*d*]pyrimidin-7-one demonstrated the presence of a large hydrophobic pocket. There was a preference for cycloalkyl groups over linear alkyl or aryl groups. Combination of the best 2-anilines with the most potent 8-position cycloalkyl moieties gave analogs with greatly improved activity, such as **30** (CDK4/cyclin D $IC_{50} = 0.008\ \mu M$).



30

Structure 30

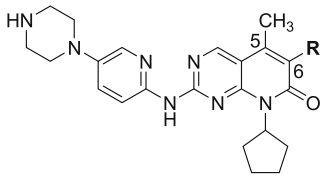
These CDK inhibitors were evaluated against CDK1/cyclin B, CDK2/cyclin A, CDK2/cyclin E and CDK4/cyclin D. In general, they have modest selectivity for CDK4 over CDK1 and 2. Compound **30** had the best profile with greater than 15-fold selectivity for CDK4/cyclin D over CDK2/cyclin E and no inhibition activity against CDK1/cyclin B. Furthermore **30** was a potent inhibitor of proliferation of the HCT116 human colon cell line ($IC_{50} = 0.213\ \mu M$). When the 2-aniline was replaced with a 2-aminopyridine substituent there was a substantial improvement in CDK4 selectivity over CDK2. Further modification with the addition of a bromine atom at the 6-position resulted in compound **31** (CDK4/cyclin D $IC_{50} = 0.016\ \mu M$, CDK2/cyclin A $IC_{50} > 5\ \mu M$, CDK2/cyclin E $IC_{50} > 5\ \mu M$), a CDK4 selective



31

Structure 31

inhibitor reported to induce G1 arrest on asynchronously growing Rb-positive MDA-MB453 breast cancer cells. A wide range of substitutions at the 6-position improved potency while maintaining good selectivity, including small polar groups such as NH_2, non-polar groups such as ethyl, and chain-extended substituents such as methoxyethoxymethyl. However, the bulky sec-butoxy group was not tolerated, suggesting a size limitation in this region of the binding pocket. A methyl group at the 5-position of the pyrido[2-3-*d*]pyrimidin-7-one template influenced selectivity and potency. This effect of the 5-methyl group was dependent on the nature of the adjacent 6-substituent. Improved potency and selectivity was observed for **32a** where the 5-methyl group was paired with a 6-acetyl group (CDK4/cyclin D IC_{50} = 0.011 μM, CDK2/cyclin A IC_{50} > 5 μM) rather than the 6-bromine atom in **32b** (CDK4/cyclin D IC_{50} = 0.16 μM, CDK2/cyclin A IC_{50} > 5 μM). This result suggested that the 6-acetyl carbonyl can form an effective H-bond interaction with the protein only when the adjacent 5-methyl group forces the carbonyl out of the plane of the pyrido[2-3-*d*]pyrimidin-7-one template. Extensive SAR studies determined that the optimal substituent for the 2-aminopyridine side chain giving superior potency, selectivity, and physicochemical properties was the piperazine of **32a** and **32b** [91]. Compound **32a** (PD0332991) was chosen for further biological evaluation. It was a highly selective inhibitor of CDK4 with weak activity against a panel of 36 protein kinases, had potent anti-proliferative effects against Rb-positive cell lines and induced G1 arrest of the cell cycle in the same cell line. Oral administration of **32a** to mice bearing the Colo-205 human colon carcinoma xenografts produced significant tumor regression. Furthermore, **32a** was orally bioavailable (F = 56%) and had moderate clearance (Cl = 37.5 mL/min/kg) in rat. This compound is currently in Phase I clinical trials.

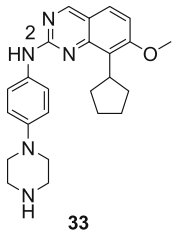


32a: (PD0332991) R=acetyl
32b: R=Br

Structure 32

2.3.9 Quinazolines

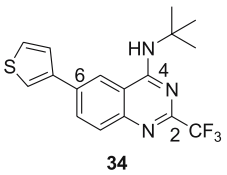
Pfizer/Warner Lambert filed patent applications covering quinazolines, such as **33** (CDK4/cyclin D IC_{50} = 0.001 μM, CDK1/cyclin B IC_{50} = 0.132 μM, CDK2/cyclin A IC_{50} = 0.28 μM, CDK2/cyclin E IC_{50} = 0.25 μM) as CDK in-



33

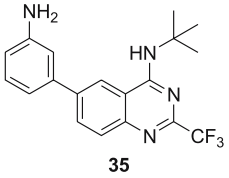
Structure 33

hibitors [93, 94]. As in the case of the pyrido[2-3-*d*]pyrimidin-7-ones, incorporation of a 2-aminopyridine side chain at the 2-position enhanced selectivity for CDK4. Dupont Pharmaceutical disclosed a series of quinazolines as inhibitors of CDK4/cyclin D and CDK2/cyclin E [95]. SAR studies on the quinazoline template demonstrated that small, non-ionizable electron-withdrawing substituents such as trifluoromethyl and trichloromethyl were preferred at the 2-position. Branched alkyl amines such as t-butyl, t-amyl and benzyl amine groups were preferred at the 4-position. The 6-position was found to be sensitive to both the size and the electronic nature of the substituent as exemplified by **34** (CDK2/cyclin E $IC_{50} = 0.54\ \mu M$) and **35** (CDK2/cyclin E $IC_{50} = 0.65\ \mu M$). This series of quinazolines had a five- to 20-fold selectivity for CDK2/cyclin E over CDK4/cyclin D. The crystal structure of **35** with CDK2 revealed that the inhibitor was bound to the ATP pocket and made an indirect hydrogen bond via a water molecule from the 1-position nitrogen of the quinazoline to the backbone carbonyl oxygen of Glu81 located in the hinge area. Compound **36** (CDK2/cyclin E $IC_{50} = 0.93\ \mu M$) inhibited the growth of HCT116 cancer cells ($IC_{50} = 5.72\ \mu M$) but had no effect on "normal" fibroblasts under identical assay conditions.



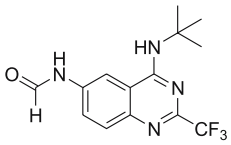
34

Structure 34



35

Structure 35

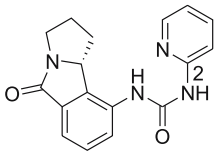


36

Structure 36

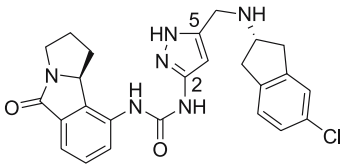
2.3.10 Diaryl Ureas

Banyu/Merck recently reported an elegant de novo design effort to optimize a novel series of diarylureas as potent and selective CDK4 inhibitors [96–100]. This effort began with the X-ray crystal structure of a potent lead compound **37** with broad CDK inhibition activity (CDK4/cyclin D IC_{50} = 0.042 μM, CDK2/cyclin A IC_{50} = 0.078 μM, CDK1/cyclin B IC_{50} = 0.12 μM) and moderate selectivity (> 50-fold) over other kinases. The non-conserved amino acids in the ATP binding site of this CDK4 crystal structure were identified from the protein alignments of 390 representative kinases. Next, using the locations of the altered amino acid residues and the binding mode of the lead compound, **37** was redesigned to produce a highly selective and potent CDK4 inhibitor. Based on these modeling studies it was determined that replacing the 2-aminopyridine urea substituent in **37** with a 2-aminopyrazole would give access to the non-conserved residues while maintaining critical intramolecular hydrogen bonding interactions. Three amino acids of CDK4, Asp99, Thr102, and Gln98, found in the p16-binding region of the ATP binding pocket, were selected as the residues to target for improved selectivity. The 5-position of the pyrazole ring was predicted to be the most appropriate site to allow optimal contact with the side chains of the targeted residues. The 5-position was modified with a variety of (alkylamino)methyl-substituents with the (5-chloroindan-2-ylamino)methyl- analog giving a highly potent and selective inhibitor **38** (CDK4/cyclin D IC_{50} = 0.0023 μM, CDK2/cyclin A IC_{50} = 0.44 μM, CDK1/cyclin B IC_{50} = 1.8 μM). Compound **38** was selective for CDK4 over CDK1 (780-fold) and CDK2 (190-fold) as well as many other kinases (> 430-fold). This compound was reported to induce G1 arrest in the



37

Structure 37



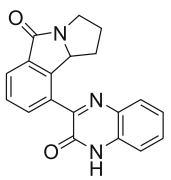
38

Structure 38

Molt-4 cancer cells, an Rb(+) cell line, at concentrations between 0.1 and 0.5 μM.

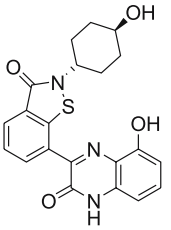
2.3.11 Quinox-2(1*H*)-ones

A Banyu patent application explored the potential of introducing a conformational restriction to the diaryl urea platform of the previous CDK4 inhibitor series (vide supra) through a series of quinoxalin-2(1H)-ones as inhibitors of CDK4 and CDK6 [101]. The quinoxalin-2(1H)-one template, exemplified by compounds **39** and **40**, was derived by cyclizing the diaryl urea between the urea-NH and the 2-pyridyl nitrogen of **37** to mimic the critical intramolecular hydrogen bonding contact between these atoms. The kinase inhibition activity for CDK4/cyclin D (**39** IC_{50} = 0.12 μM; **40** IC_{50} = 0.005 μM) and CDK6/cyclin D (**39** IC_{50} = 0.09 μM) was reported in the patent application; however, no selectivity data was presented. Banyu expanded on the theme of introducing conformational restrictions with a series of macrocyclic quinoxaline derivatives [102]. Compound **41** was a very potent inhibitor of both CDK4/cyclin D (IC_{50} = 0.012 μM) and CDK6/cyclin D (IC_{50} = 0.024 μM) in vitro and prevented the proliferation of HTC-116 human colon cancer cells (IC_{50} = 0.016 μM).



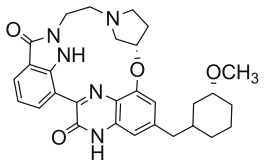
39

Structure 39



40

Structure 40

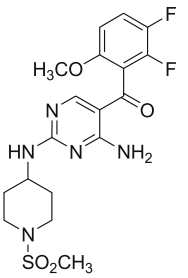


41

Structure 41

2.3.12 Pyrimidines

Roche has reported a 2,4-diaminopyrimidine **42** (Ro-4584820) to be a Phase I clinical candidate [48, 103]. Compound **42** is a pan-CDK inhibitor (CDK1 K_i = 0.001 μM; CDK2 K_i = 0.003 μM; CDK4 K_i = 0.001 μM), consequently it induces both G1 and G2 cell cycle arrest in tumor cell lines. It is a potent inhibitor of tumor cell proliferation in both the HCT-116 colon cancer (IC_{50} = 0.080 μM) and the H460A (IC_{50} = 0.055 μM) lung cancer cell lines.

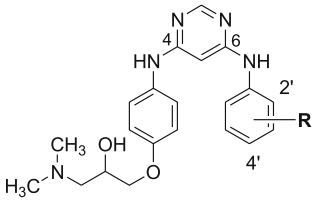


42

Structure 42

This compound is effective in tumor xenograft models when dosed orally or IV. Compound **42** is in safety and tolerability studies in humans.

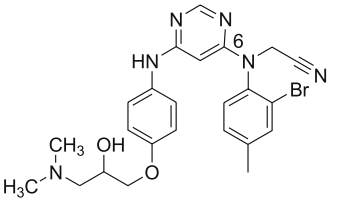
A high throughput screening campaign carried out by AstraZeneca identified a 4,6-bis-anilino pyrimidine compound **43a** that was a weak inhibitor of CDK4 activity (IC_{50} = 15 μM) and was selective against CDK2 (IC_{50} = 93 μM) [104]. Following this discovery, efforts focused on the goal of improving potency to identify a compound suitable for X-ray crystallography. Initial SAR studies evaluated the effect of substitution on the 6-aniline. It was found that many substituents were tolerated with the 2′-bromo (**43b** CDK2/cyclin E IC_{50} = 28 μM, CDK4/cyclin D IC_{50} = 4 μM), and 2′-nitro (**43c** CDK2/cyclin E IC_{50} = 25 μM, CDK4/cyclin D IC_{50} = 2 μM) the most potent. Disubstituted derivatives were also prepared with two compounds, **43d** (CDK2/cyclin E IC_{50} = 38 μM, CDK4/cyclin D IC_{50} = 9 μM) and **43e** (CDK2/cyclin E IC_{50} = 35 μM, CDK4/cyclin D IC_{50} = 6 μM), having sufficient potency and solubility to obtain protein crystal structures. In this case, the inactive CDK2 monomer was used as a structural surrogate for CDK4. This tactic successfully determined the bound conformation for this series and was used to construct a binding model for the CDK4 target. The observed interactions were consistent with those found for other pyrimidine kinase inhibitors. The pyrimidine N1 was a hydrogen bond acceptor with the – NH – of Leu83 while the 6-aniline – NH – formed a hydrogen bond donor contact with the backbone carbonyl of Leu83. The *para*-substituent of the 4-aniline was oriented towards the solvent interface at the edge of the ATP binding pocket while 6-aniline was buried inside the binding site. In the CDK2/**43e** structure a water molecule was situated between the 4-aniline – NH – and the Asp145 residue, while in the CDK2/**43d** structure this water molecule appeared to be displaced by the *ortho*-fluorine atom of **43d**. From the X-ray structure data it was determined that N-alkylation of the 6-aniline NH would force the aniline into a conformation similar to the CDK2/**43e** structure and displace the bound water molecule. N-Alkylation improved the potency of the series with the *N*-cyanomethyl analog giving a 20-fold increase in CDK4 activity versus an



43a: R = 2',4'-difluoro
43b: R = 2'-Br
43c: R = 2'-NO_2
43d: R = 2',6'-difluoro
43e: R = 2'-F, 5'-CF_3

Structure 43

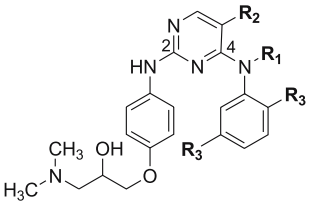
unsubstituted compound. Increased chain length or removal of the cyano group resulted in a loss of activity, indicating that this group made a specific interaction with the protein. An X-ray structure was obtained with compound **44** (CDK2/cyclin E IC_{50} = 3 μM, CDK4/cyclin D IC_{50} = 0.1 μM) complexed with CDK2. As predicted, the 6-aniline was situated in a conformation similar to that observed for compound **43e**, the cyanomethyl group displaced the bound water molecule and the carbon-nitrogen triple bond made a stacking interaction with the π-cloud of the Phe80 side chain. This binding mode was consistent with the observed loss of activity upon extending the chain length and the removing the nitrile.



44

Structure 44

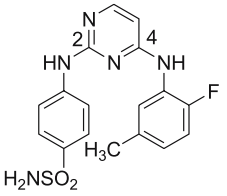
In comparative SAR studies, the 2,4-bis-anilinopyrimidines (**45a–d**) were found to be about tenfold more potent for CDK4 and more selective against CDK2 than the corresponding 4,6-bis-anilinopyrimidine analogs [105]. Unlike the 4,6-derivatives, there was little change in potency over a wide range of substituents for the 2,4-derivatives: disubstitution on the corresponding 4-aniline of the 2,4-bis-anilino series did not increase potency but caused a loss in CDK4/CDK2 selectivity. An X-ray structure of the 2,4-bis-anilinopyrimidine **45a** (CDK2/cyclin E IC_{50} = 1.0 μM, CDK4/cyclin D



45a: R_1, R_2 = H, R_3 = Cl
45b: R_1= CH_2CN, R_2 = H, R_3= Cl
45c: R_1= -CH_2CCH, R_2= H, R_3 = Cl
45d: R_1= H, R_2= Br, R_3= Cl
45e: R_1= -$CH_2CH_2CH_2CF_3$, R_2 = Br, R_3 = H

Structure 45

IC_{50} = 0.8 μM) complexed with CDK2 adopted a different conformation in the binding site compared to the 4,6-series, with a change in the tilt of the 4-aniline relative to the pryrimidine core. N-Alkylation, which improved potency in the 4,6 series, had a less dramatic effect on the 2,4-bis-anilinopyrimidines. Once again, the *N*-cyanomethyl **45b** was a potent inhibitor (CDK2/cyclin E IC_{50} = 0.7 μM, CDK4/cyclin D IC_{50} = 0.1 μM) and the *N*-propyne analog **45c** (CDK2/cyclin E IC_{50} = 2.0 μM, CDK4/cyclin D IC_{50} = 0.2 μM) was equally effective. Following examination of the CDK2/**44** X-ray structure it was suggested that a substituent at the 5-position of the 2,4-dianilino-pyrimidine core would reach the same area occupied by the *N*-cyanomethyl substituent. It was found that the 5-halo analogs, such as **45d** (CDK2/cyclin E IC_{50} = 10.0 μM, CDK4/cyclin D IC_{50} = 0.1 μM), gave compounds as potent against CDK4 as the *N*-alkyl series but with improved selectivity against CDK2. A crystal structure of compound **45d** bound to CDK2 revealed that the water molecule evicted by the *N*-cyanomethyl group in the CDK2/**44** structure was still in position and the bromine atom of **45d** was packed against the side-chain phenyl of the Phe80 residue. Based on the SAR and X-ray structure analysis it was suggested that more polar, larger substituents at the 5-position would force the 4-aniline into a more favorable conformation and improve the packing interaction with the phenyl of Phe80. If this hypothesis was correct then combining both features should yield compounds with increased potency. Indeed, this was confirmed by compound **45e** with a bromine at the 5-position and a *N*-(4,4,4-trifluoro)butyl group appended to the nitrogen atom at the 4-position, this compound had improved potency against CDK4 (IC_{50} = 0.01 μM) and CDK2 (IC_{50} = 0.2 μM). The selectivity profile can be shifted in favor of CDK2 over CDK4 in the 2,4-bis-anilinopyrimidine series by altering the *para*-substituent on the 2-aniline [106]. Where the 3-dimethylaminopropan-2-ol of **45e** gave enhanced inhibition activity against CDK4, the *para*-sulfonamide group favored inhibition of CDK2. Compound **46** nicely illustrated this reversal of selectivity (CDK2/cyclin E IC_{50} = 0.006 μM, CDK4/cyclin D IC_{50} = 2.7 μM).

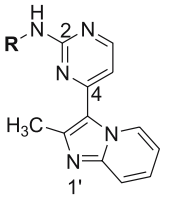


46

Structure 46

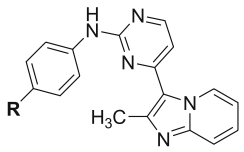
2.3.13
3-(Pyrimidin-4-yl)imidazo[1,2-*a*]pyridine and 3-(Pyrimidin-4-yl)imidazo[1,2-*b*]pyridazines

A high-throughput screen of the AstraZeneca compound library identified the 3-(pyrimidin-4-yl)imidazo[1,2-*a*]pyridine **47a** as an inhibitor of CDK2 (IC_{50} = 4 μM) and CDK4 (IC_{50} = 8 μM) [106]. Substitution of the 2-amino group led to **47b**, a compound with sufficient potency (CDK2/cyclin E IC_{50} = 2.5 μM) and solubility to obtain a crystal structure with the inactivated CDK2 enzyme. The imidazo[1,2-*a*]pyridine **47b** formed a hydrogen-bonding network between the pyrimidine N1 and Leu83 NH, the 2-acetamido NH of **47b** and the amide carbonyl of Leu83, and the imidazo[1,2-*a*]pyridine *N*1′ and the Lys33 side-chain amine. A water molecule acts as a bridge between the 2-acetamide carbonyl of **47b** and the Asp86 carboxylate. Addition of an aniline at the 2-position to the 3-(pyrimidin-4-yl)imidazo[1,2-*a*]pyridine core resulted in **48a**, a compound with a 100-fold improved inhibition activity against CDK2 (IC_{50} = 0.036 μM) and selective against CDK4 (IC_{50} = 3.6 μM). As with previous series, adding a sulfonamide as a *para*-substituent on the 2-aniline improved the CDK2 selectivity. For **48b** (CDK2/cyclin E IC_{50} = 0.032 μM, CDK4/cyclin D IC_{50} = 0.15 μM) with a 3-(dimethylamino)-2-(hydroxy)propoxyl *para*-substituent, there was little change in the CDK2 activity and CDK4 potency was improved 20-fold. For **48c** (CDK2/cyclin E IC_{50} < 0.003 μM, CDK4/cyclin D IC_{50} = 2.5 μM) adding the *para*-sulfonamide substituent resulted in a highly CDK2-selective inhibitor. The X-ray structure of **48c** with CDK2 showed that the *para*-sulfonamide group formed a hydrogen bond network with the Asp86 NH and its side-chain carboxylate. Because the Asp86 residue is conserved in both CDK2 and CDK4, a facile rationalization of the observed CDK2 selectivity of **48c** is not possible. However, acidic or neutral residues in the CDK4 enzyme replace several basic residues that line the ATP binding site of CDK2. These changes were invoked to explain the preference of CDK4 for **48b**, a compound with a basic side chain.



47a: R = H
47b: R = acetyl

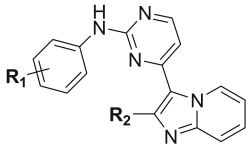
Structure 47



48a: R = H
48b: R = $OCH_2CH(OH)CH_2N(CH_3)_2$
48c: R = SO_2NH_2

Structure 48

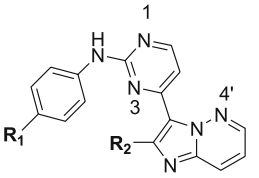
SAR studies on the series illustrated by compounds **49a–d** demonstrated that substitution at $-R_2$ had a profound effect on CDK2 inhibition activity [107]. The $-R_2$ methyl analog, **49a** (CDK2/cyclin E IC_{50} = 4.0 μM) was a poor inhibitor of CDK2 while the desmethyl **49b** (CDK2/cyclin E IC_{50} < 0.003 μM) suppressed activity to the lowest limit of detection in the kinase assay. This effect at $-R_2$ rendered all variations at $-R_1$, the *meta*-Cl analog **49c** (CDK2/cyclin E IC_{50} < 0.003 μM) and the *para*-sulfonamide analog **49d** (CDK2/cyclin E IC_{50} < 0.003 μM), equipotent. Consequently, in the $-R_2$ desmethyl series (**49b–d**) CDK2 activity was insensitive to substitution on the *para*-sulfonamide group. This was consistent with the binding mode observed for **48c** in the CDK2 crystal structure with the *para*-sulfonamide directed to the solvent–protein interface. This provided an avenue for modification of the physical properties of the lead by the incorporation of polar and basic side chains to improve solubility, reduce serum protein binding and increase cell potency. The introduction of the sulfonamide group in this series led to a decrease in cytotoxicity in non-proliferating cells, attributed to the reduction of off-target activity by the improved CDK2 selectivity. One example of this series, **49e** (CDK2/cyclin E IC_{50} = 0.005 μM) has threefold selectivity over CDK1 (IC_{50} = 0.015 μM) and is 52-fold selective over CDK4 (IC_{50} = 0.26 μM). Furthermore, **49e** was a potent inhibitor of the growth of MCF-7 cells with an (IC_{50} = 0.070 μM). Optimization to improve the physico-chemical properties of the imidazo[1,2-*a*]pyridine series for oral activity required the re-



49a: R_1 = H, R_2 = CH_3
49b: R_1 = H, R_2 = H
49c: R_1 = *meta*-Cl, R_2 = H
49d: R_1 = *para*-SO_2NH_2, R_2 = H
49e: R_1 = *para*-$SO_2NH(CH_2)_2N(CH_3)_2$, R_2 = H

Structure 49

duction of the lipophilicity of the core structure. This was accomplished by adding an additional nitrogen atom to the pyridine ring of the imidazo[1,2-*a*]pyridine system, giving a new series of CDK inhibitors: the 3-(pyrimidin-4-yl)imidazo[1,2-*b*]pyridazines [108]. These compounds had CDK2 activity similar to the corresponding imidazo[1,2-*a*]pyridines. However, as exemplified by **50a** (CDK2/cyclin E IC_{50} = 1.0 μM) the presence of a *para*-sulfonamide substituent was required for potency. Comparison of **50b** (CDK2/cyclin E IC_{50} < 0.003 μM) with **50c** (CDK2/cyclin E IC_{50} = 0.003 μM) demonstrated that, unlike the analogous imidazo[1,2-*a*]pyridines, introduction of a methyl group at – R_2 did not adversely affect CDK2 inhibition activity.



50a: R_1 = H, R_2 = H
50b: R_1 = $SO_2NH(CH_2)_2OCH_3$, R_2 = H
50c: R_1 = $SO_2NH(CH_2)_2OCH_3$, R_2 = CH_3
50d: R1 = $SO_2NH(CH_2)_3N(CH_3)_2$, R_2 = H

Structure 50

The divergence of the SAR between these two series, namely the tolerance to substitution at – R_2 exhibited by the imidazopyridazine **50a–d** compared to the imidazopyridines **49a–e**, indicated a significant change in the binding conformation between the two heteroaryl rings. This was confirmed by comparison of the crystal structures of the parent imidazo[1,2-*a*]pyridine **49d** and the imidazo[1,2-*b*]pyridazine **50d**. Both structures had key hydrogen bonding interactions between the pyrimidine N1 and Leu83 NH and between the aniline NH and Leu83 amide carbonyl oxygen. However, the orientation of the imidazo[1,2-*b*]pyridazine ring in **50d** was "flipped" 180° relative to the imidazo[1,2-*a*]pyridine ring of **49d**. The reorientation of **50d** directed the – R_2 of the imidazo[1,2-*b*]pyridazine ring to the open end of the binding pocket, thus the tolerance to substitution at this position. Furthermore, reorientation of the imidazo[1,2-*b*]pyridazine **50d** led to the formation of a new edge-to-face interaction with the phenyl side chain of Phe80. The "driving force" behind the reorientation of the imidazo[1,2-*b*]pyridazine was attributed to the minimization of electrostatic repulsion between the *N*4′ of the imidazo[1,2-*b*]pyridazine and the N3 of the adjacent pyrimidine ring that would result if **50d** were to adopt the same orientation as the imidazo[1,2-*a*]pyridine of **49d**. The most potent compounds in the imidazo[1,2-*b*]pyridazine series, **50b** and **50c**, were characterized in more detail. Both **50b** (> 13-fold) and **50c** (100-fold) were selective for CDK2

over CDK1. Compound **50b** had broad kinase selectivity with IC_{50} values of $> 10\ \mu M$ against the following kinases: CSK, EGFR, FAK, FGFR-1, IGF-1R, JAK3, Src, vAbl, Zap70, p38α, JNK1, and PKA. Furthermore, these compounds had improved plasma levels and their half-lives following oral dosing compared to the imidazo[1,2-*a*]pyridine series.

2.3.14 Aminoimidazo[1,2-*a*]pyridines

Screening of the Lilly compound library led to the identification of aminoimidazo[1,2-*a*]pyridine **51a** (CDK2/cyclin A $IC_{50} = 0.122\ \mu M$) as an ATP-competitive inhibitor scaffold [109, 110]. X-ray crystal structures of **51a** and **51b** (CDK2/cyclin A $IC_{50} = 0.324\ \mu M$) bound to the inactive form of CDK2 revealed two unique binding modes (Figs. 4 and 5). Both inhibitors occupied the ATP binding site and formed a similar H-bonding network with the hinge area of the kinase: (i) the N-1 of the imidazopyridine was a hydrogen bond acceptor with the backbone amide NH of Leu83, and (ii) the NH of the C2 amino group donated a hydrogen bond to the backbone carbonyl oxygen of Leu83. In addition, there was a hydrogen bond between the Asp145 amide NH and the carbonyl oxygen of the benzoyl group at the 6-position of the imidazopyridine scaffold of both inhibitors. The difference in the binding modes between compound **51a** (Fig. 4) and **51b** (Fig. 5) lay in a twist resulting from a competition between intra- and intermolecular interactions. In compound **51b**, an intramolecular H-bond between the 2-amino group and the carbonyl oxygen of the 3-benzoyl forced the two pendant aromatic rings into a face–face stacking arrangement. Compound **51a**, which did not have this intramolecular hydrogen bond, adopted an orientation where one aromatic ring was rotated 90°, placing the ring into a hydrophobic space in the ATP binding pocket.

SAR studies on **51a** revealed that at least one *ortho*-substituent on the 6-benzoyl was required to obtain IC_{50} values $< 1\ \mu M$. Disubstitution with

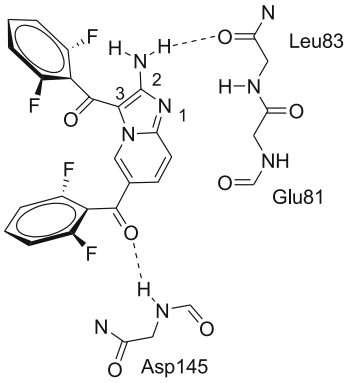


Fig. 4 Binding interactions of compound **51a** in crystal structure

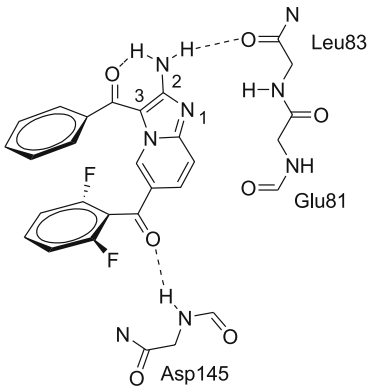
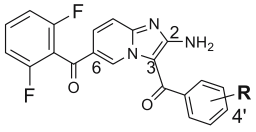


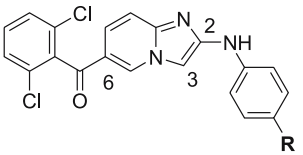
Fig. 5 Binding interactions of compound **51b** in crystal structure



51a: R = 2',6'-difluoro
51b: R = H
51c: R = 2',6'-difluoro-4'-methoxy

Structure 51

electron-withdrawing groups at both *ortho*-positions gave the most active compounds. The preferred electron-withdrawing substituents at the 2,6-positions of the 6-benzoyl were fluorine and chlorine. Most compounds of this series were selective for the CDKs versus PKA, CAMKII and GSK3β, with the selectivity ratio ranging from four- to > 400-fold. All were less active against CDK4 than CDK1 and 2. Compound **51c** (CDK2/cyclin A IC_{50} = 0.091 μM) inhibited the proliferation of HCT-116 cancer cells in tissue culture (IC_{50} = 0.47 μM). Further modifications to the aminoimidazo[1,2-*a*]pyridine template included exchanging the 2,6-difluorobenzoyl group at the 3-position of **51a–c** for a *para*-substituted aniline at the 2-position in **52a–c**. This maneuver compensated for the loss of the internal hydrogen bond interaction and gave moderately potent inhibitors of the CDKs [111]. A crystal structure of **52a** (CDK2/cyclin E IC_{50} = 0.56 μM) revealed the basis for this behavior (Fig. 6). As expected, **52a** was bound in the ATP binding site with the previously observed H-bonding network, namely, the N-1 of the imidazopyridine was a H-bond acceptor with the backbone amide NH of Leu83 and the 2-aniline NH donated a hydrogen bond to the backbone carbonyl of Leu83. However, the *para*-sulfonamide substituent of **52a** made two new hydrogen bond interactions, one between a sulfonamide oxygen and Lys89, the other through the sulfonamide NH to Asp86. These new interactions were



52a: R = SO_2NH_2
52b: R = SCH_3
52c: R = $SO_2N(CH_3)_2$

Structure 52

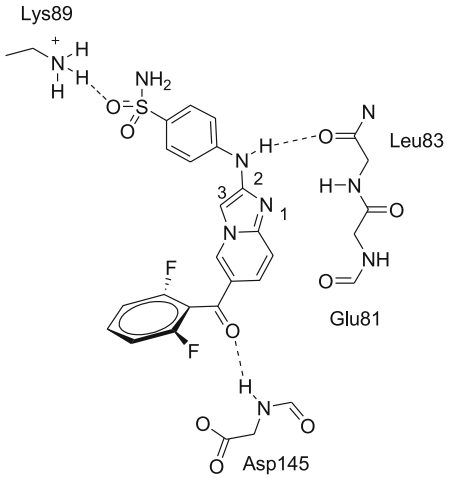
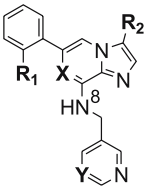


Fig. 6 Binding interactions of **52a** in crystal structure

proposed to be responsible for maintaining the inhibition activity of this series. This was confirmed experimentally: removal of the sulfonamide oxygens to give the methylsulfide **52b** resulted in a precipitous loss of activity (CDK2/cyclin E IC_{50} = 13.84 μM) and the *N*-dimethyl sulfonamide **52c** lost potency (CDK2/cyclin A IC_{50} = 1.54 μM). Compound **52a** was selective when tested against a panel of other serine/threonine kinases including GSK3β, CAMKII, PKA, and the PKCs-α,β,ε,γ.

2.3.15 Imidazo[1,2-*a*]pyrazines, Imidazo[1,2-*a*]pyridines, Pyrazolo[1,5-*a*]pyridines and Pyrazolo[1,5-*a*]pyrimidines

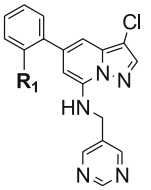
Schering-Plough published patents covering four structurally related series as CDK2, ERK and GSK-3β inhibitors: imidazo[1,2-*a*]pyrazines **53a** (CDK2/cyclin E IC_{50} = 0.240 μM) [112], imidazo[1,2-*a*]pyridines **53b** (CDK2/cyclin E IC_{50} = 0.036 μM) [113], pyrazolo[1,5-*a*]pyridines **54** (CDK2/cyclin E IC_{50} = 0.078 μM) [114], and pyrazolo[1,5-*a*]pyrimidines **55** (CDK2/cyclin E



53a: X = N, Y = CH, R_1 = Cl, R_2 = Br
53b: X = CH, Y = N, R_1 = Cl, R_2 = Br

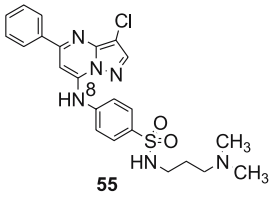
Structure 53

IC_{50} = 0.011 μM) [115, 116]. The SAR gleaned from the patent for the imidazo[1,2-*a*]pyrazines **53a** suggested that a chlorine atom at – R_1 increased CDK2 inhibition activity 28-fold over an unsubstituted analog, while bromo- and iodo- at – R_2 were more potent than the corresponding chloro-analog. The (pyridin-3-yl)methylamine was reported to be the preferred group at the 8-position. For the imidazo[1,2-*a*]pyridines **53b** the best compounds had a chlorine atom at – R_1. The optimal group at the 8-position was the (pyrimidin-5-yl)methylamine; however, substitution on the pyrimidine ring was not tolerated. Unlike the imidazo[1,2-*a*]pyrazines and imidazo[1,2-*a*]pyridines, the pyrazolo[1,5-*a*]pyrimidines **54** with the phenyl ring unsubstituted at – R_1 gave the most potent compounds. In the case of the pyrazolo[1,5-*a*]pyrimidines **55**, activity was further enhanced by the addition of a 4-(sulfonamido)aniline at the 8-position. Substitution of the sulfonamide



54: R_1 = H

Structure 54



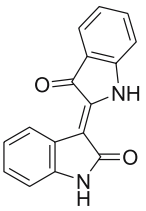
55

Structure 55

with an alkylamine improved activity. By varying the chain length from a *N*-2-(dimethylamino)ethyl to a *N*-2-(dimethylamino)propyl, CDK2 activity was improved twofold.

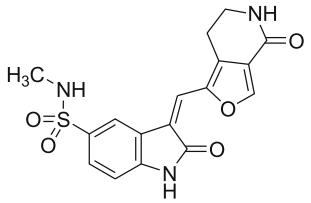
2.3.16 Oxindoles

Oxindole CDK inhibitors related to the pan-kinase inhibitor indirubin **56** isolated from Chinese herbal medicine [117, 118] have been reported by several companies: Sugen (now Pfizer) [119, 120] (**57**), Hoffman La Roche [121] (**58**), Boehringer Ingelheim [122] (**59**), and GlaxoSmithKline [123] (**60**). Many of these analogs were very potent inhibitors of the CDKs and exhibited excellent anti-proliferative activity against tumor cell lines. The series from GlaxoSmithKline [123] is selected here to exemplify this class of inhibitor and illustrate how, through the use of X-ray crystallography, modeling, and SAR studies, activity and selectivity were optimized.



56

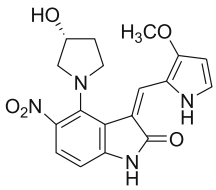
Structure 56



57

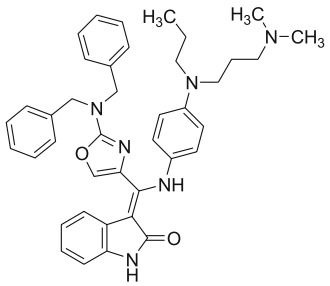
Structure 57

The indolin-2-one template, a feature of inhibitors of the receptor tyrosine kinases of Her-2, VEGF, and EGF, was used by GlaxoSmithKline scientists as a starting point to design a series of analogs from which the selective CDK-2 inhibitor **60** (IC_{50} = 0.06 μM) was identified. A thorough analysis of



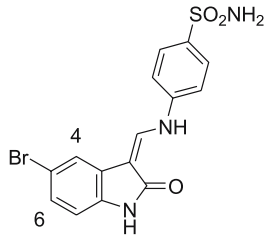
58

Structure 58



59

Structure 59



60

Structure 60

the crystal structure of **60** complexed with the inactive form of CDK2 (Fig. 7) showed that the oxindole ring occupied the ATP binding pocket in a manner similar to previous compounds of this class: the oxindole NH donated a H-bond to the backbone carbonyl of Glu81 and the oxindole carbonyl oxygen of **60** accepted a H-bond from the backbone NH of Leu83. The 7-position of the oxindole was in close proximity to the Phe80 side chain and appeared to be too crowded to permit further substitution, while the 6-position projected toward a cavity in the back of the pocket into the region affected by cyclin A association. This cavity could accommodate only small substituents

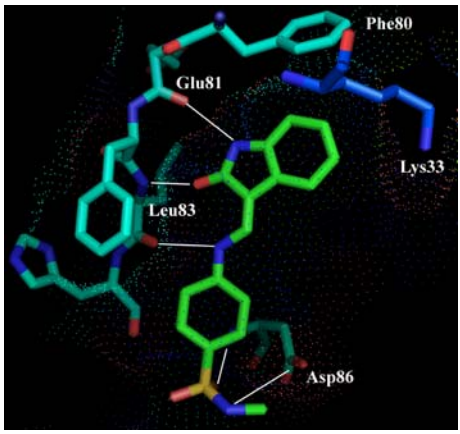
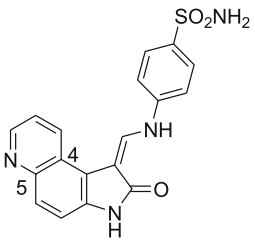


Fig. 7 Compound **60** bound to CDK2

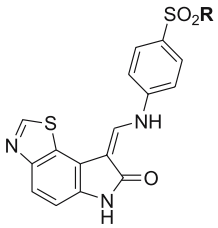
due to the bulk of the Phe80 side chain. The 5-position of the oxindole was close to Lys33, which suggested the possibility of a beneficial H-bond interaction at this location. A lipophilic substituent at the 4-position of the oxindole could result in a favorable interaction with the adjacent hydrophobic environment. The sulfonamide group, which interacted with Asp86 at the opening of the binding cleft of CDK2, provided a site for substitution that would project into solution and could be used to adjust the physical properties of this series.

Disubstitution at the 4- and 5-positions provided potent inhibitors of CDK2. Especially effective were compounds with a fused heterocycle such as **61**. The quinoline nitrogen was designed as a hydrogen bond acceptor to interact with the γ-amino group of the Lys33 side chain. Compound **61**, had excellent activity against CDK1 (IC_{50} = 0.0015 μM) and CDK2 (IC_{50} = 0.015 μM). In the crystal structure of **60**, the sulfonamide moiety formed two H-bonds, one with the backbone NH and one with the side-chain carboxylate of Asp86 at the opening to the binding cleft of CDK2. However, the



61

Structure 61



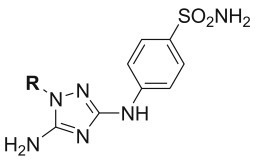
62a: R= NH_2
62b: R= CH_3

Structure 62

SAR demonstrated by sulfonamide **62a** (CDK2 IC_{50} = 0.003 μM; CDK1 IC_{50} = 0.029 μM) and sulfone **62b** (CDK2 IC_{50} = 0.008 μM; CDK1 IC_{50} = 0.100 μM) suggested that the hydrogen bond between the sulfonamide NH and the Asp86 carboxylate did not play a significant role in binding. These compounds selectively inhibited proliferation of tumor cells over a human fibroblast "normal" cell line and arrested tumor cell proliferation at the G1/S phase check point, consistent with the CDK activity.

2.3.17 Diaminotriazoles

Johnson & Johnson claimed a series of diaminotriazoles, exemplified by compound **63a** (JNJ-7706621), to be potent CDK inhibitors [124–126]. As a CDK inhibitor, **63a** was selective for CDK1/cyclin B (IC_{50} = 0.009 μM) and CDK2/cyclin A (IC_{50} = 0.004 μM) over CDK3 (15-fold versus CDK2), CDK4/cyclin D1 (63-fold versus CDK2), and CDK6/cyclin D1 (44-fold versus CDK2). Against other kinase families, **63a** had a "multitargeted" profile. It was a moderate inhibitor of the Aurora kinases and several receptor tyrosine kinases involved in angiogenesis: VEGF-R2, FGF-R2, VEGF-R3, Tie2, and FGF-R1. The compound was anti-proliferative towards tumor cell lines in vitro (HeLa IC_{50} = 0.28 μM; HCT-116 IC_{50} = 0.25 μM). In flow cytometry



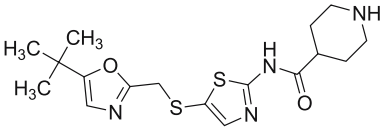
63a R = 2,6-di-F-benzoyl-
63b R = 2,6-di-F-benzyl-
63c R = 3,5-di-Me-thien-2-yl-(CO)-
63d R = 2,6-di-F-phenyl-NH-(CS)-

Structure 63

studies, **63a** induced cell cycle arrest in the G2/M phase consistent with the CDK inhibition profile. This inhibitor was efficacious in an A375 melanoma human tumor xenograft model in nude mice. The SAR of the series demonstrated the importance of the carbonyl group of the 2,6-difluorobenzoyl for CDK1 activity: substitution by a 2,6-difluorobenzyl group at -R resulted in an inactive compound (**63b**, CDK1 $IC_{50} > 100\,\mu M$). Replacing the benzoyl with a 2-carboxylthienyl, such as with **63c**, gave a potent inhibitor (CDK1 $IC_{50} = 0.003\,\mu M$) with good tumor cell anti-proliferative activity (HeLa $IC_{50} = 0.072\,\mu M$; HCT-116 $IC_{50} = 0.027\,\mu M$). An effective surrogate for the carbonyl group of **63a** and **63c** was the thiourea group in **63d**. This compound retained good enzyme inhibition (CDK1 $IC_{50} = 0.0006\,\mu M$) and tumor cell anti-proliferative activity (HeLa $IC_{50} = 0.035\,\mu M$; HCT-116 $IC_{50} = 0.02\,\mu M$).

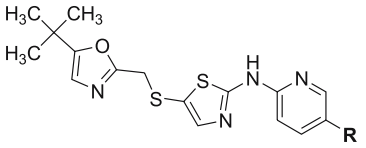
2.3.18 *N*-Acyl- and *N*-Aryl-2-aminothiazoles

Bristol Myers Squibb reported a series of *N*-acyl-2-aminothiazoles to be potent and selective inhibitors of CDK2/cyclin E with anti-tumor activity in mice. Compound **64** (BMS-387032), now licensed to Sunesis (SNS-032), is the Phase 1 clinical candidate from this series [127]. In a cell-free enzyme assay, **64** inhibited CDK2/cyclin E ($IC_{50} = 0.048\,\mu M$), was tenfold selective over CDK1/cyclin B, and 20-fold selective over CDK4/cyclin D. Furthermore, **64** had excellent selectivity in a panel of 12 unrelated kinases and had potent anti-proliferative activity against the A2780 tumor cell line ($IC_{50} = 0.095\,\mu M$). In pharmacokinetic studies **64** had a plasma half-life of 5–7 h in three species and low protein binding in both mouse (69%) and human (63%) serum. When administered orally, **64** exhibited good bioavailability across three species: mouse (100%), rat (31%), and dog (28%). Compound **64** was efficacious in both a P388 murine tumor model and an A2780 human ovarian carcinoma xenograft model. The three-dimensional solid-state structure of **64** complexed with CDK2 in the absence of cyclin was determined by X-ray crystallography. The crystal structure confirmed that **64** was bound in the ATP binding site and the inhibitor adopts the same orientation and "folded" conformation previously described for this series [128]. While the *N*-acylaminothiazoles analogs exhibited selectivity for CDK2 over both CDK1



64

Structure 64



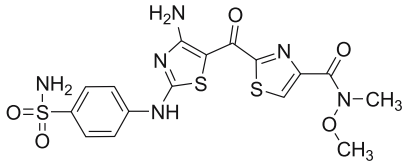
65a: R = -$CH_2NHCH_2C(OH)(CH_3)_2$
65b: R = H

Structure 65

and CDK4, a related series of *N*-arylaminothiazoles have been reported and characterized by the workers at BMS as pan-CDK inhibitors [129]. Compound **65a** (BMS-357075) is a potent inhibitor of CDK1 (IC_{50} = 0.018 μM), CDK2 (IC_{50} = 0.003 μM), and CDK4 (IC_{50} = 0.026 μM). In a P388 murine leukemia model **65a** produced a 56% increase in survival time over an untreated control. The structure of a related analog **65b** complexed with CDK2 in the absence of cyclin was determined by X-ray crystallography. The crystal structure showed that **65b** binds in the ATP binding site in a "folded" conformation similar to **64** [127].

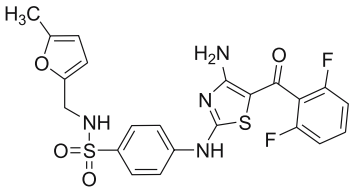
2.3.19
5-Benzoyl-2,4 diaminothiazoles

Agouron (now Pfizer) disclosed in a series of patents the discovery of potent CDK inhibitors based on the 5-benzoyl-2,4 diaminothiazole template [130, 131]. Compounds **66** and **67** were both potent CDK2 inhibitors (**66** K_i < 0.005 μM; **67** K_i = 0.10 μM), but these compounds varied in their activity



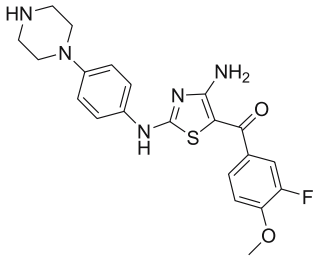
66

Structure 66



67

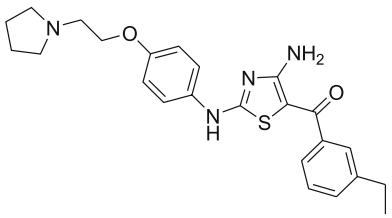
Structure 67



68

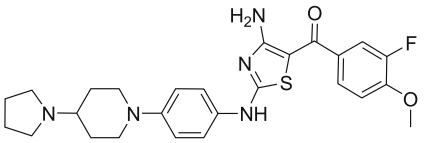
Structure 68

against CDK4. Compound **67** had potent inhibition activity for CDK4 (K_i = 0.028 μM) while compound **66** was much less potent for CDK4 (K_i = 1.1 μM). Compound **67** was anti-proliferative against the HCT-116 tumor cell line (IC_{50} = 0.043 μM). Hoffman La Roche patented a series of 5-benzoyl-2,4-diaminothiazoles as potent and selective CDK4 inhibitors [132–134]. Compounds **68** (CDK4 IC_{50} = 0.014 μM), **69** (CDK4 IC_{50} = 0.022 μM), and **70** (CDK4 IC_{50} = 0.012 μM) were also claimed to have selectivity over CDK1 (**68** 71-fold; **69** 136-fold; and **70** 107-fold) and CDK2 (**68** 57-fold; **69** 42-fold; and **70** 240-fold).



69

Structure 69



70

Structure 70

3
Aurora Kinases

3.1
Biology of the Aurora Kinases

The Aurora family of serine/threonine kinases plays a central role in regulating mitosis, with each member having unique functions and localization patterns. This family is crucial in controlling chromosome segregation and condensation, spindle assembly and cytokinesis during mitosis [135]. Overexpression of members of this kinase family has been observed in a variety of human cancers, including hepatocellular, pancreatic and ovarian cancer (for Aurora-A, [136–139]) and thyroid cancer and astrocytomas (for Aurora-B, [140, 141]). Additionally, the Phe31Ile polymorphism of Aurora-A, associated with enhanced transforming capability and increased polyploidy, has been detected in colorectal, esophageal, and breast cancer samples [142–144].

Aurora-A (also known as Aurora-2, STK15/STK6, BTAK), functions through its association with centrosomes and microtubules to regulate sister chromatid migration and spindle assembly and maintenance [145]. Although this association with centrosomes does not require kinase activity, phosphorylation of a key substrate, cdc25B, which activates CDK1/cyclin B1, occurs only when Aurora-A is associated with the centrosome [146]. Aurora-A also directs the phosphorylation of p53 at Ser315, which targets p53 for Hdm2-mediated ubiquitination and destruction by the proteosome [147]. The ability of Aurora-A to phosphorylate its substrates in cells is linked to its association with TPX2, which prevents dephosphorylation of Aurora-A by PP1 and allows for additional autophosphorylation of Aurora-A [148, 149]. Consistent with these activities, functional disruption of Aurora-A activity arrests cells in mitosis.

Aurora-B (also known as Aurora-1) regulates cytokinesis and chromosome architecture. Aurora-B, a chromosome passenger protein, has a distinct localization pattern from Aurora-A. Aurora-B phosphorylates its binding partner INCENP and together with Survivin forms a complex that phosphorylates histone H3 [150–152]. Although Aurora-A and Aurora-B have a different spectrum of activities, dual inhibitors cause tumor cells to display a phenotype derived from Aurora-B disruption. Studies to investigate the basis of this revealed that the need for Aurora-A is bypassed when Aurora-B is not functional [153] therefore, cellular inhibition of Aurora-B may be sufficient. Several features of Aurora-C are shared with Aurora-B, including localization and interaction with Survivin; this would be expected from the late evolutionary divergence between the two family members and the ability of Aurora-C to complement Aurora-B function in cells [154]. The altered expression of Aurora kinases in clinical samples and evidence that genetic neutralization of this family of kinases disrupts the maintenance of a stable genome has led to the development of chemical inhibitors of the Aurora kinases at several com-

panies. As a result of this work, the first Aurora kinase inhibitors, VX-680 (**71**), AZD1152 (**92**), and MLN8054 (**97**), have now progressed to clinical trials and data from these studies are awaited.

3.2 Structural Biology of the Aurora Kinases

3.2.1 Aurora-A

AstraZeneca, Pharmacia, Vertex, Syrrx, and the European Molecular Biology Laboratory (EMBL) have reported crystal structures of Aurora-A [149, 155–160]. All the crystal structures of Aurora-A are of N-terminal truncated catalytic domains. The structures published by Vertex and Syrrx are of unphosphorylated Aurora-A co-crystallized with adenosine and ADP, respectively. Three crystal structures reported from EMBL are of doubly phosphorylated Aurora-A (Thr287, Thr288), one with bound ATP, one with ADP, and one consisting of an ADP complex with the activating microtubule-associated protein TPX2. AstraZeneca and Pharmacia (Nerviano Medical Sciences) have both reported crystal structures with small molecule inhibitors bound to the active sites of mutant forms of Aurora-A with point mutations at T287D and T288D. The structure of Aurora-A has the typical bilobal kinase motif, consisting of a β-sheet containing the N-terminal domain and a C-terminal domain comprised mainly of α-helices. These domains are linked together by the typical "hinge" region that plays an important role in forming the catalytic active site (Fig. 8) [161, 162].

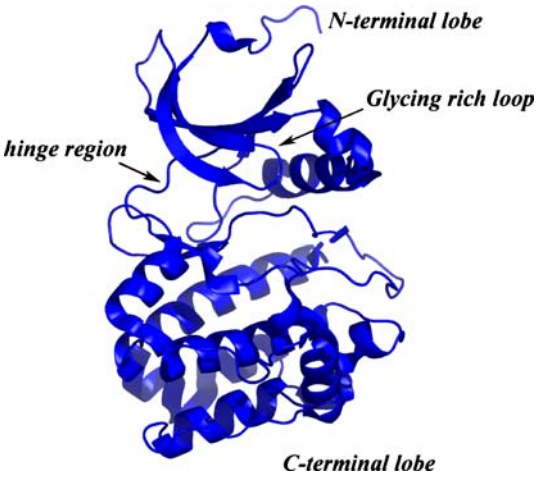


Fig. 8 Ribbon structure of Aurora-A

ATP is bound in a hydrophobic pocket created, in part, by the residues Leu137 and Val147 in the glycine-rich loop, Ala160 and Leu263 located in the active site. The 6-amino and 1-imido groups of adenosine bind to the "hinge" peptide through direct hydrogen bonds with the amide residues Glu211 and Ala213, respectively. The 6-amino group of the adenosine points toward a hydrophobic pocket, a "fluorophenyl pocket", so-named from a series of p38 MAP kinase inhibitors which contained a fluorophenyl group and interacted with a similar active site pocket [163], which is created by residues Ala160, Leu194, Leu210, and Glu211 from the hinge region and Val147 from the glycine-rich loop (Fig. 9).

Aurora-A displays several unique features that can be exploited in the design of selective inhibitors. In the vicinity of the purine base of adenosine, there are two regions that could be used for the design of potent and selective inhibitors of Aurora-A. The first is the deep hydrophobic "fluorophenyl pocket" formed by the flexible glycine-rich loop and the hinge region. This pocket contains several residues that are not conserved in Src [164, 165], IRK [166, 167], and the CDKs [168, 169]. Corresponding to Leu210 in Aurora-A, Src, IRK, and the CDKs have Thr, Met, and Phe residues at the corresponding position (Fig. 10). The Thr of Src presents a hydrogen-bonding group and a less hydrophobic surface at the entrance to this pocket while the Phe in the CDKs and Met of IRK restrict the access to this pocket. As with the MAP

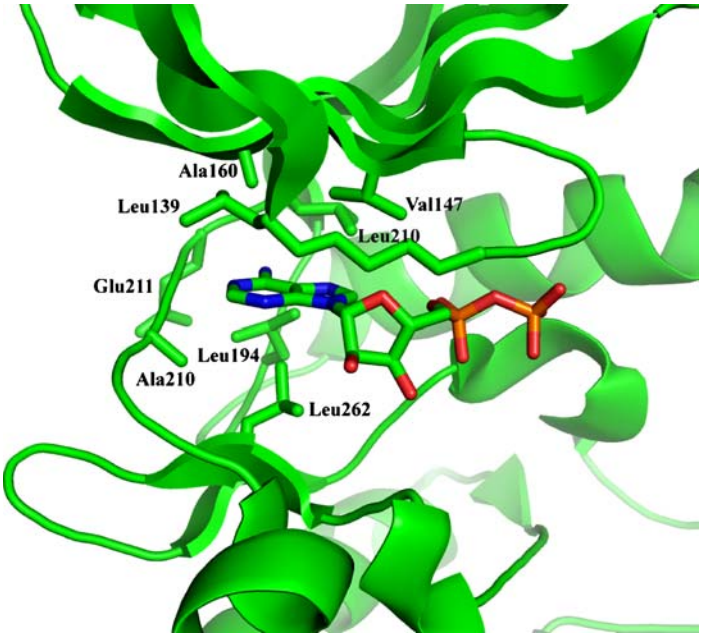


Fig. 9 ATP binding pocket of Aurora-A

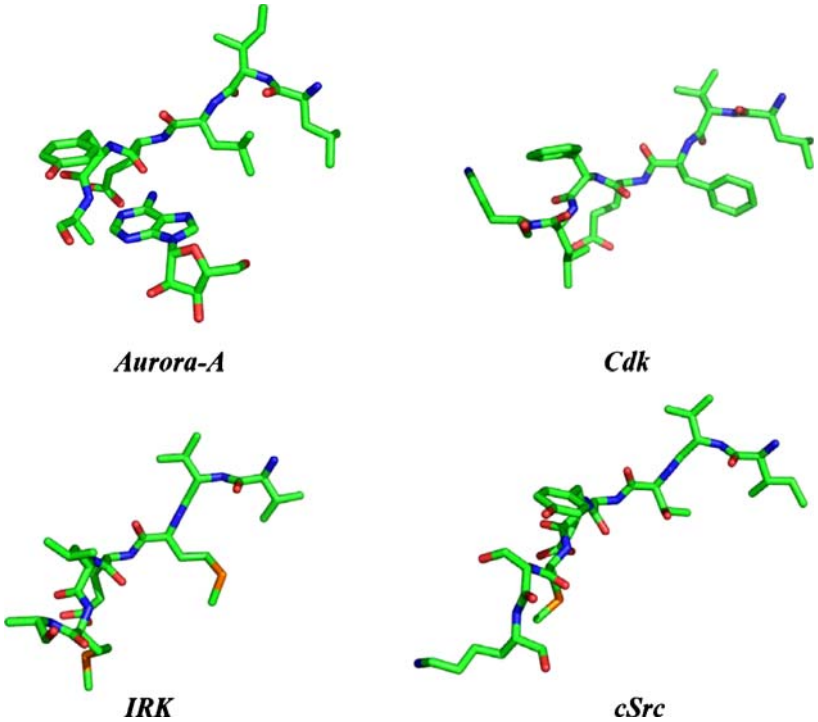


Fig. 10 Differences in the "fluorophenyl pocket" of Aurora-A, CDK, IRK, and Src kinases

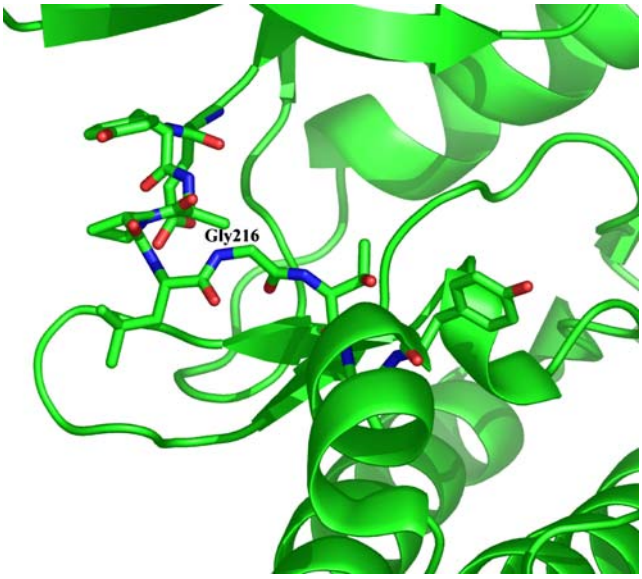


Fig. 11 Gly 216 insertion in the "hinge peptide" of Aurora-A

kinases, the "fluorophenyl pocket" offers the possibility of exploiting differences in shape and charge to obtain Aurora-A selectivity.

Another opportunity for differentiation lies in the hinge region of Aurora-A. This polypeptide contains a single Gly insertion, changing the size and conformation of the adenosine-binding pocket. The conformational change induced by the insertion of Gly216 in Aurora-A at a point where the hinge meets the C-terminal lobe creates a smaller ATP-binding pocket (Fig. 11).

3.2.2 Aurora-B

Crystal structures of Aurora-B have been reported by the European Institute of Oncology working in collaboration with the FIRC Institute of Molecular Oncology Foundation and University of Virginia Medical School [170]. The crystal structures of Aurora-B consists of one bound with the "IN-box" segment of the inner centromere protein (INCENP) activator and another co-crystallized with INCENP and the small molecule inhibitor Hesperadin (**96**) (Fig. 12).

Aurora-B, like Aurora-A, has the classical bilobal protein kinase fold consisting of an N-terminal lobe, rich in β-strands, and a C-terminal lobe comprised mainly of α-helices. The ATP binding pocket lies in the "hinge" region at the interface between the lobes. There is about a 60% sequence homology between Aurora-A and Aurora-B in mammals. Comparison of the ATP-binding domains revealed that the 26 amino acids in the active site of the human homologues of Aurora-B and Aurora-C varied by only

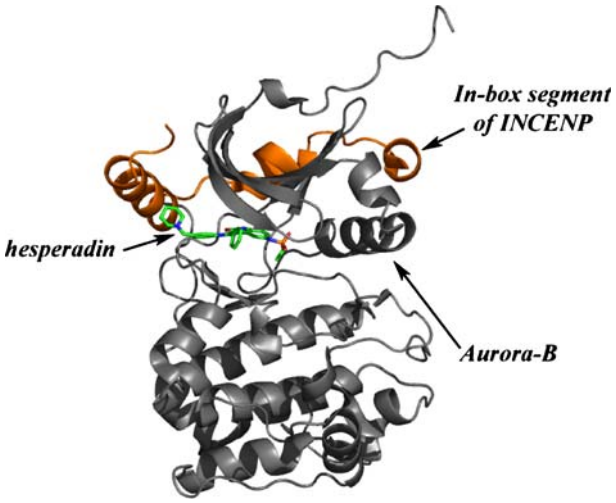


Fig. 12 Aurora-B–INCENP–Hesperadin complex

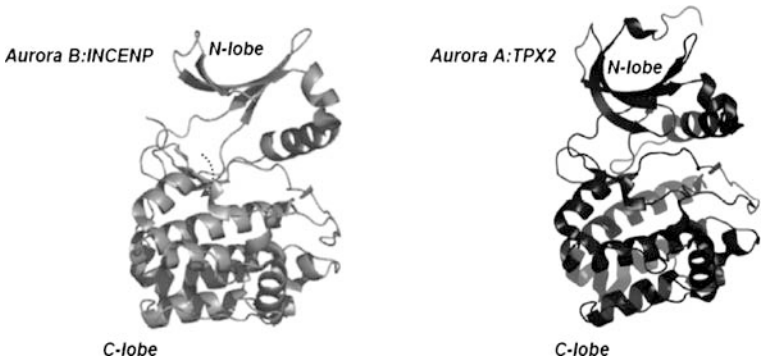


Fig. 13 Comparison of the catalytic clefts of Aurora-B: INCENP and Aurora-A: TPX2

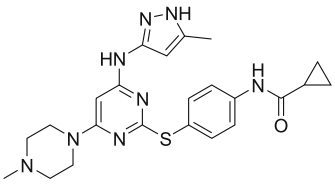
three residues from Aurora-A [162] and are located at the cleft in the direction of the solvent-accessible region. Potential selectivity for Aurora-A over Aurora-B may be possible by targeting these non-conserved residues. On the other hand, comparison of the Aurora-A/TPX2 [149] and Aurora-B/INCENP structures revealed that the Aurora-B/INCENP catalytic cleft is open $\sim 15^{\circ}$ wider than in Aurora-A/TPX2 (Fig. 13). The size difference between the catalytic clefts may offer the means to prepare Aurora-B selective inhibitors and account for the Aurora-B selectivity observed with some inhibitors.

3.3
Structure–Activity Relationships of Aurora Kinase Inhibitors

The patent literature has reports of many compounds that are claimed to have Aurora kinase inhibition activity but in some of these cases it is not possible to determine whether these compounds are pan-kinase inhibitors or if the compounds are selective for the Aurora kinases. We limited this review to publications that give specific data for the Aurora kinases and have pertinent selectivity data. Another review has appeared recently [171].

3.3.1
Pyrazoles and Pyrazolones

VX-680 (**71**) is the most advanced pyrazole class Aurora inhibitor, disclosed by Vertex. The compound is reportedly in Phase I clinical development in patients with relapsed or refractory acute myelogenous leukemia, myelodysplastic syndrome, acute lymphocytic leukemia, and chronic myelogenous leukemia and in Phase II for advanced colorectal cancer [172, 173]. VX 680 (**71**) was a potent inhibitor of all three Aurora kinase enzymes (Aurora-A $K_i = 0.0006\ \mu M$; Aurora-B $K_i = 0.018\ \mu M$; Aurora-C $K_i = 0.0046\ \mu M$). Com-

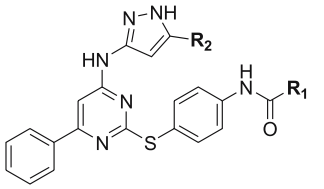


71

Structure 71

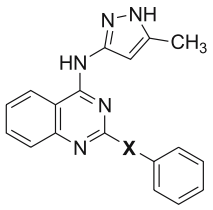
pound **71** was selective against 56 kinases ($IC_{50} > 1\,\mu M$) but was an inhibitor of FLT-3 ($IC_{50} = 0.03\,\mu M$), Fyn ($IC_{50} = 0.52\,\mu M$), ITK ($IC_{50} = 0.22\,\mu M$), Lck ($IC_{50} = 0.08\,\mu M$), and Src kinases ($IC_{50} = 0.35\,\mu M$). The compound inhibited the phosphorylation of Histone-H3 in MCF7 cells at concentrations of 0.003–0.3 μM and blocked tumor cell proliferation in a wide panel of tumor cell lines (colorectal, leukemia, breast, prostate, pancreatic, melanoma, cervical) with EC_{50} values ranging from 0.015 to 0.113 μM. In MCF7 cells **71** induced polyploidy in cells with 4N DNA content. Compound **71** inhibited HeLa cell division; however, these cells were still able to enter mitosis and proceed through S-phase. Compound **71** had no effect on the viability of non-cycling cells or peripheral blood mononuclear cells at concentrations up to 10 μM. The inhibitor promoted a dose-proportional inhibition of tumor growth in in vivo human xenograft tumor studies in nude mice following either intraperitoneal or intravenous administration in HL-60 acute myleogenous leukemia, MIA PaCa-2 pancreatic and HCT-116 colon cancer models. In **71**-treated HCT-116 bearing mice there was a marked reduction in histone H3 phosphorylation and a higher incident of apoptosis compared to control untreated tumor. Although efficacy was observed in a mouse syngeneic leukemia model using the BaF3 murine leukemia line with an activating human FLT-3 internal tandem deletion mutation it is unclear whether the FLT-3 ($IC_{50} = 0.03\,\mu M$) or Aurora kinase inhibitory activities of **71** led to the antitumor effects in this study. One initial challenge in the discovery of **71** was to control the inherent GSK3 activity of this series. SAR studies illustrated that GSK3 selectivity could be managed by appropriate substitution at R_1 and R_2. Compounds **72a–c** demonstrated excellent activity against Aurora-A ($K_i < 0.1\,\mu M$), while the R_1 cyclopropylamide **72b**, the substitution pattern adopted for **71**, was more selective against GSK3 ($K_i = 1.0$ to $7.0\,\mu M$) than the acetamide **72a** (GSK3 $K_i = 0.1$ to $1.0\,\mu M$) [174, 175]. When R_2 was varied, greater selectivity against GSK3 was observed for the 5-methylpyrazole **72a** (GSK3 $K_i = 0.1$ to $1.0\,\mu M$) than the 5-cyclopropylpyrazole **72c** (GSK3 $K_i < 0.1\,\mu M$).

Vertex has reported several variations of the pyrazole class with activity against Aurora-A and a number of other protein kinases [174–187]. Patent applications covering (1H-pyrazol-3-yl)quinazolines (**73a,b**) and a series exemplified by **74** were described as having protein kinase inhibitor activity



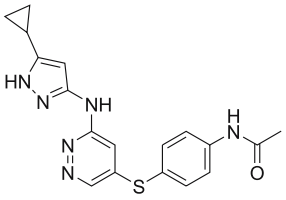
72a: R_1 = -CH_3; R_2 = -CH_3
72b: R_1 = cyclopropyl; R_2 = -CH_3
72c: R_1 = -CH_3; R_2 = cyclopropyl

Structure 72



73a: X = -S-
73b: X = bond

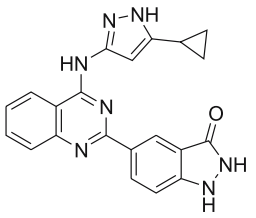
Structure 73



74

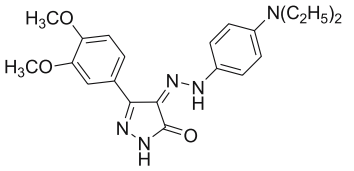
Structure 74

against Aurora-A, GSK3, and Src kinases along with CDK2, Akt, and ERK in some cases. Furthermore, a wide variety of five-membered heterocyclic replacements for the pyrazole were claimed although no biological data was included for these substitutions [188–190]. Some pyrazoles closely related to **71** but substituted with an indazolinone ring (**75**) have also been reported [191]. No compound-specific data was provided but it was claimed that compounds with inhibitory constants < 0.1 μM for Aurora-A and GSK3 had been prepared. A series of pyrazolone derivatives were reported to be useful as inhibitors of GSK3, Aurora-A, and CDK2. For example, **76** has an inhibitory constant of < 0.1 μM for Aurora-A and GSK3β and between 1–20 μM for CDK2. No additional selectivity data against other kinases was reported for these analogs [192].



75

Structure 75

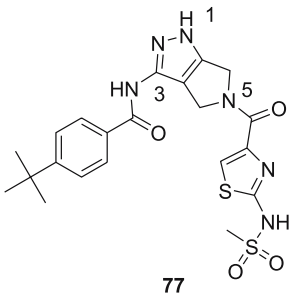


76

Structure 76

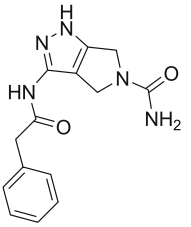
3.3.2 Pyrrolopyrazoles

Pfizer (Pharmacia/Nerviano) has described the versatile 1,4,5,6-tetrahydropyrrolopyrazole scaffold as both a potent Aurora-A (**77** Aurora-A $IC_{50} < 0.01\,\mu M$; CDK2/cyclin A $IC_{50} > 10\,\mu M$)) and CDK2 (**78** Aurora-A $IC_{50} > 10\,\mu M$; CDK2/cyclin A $IC_{50} = 0.03\,\mu M$) kinase inhibitor [157, 193, 194]. According to the binding model for both kinases, the aminopyrazole of the pyrrolopyrazole core would interact with the "hinge" peptide, the 3-substituent was directed toward the solvent while the 5-substituent enters the phosphate binding region of the ATP pocket. As illustrated by **77**, 3-benzamide derivatives were potent Aurora-A inhibitors while selectivity for CDK2 was achieved with 3-phenylacetamide substitution in **78.** This se-



77

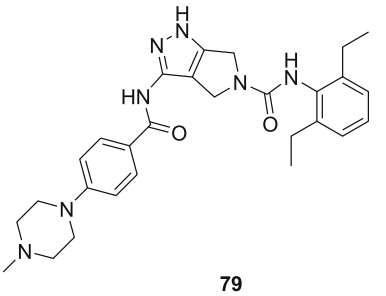
Structure 77



78

Structure 78

lectivity was believed to be due to a difference in protein conformation between the hinge region and the beginning of the C-terminal tail, which disfavored non-planar inhibitors for Aurora-A. The 4′-*tert*butylbenzamido and 4′-(4-methyl-piperazin-1-yl)benzamido moieties emerged as the 3-position substituents that gave the highest inhibitory activity for Aurora-A. Optimization of the 5-position led to increased enzymatic potency for Aurora-A, but the cellular activity was not correlated with this improved enzyme inhibition. Compound **79** (PHA-680632) inhibited all three Aurora kinases (Aurora-A IC_{50} = 0.027 μM; Aurora-B IC_{50} = 0.135 μM; Aurora-C IC_{50} = 0.120 μM) and had potent anti-proliferative activity against five tumor cell lines (HCT-116 IC_{50} = 0.045 μM; HL-60 IC_{50} = 0.130 μM; A-2780 IC_{50} = 0.110 μM; HT-29 IC_{50} = 0.080 μM; HeLa IC_{50} = 0.410 μM). In a selectivity panel, **79** was 25-fold more potent for Aurora-A over 19 of the 20 unrelated kinases evaluated.

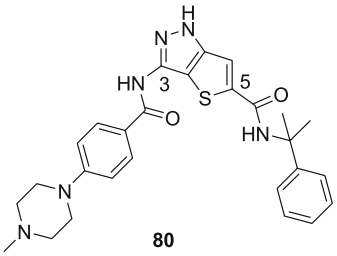


79

Structure 79

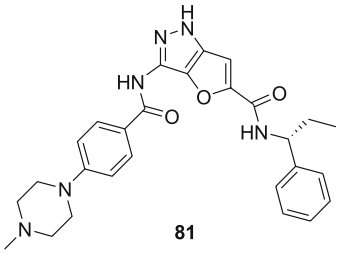
3.3.3 Thienopyrazoles, Furopyrazoles, Indazoles

Pfizer (Pharmacia) disclosed the development of potent Aurora-A kinase inhibitors containing the 3-aminothieno[3,2-*c*]pyrazole (**80**) and 3-aminofuro[3,2-*c*]pyrazole (**81**) scaffolds [195, 196]. Extensive data was not reported



80

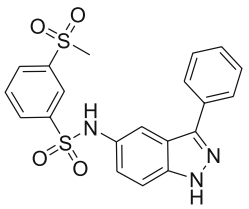
Structure 80



81

Structure 81

for the furopyrazole series, however, compound **81** had CDK2 and PAK4 inhibition activity ($K_i < 0.5\ \mu M$) as well as Aurora-A ($K_i < 0.1\ \mu M$). The SAR of the 3-aminothieno[3,2-*c*]pyrazole series showed that a wide variety of *para*-substituted 3-benzamides were tolerated with the 4-methylpiperazin-1-yl, found in **80** (Aurora $K_i = 0.001\ \mu M$; HeLa $IC_{50} = 0.002\ \mu M$), and 4-morpholin-4-yl groups the most preferred. The gem-dimethyl group in **80** can be replaced with cyclopropyl, *R* or *S* methyl or ethyl, and *S*-methyl(pyrollidin-1-yl) or *S*-methyl(morpholin-4-yl) with little consequence to the Aurora inhibition ($K_i = 0.001$ to $0.018\ \mu M$) or tumor cell anti-proliferative activity (HeLa $IC_{50} = 0.002$ to $0.18\ \mu M$). Aventis has published a patent application describing indazoles as inhibitors of Aurora-A [197]. These indazoles had broad activity against kinases, as exemplified by compound **82**, which inhibited Aurora-A (97% inhibition at $10\ \mu M$), FAK (98% inhibition at $10\ \mu M$), KDR



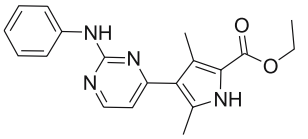
82

Structure 82

(70% inhibition at 10 μM), Src (89% inhibition at 10 μM), and TIE-2 (86% inhibition at 10 μM).

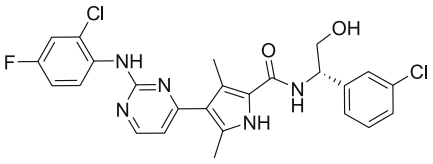
3.3.4 Pyrrolopyrimidines, Thiazolopyrimidines, Imidazolopyrimidines

Vertex disclosed a series of pyrrolopyrimidines, represented by **83** and **84**, as kinase inhibitors active against Aurora-A, GSK3β, JNK3, Src, and Erk [198]. No compound-specific data was given but it was claimed that IC_{50} values ranged from < 1 to 5 μM. A patent application covering thiazololopyrimidines as inhibitors of Aurora-A, GSK3β and SYK has been published by Vertex [199]. For example, compound **85** was reported to have an inhibitory constant < 0.5 μM for Aurora-A, > 1 μM for GSK-3β, and 0.5–2 μM for Syk. Cyclacel disclosed a series of thiazolopyrimidines as Aurora-A kinase inhibitors [200–202]. Compound **86** had broad CDK kinase activity, inhibiting CDK2 (IC_{50} = 0.5 μM), CDK4 (IC_{50} = 3.3 μM), CDK7 (IC_{50} = 1.6 μM), and CDK9 (IC_{50} = 0.69 μM) in addition to Aurora-A (IC_{50} = 0.033 μM). The SAR provided for the thiazole-2-one analogs suggested that the N-ethyl group had a small but beneficial effect on the selectivity profile. Imidazolyl pyrimidines, which had been initially developed as JNK protein kinase inhibitors, also had potent activity for Aurora-A, Src, and Lck [203]. Compound **87** had an inhibition constant of < 0.1 μM for Aurora-A and 0.1–1 μM for JNK3. The SAR gleaned from this patent illustrated the importance of the imidazole NH for Aurora activity in this series. Exchanging the imidazole for a thiazole resulted in the loss of Aurora inhibition while maintaining moderate activity for JNK3. Analogs with the most potent inhibition ac-



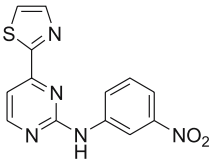
83

Structure 83



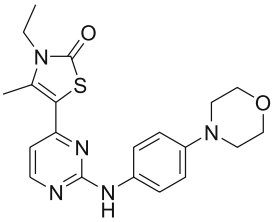
84

Structure 84



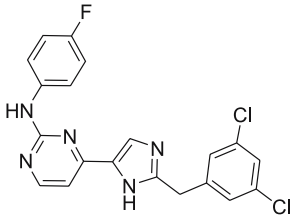
85

Structure 85



86

Structure 86



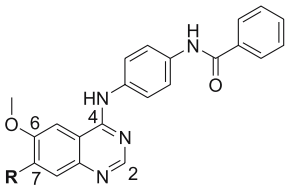
87

Structure 87

tivity for the Aurora kinase had a 3,5-dichlorobenzyl group as a common feature.

3.3.5 Quinazolines

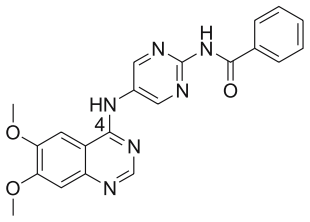
A series of 4-anilinoquinazolines, exemplified by compound **88a** (Aurora IC_{50} = 0.374 μM), was identified through a high through-put screening campaign and used as a starting point for AstraZeneca's medicinal chemistry efforts. These inhibitors were notable for both their potency and selectivity for Aurora kinases [155, 156, 204–206]. The binding mode for quinazoline-based kinase inhibitors is well understood. They are known to orient in the ATP binding site by making a critical H-bond interaction with the "hinge" peptide of the protein through the quinazoline N-1, causing the 4-benzamide to extend into the selectivity pocket and the 6,7-dimethoxy group of **88a**



88a: R = -OCH_3
88b: R = 3-(morpholin-4-yl)propoxy-

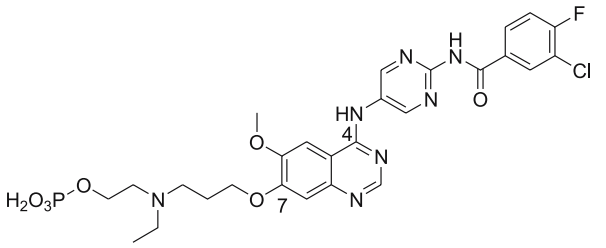
Structure 88

to be directed towards the solvent-accessible region of the enzyme. Replacing the 7-methoxy of **88a** with 3-(morpholin-4-yl)propoxy gave **88b**, which had improved activity in the in vitro kinase inhibition assays (Aurora-A, IC_{50} = 0.11 μM, Aurora-B IC_{50} = 0.13 μM) and cellular potency (MCF7 IC_{50} = 1.06 μM). Compound **88b** was a relatively weak inhibitor of MEK1 (IC_{50} = 1.79 μM), Src (IC_{50} = 1.03 μM), Lck (IC_{50} = 0.88 μM) and inactive (IC_{50} > 10 μM) against CDK1, CDK2, CDK4, CHK1, Flt, KDR2, IKK1/2, PLK1, and FAK kinases. A FACS study demonstrated that **88b** caused a concentration-dependent increase in 4n DNA and induced apoptosis in cycling cells, consistent with the proposed mechanism of action. Compounds **88a** and **88b**, had low aqueous solubility (1–10 μM at pH 7.4) and were highly protein bound in rat plasma (**88a** 0.01% free drug; **88b** 0.3% free drug). Exchanging the 4-aniline for six-membered heterocycles led to analogs with increased potency and selectivity, and with improved physical properties [205, 207]. While both pyridin-3-yl and pyrimidin-5-yl analogs had improved potency and selectivity for Aurora kinases, the most notable was the pyrimidin-2-yl **89** (Aurora-A IC_{50} = 0.008 μM; Aurora-B IC_{50} = 0.025 μM). The physicochemical properties of **89** also led to a substantial improvement in rat plasma protein binding (4.5% free drug) over **88a,b**. Extensive work on this scaffold led to the phosphate prodrug **90** (Aurora-A IC_{50} = 0.003 μM; Aurora-B IC_{50} = 0.012 μM; MCF7 IC_{50} = 0.30 μM) where the complementarity of the ben-



89

Structure 89

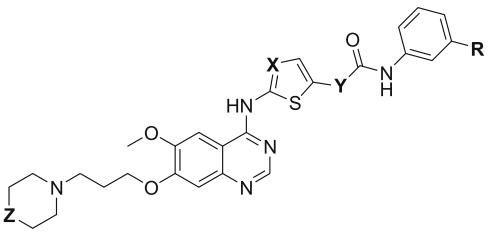


90

Structure 90

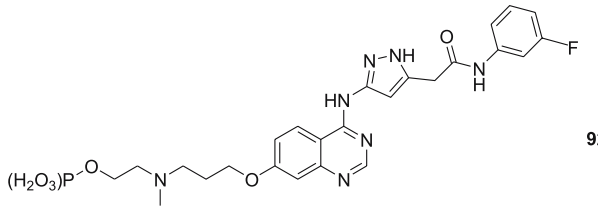
zamide was carefully fine-tuned with a 3-chloro-4-fluorophenyl substituent and the 7-alkoxy interaction was optimized with a 3-(*N*-hydroxyethyl-*N*-ethyl)aminopropoxy group [207] to provide an effective lipophilic contact with the protein, while gaining access to the solvent channel with a polar group.

In the previous study, replacement of the aniline ring with six-membered heterocycles resulted in potent inhibitors, therefore, replacing the aniline group with five-member heterocycles was also explored. Quinazolines with five-membered rings of various structures were prepared and evaluated. The 5-thiophene **91a** (Aurora-A IC_{50} = 0.011 μM; Aurora-B IC_{50} = 0.057 μM) and the 2-thiazole **91b** (Aurora-A IC_{50} = 0.004 μM; Aurora-B IC_{50} = 0.042 μM) were potent inhibitors with good activity against both Aurora-A and Aurora-B kinases [208–210]. Inserting a methylene group between the amide carbonyl and the five-membered heterocycle resulted in analogs with excellent potency against both Aurora-A and Aurora-B. This could be clearly seen through the direct comparison of compound **91c** (Aurora-A IC_{50} < 0.001 μM; Aurora-B IC_{50} < 0.001 μM) with **91b**, where insertion of the methylene linkage between the thiazole ring and the amide carbonyl lead to improved activity against Aurora-A and Aurora-B [209–211]. In addition, **91c** had increased potency in the MCF7 (IC_{50} = 0.008 μM) and SW620 (IC_{50} = 0.012 μM) cell proliferation assay. In vivo, compound **91d** induced dose-dependent inhibition of histone H3 phosphorylation (25–45% at 25–50 mg/kg i.p.) in nude mice inoculated with SW620 tumors [212]. From this series, the Phase I clinical candidate AZD-1152 (**92**) was identified. Compound **92** is



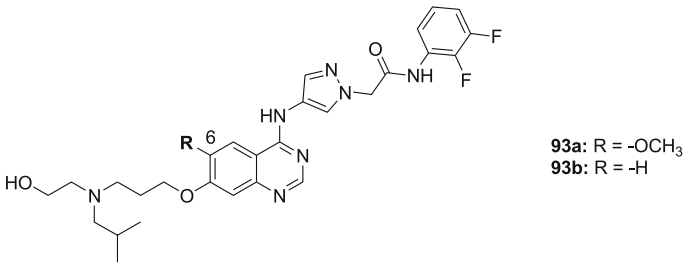
91a: R = H; X = CH; Y = bond; Z = O
91b: R = H; X = N; Y = bond; Z = O
91c: R = H; X = N; Y = CH_2; Z = O
91d: R = Cl; X = N; Y = CH_2; Z = NCH_3

Structure 91



Structure 92

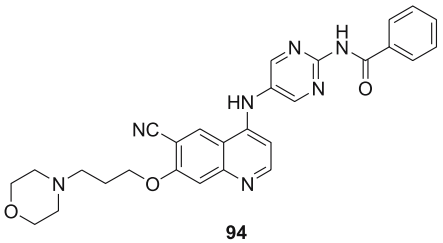
a phosphate prodrug that is soluble in basic vehicles and readily hydrolyzed to the parent in vivo. The parent compound of **92** is a potent inhibitor of Aurora-A (IC_{50} < 0.001 μM) Aurora-B (IC_{50} = 0.007 μM) and Aurora-C (IC_{50} = 0.005 μM); **92** suppressed histone H3 phosphorylation in human tumor cell lines with IC_{50} values from 0.005 μM to 0.035 μM, and had a potent anti-proliferative effect on SW620 cells (IC_{50} = 0.002 μM). In colorectal and lung human xenografts, **92** inhibited tumor growth by 69% and 100%, respectively, after 48 h of dosing at 150 mg/kg/day sc [213–219]. Compounds where the five-membered heterocycle was a pyrazole, triazole, or imidazole were also potent inhibitors of the Aurora kinases when this methylene linkage was present in the molecule [220–223]. Removing the 6-methoxy substituent from compound **93a** (Aurora-A IC_{50} = 0.010 μM; Aurora-B IC_{50} = 0.008 μM) yielded **93b** (Aurora-A IC_{50} = 0.500 μM; Aurora-B IC_{50} = 0.009 μM) an analog with > 50-fold selectivity for Aurora-B over Aurora-A. These compounds had excellent cellular activity (**93a** SW620 IC_{50} = 0.001 μM; **93b** SW620 IC_{50} = 0.001 μM).



Structure 93

3.3.6 Quinoline

Scientists at AstraZeneca replaced the quinazoline core of the previous series with a quinoline to give analogs such as **94** with good selectivity for the Aurora kinases [224]. Compound **94** (Aurora-A IC_{50} = 0.052 μM; Aurora-B IC_{50} = 0.012 μM) was potent in the enzymatic assays and had good solubility.



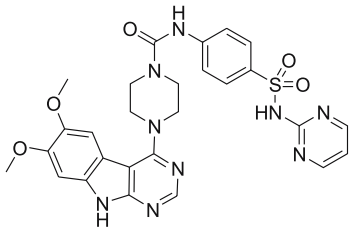
94

Structure 94

However, while the quinoline analogs were more potent in cell assays (MCF7 IC_{50} = 0.360 μM) they exhibited shorter plasma half-lives compared to the corresponding quinazolines.

3.3.7 Indolopyrimidines

Montigen has disclosed a series of indolopyrimidine Aurora inhibitors [225]. The lead compound, MP-235 (**95**), was reported to inhibit Aurora kinases at nanomolar concentrations (Aurora-A IC_{50} = 0.090 μM)). Further modifications identified analogs with greater potency and selectivity; these inhibitors had anti-proliferative effects in the human pancreatic cell lines MiaPaCa-2 and Panc-1 and other cancer cell lines but at relatively high concentrations (IC_{50} values from 125 to 300 μM). Compound **95** was selective for Aurora-A when tested against a panel of 20 kinases (IC_{50} > 0.5 μM).

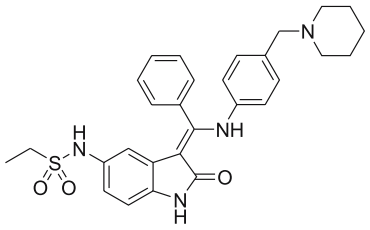


95

Structure 95

3.3.8 Oxindole

Boehringer Ingelheim reported on the novel oxindole, Hesperadin (**96**). Compound **96** induced aneuploidy, caused defects in mitosis and cytokinesis, and inhibited Aurora-B (IC_{50} ~ 0.25 μM) [226, 227].The specificity of the compound against other members of the Aurora family has not been reported;



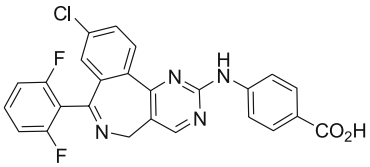
96

Structure 96

however, **96** was found to be an effective inhibitor of six different kinases (AMPK, Lck, MKK1, MAPKAP-K2, CHK1, and PHK2) out of a panel of 25 kinases. In addition, **96** was a weak inhibitor of CDK1/cyclin B (IC_{50} = 2.8 μM) but did not inhibit CDK2/cyclin E and CDK4/cyclin D1 (IC_{50} > 10 μM). Compound **96** inhibited the phosphorylation of histone H3 on Ser10 in HeLa tumor cells (IC_{50} ~ 0.250 μM), a process catalyzed by Aurora-B during mitosis. A crystal structure of **96** complexed to Aurora-B/INCENP has been reported [170]. The oxindole was situated within the ATP binding pocket with the oxygen and nitrogen atoms forming hydrogen bonds to the main chain carbonyl and amide NH of Glu171 and Ala173, respectively (Fig. 11). The observed orientation is similar to other oxindole-based inhibitors bound in the active sites of FGFR1 and CDK2. The piperidine was exposed to solvent while the sulfonamide was directed into the active site. The sulfonamide oxygen atoms made two H-bonds to Lys103 and Lys122.

3.3.9
5*H*-pyrimidino[5,4-*d*]benzazepines

The group at Millennium Pharmaceuticals has claimed MLN-8054 (**97**) to be the first kinase inhibitor selective for Aurora-A over Aurora-B, which gives robust inhibition of human tumor xenografts [228–230]. Treatment of cultured human tumor cells with **97** resulted in the accumulation of mitotic cells with spindle abnormalities, a phenotype consistent with selective Aurora-A inhibition. In a pharmacodynamic model the time-dependent accumulation of



97

Structure 97

mitotic cells was detected in SW480 and HCT-116 xenografts in nude mice 4 h after the administration of a single oral dose of **97**. In a study examining the effect of **97** on centrosome maturation and spindle formation in mitotic tumor cells, **97** was found to cause a high degree of defects in spindle pole formation with the majority of spindle fibers abnormally formed and the DNA not tightly aligned to the metaphase plate. Using time-lapsed video microscopy with fluorescent α-tubulin and histone H2B, **97**-treated tumor cells were shown to undergo a significantly delayed anaphase relative to untreated cells. A dosing scheduling study in the HCT-116 human tumor xenograft model compared continuous oral dosing over 20 days (30 mpk BID) with 5 days on/5 days off repeated twice (30 mpk BID), 10 days on/10 days off (30 mpk BID) and 3 days on/7 days off, repeated twice (60 mpk BID). Continuous dosing was the most effective with 103% tumor growth inhibition (TGI), while the 5/5 twice (77% TGI) and 10/10 (83% TGI) and 3/7 twice at 60 mpk bid (73% TGI) schedules were slightly less efficacious. MLN-8054 (**97**) is now in Phase I clinical trials.

4
Polo-Like Kinases

4.1
Biology of the Polo-Like Kinases

The Polo-like kinases (PLKs) are serine/threonine kinases that participate in the control of mitotic progression. There are four family members that possess a conserved catalytic domain and a defining Polo-box domain (PBD) [231, 232]. The PBD, located in the non-catalytic C-terminus of the proteins, is critical for the interaction of PLK family enzymes with their substrates. Although mutations in the PBD do not affect the kinase activity of the PLKs, these altered proteins lack biological activity in cells, highlighting the critical contribution of the PDB for substrate recognition [233]. Active PLKs regulate bipolar spindle formation, chromosome segregation, centrosome maturation, and execution of cytokinesis through the phosphorylation of substrate proteins such as cyclin B1, cdc2, myt1, BRCA2, and cdc27 (reviewed in [234]).

PLK-1, the most extensively studied member of this kinase family, exhibits peak expression during late G2 and M phases. Specifically, PLK-1 drives the transition into mitosis by phosphorylating cyclin B1, promoting its subsequent translocation to the nucleus [235, 236] and by phosphorylating cdc25C, activating its phosphatase activity [237]. In addition, PLK-1 plays a critical role in the response to DNA damage, where DNA damage inhibits the activity of PLK-1 and therefore prevents the activation of cdc25C and the progression into M phase [238]. PLK-1 overexpression has been linked to induction

of DNA synthesis in quiescent cells and transformation of NIH-3T3 cells and their subsequent tumorigenesis in nude mice [239, 240]. The role of PLK-1 in driving cancer cell proliferation has been examined using *si*RNA, and the reduction in PLK-1 levels concomitantly reduces the viability of cells and induces apoptosis and G2/M arrest [241]. PLK-1 is overexpressed in a wide variety of human tumors, including colorectal, ovarian, breast, prostate, and pancreatic cancers and melanomas [242–247], and as such, may play a significant role clinically.

The other PLK family members, PLK-2 (a.k.a. SNK, serum inducible kinase), PLK-3 (a.k.a. FNK, FGF-inducible kinase) or PRK (proliferation related kinase) and PLK-4 (SAK), exert their effects on cell cycle control due to differential expression at distinct stages in the cycle. Similarly to PLK-1, both PLK-2 and PLK-3 play a role in the cellular responses to DNA damage. However, in clinical samples, PLK-3 appears to be negatively regulated with disease, as it is down-regulated in lung, and head and neck tumor tissue [248, 249]. These results correlate with the induction of apoptosis seen when PLK-3 is overexpressed in cells. PLK-4 plays a role in centriole duplication but in animal models of haploinsufficiency, greater spontaneous tumor development is seen in mice with lower PLK-4 gene dosage [250]. This information suggests that small-molecule inhibitors should selectively target PLK-1 to be therapeutically useful.

4.2 Structural Biology of the Polo-Like Kinases

No crystal structures are currently available of a PLK kinase domain; however, a recent structural study has been reported for the Polo-box domain of the C-terminal non-catalytic region, which forms the basis for a hypothesis on the autoregulation of PLK kinase activity [251]. Since there is high degree of structural similarity between kinase catalytic domains, homology models based on the available crystal structures offer an alternative method for identifying features in the active site that are important for activity and selectivity. Two homology models for human PLK-1 have been reported based on a combination of homologous crystal structures: the first used PKA, CDK2, ERK2 [232, 252] and the second, Aurora-A, Aurora-B and Akt/PKB [253]. The second PLK-1 homology model was also used as a template to model the PLK-2, PLK-3, and PLK-4 kinase domains.

Analysis of these homology models provided insight into the nature of the ATP binding pocket, revealing unique features not apparent in other kinases (Fig. 14). In PLK-1 the residue at position 67, Val in most kinases, is a Cys. This residue offers the possibility of designing inhibitors targeting the reactive thiol group of Cys67 to covalently modify the enzyme active site. The concept of designing an irreversible inhibitor with an electrophilic group to react with a Cys thiol in the active site has been used successfully for in-

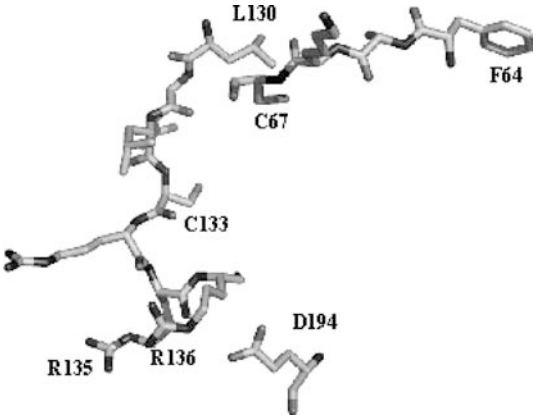


Fig. 14 Model of the ATP binding site of PLK-1

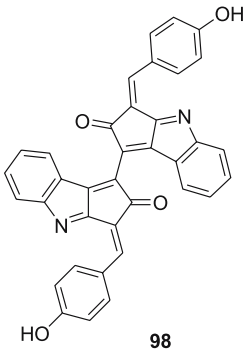
hibitors of erbB2 tyrosine kinase [254, 255]. Other residues found in the ATP binding pocket of PLK-1 that might be exploited to generate selective inhibitors, include Leu130, corresponding to the so-called "gatekeeper residue", Phe183, D194, Arg135, and Arg136.

4.3
Structure–Activity Relationships of Polo-Like Kinase Inhibitors

PLK-1 is a relatively novel kinase target and a number of naturally occurring, broadly active kinase inhibitors are being considered as starting points for the design of more selective inhibitors of this enzyme. These compounds will be presented first, followed by a summary of the data available for the only clinical candidate reported up to this time, BI-2536 (**103**), and a survey of the literature on the chemical series now being pursued by the pharmaceutical industry. A recent review of this topic is also available [256].

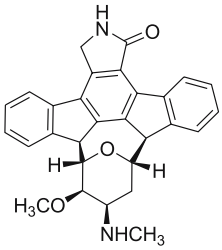
4.3.1
Scytonemin, Staurosporine, Purvalanol A and Flavinoids

Scytonemin (**98**) [257–259] inhibited the phosphorylation of cdc25C by recombinant PLK-1 (IC_{50} = 1.95 μM). In ATP competition assays **98** showed the profile of a mixed inhibitor. The compound was not selective and had similar potency against other kinases, including MYT1 (IC_{50} = 1.17 μM), CHK1 (IC_{50} = 1.42 μM), CDK1/cyclin B (IC_{50} = 3.02 μM), and PKC (IC_{50} = 2.73 μM). Staurosporine (**99**) was found to be a moderately potent inhibitor of PLK-1 (IC_{50} = 0.8 μM), however, it was non-selective and showed activity against a wide range of kinases, especially CDK2 (IC_{50} = 0.004 μM) [253]. Purvalanol A, (**5a**), a relatively selective and potent inhibitor of CDKs, cdc2/cyclin B



98

Structure 98

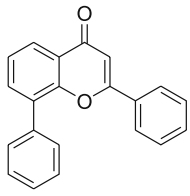


99

Structure 99

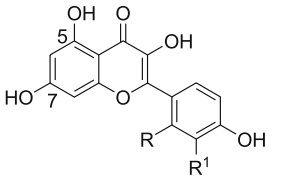
(IC_{50} = 0.004 μM), CDK2/cyclin A (IC_{50} = 0.07 μM), CDK2/cyclin E (IC_{50} = 0.035 μM), CDK4/cyclinD1 (IC_{50} = 0.85 μM), and CDK5/p35 (IC_{50} = 0.075 μM) was found to inhibit PLK-1 with micromolar potency (IC_{50} = 5.0 μM) [253, 260, 261].

The flavonoids, LY294002 (**100**, PLK-1 IC_{50} = 5.25 μM) [253, 262], morin (**101a**, PLK-1 IC_{50} = 12.6 μM), robinetin (**101b**, PLK-1 IC_{50} = 60 μM), and quercetin (**102**, PLK-1 IC_{50} = 64 μM) were modest inhibitors of PLK-1, whereas other flavonoid compounds, including myricetin, datescetin, luteolin, galangin, daidzein, fisetin, kaemperfol, and kaempferide, were found



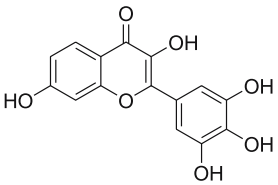
100

Structure 100



101a: R = OH, R^1 = H
101b: R = H, R^1 = OH

Structure 101



102

Structure 102

to be inactive (IC_{50} > 100 μM). The inactive flavonoids contain the same chromenone scaffold, such as **101**, **102**, and differ only by their hydroxylation pattern. Docking experiments using a PLK-1 homology model showed that the 5- and 7-hydroxyls could act as hydrogen bond donors to the carbonyls of Cys133 and Leu130 located in the "hinge" peptide of the kinase, while the catechol hydroxyl group formed hydrogen bonds to Asp194 and to the backbone N – H of Ala65 (Fig. 15) [253, 263, 264]. However, this model does not make it clear how to distinguish an "active" hydroxylation pattern from an inactive analog.

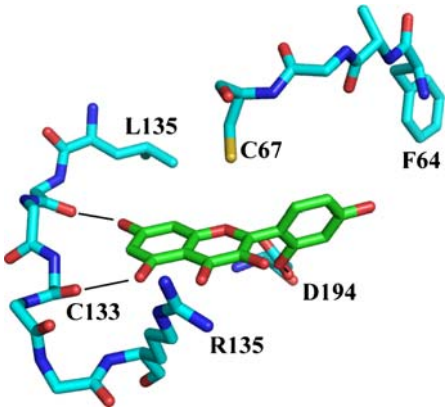
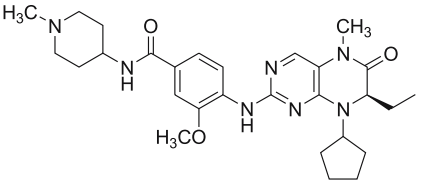


Fig. 15 Model of **101a** in the PLK-1 active site

4.3.2
Pyrimidinopyrazines

Boehringer Ingelheim has reported data on their clinical candidate, BI-2536 (**103**). Compound **103** is a potent PLK-1 inhibitor (IC_{50} = 0.0008 μM) and is highly selective for PLK-1 (> 10 000-fold) against a large panel of serine and threonine kinases. In cell culture **103** inhibits the proliferation of a range of tumor cell lines with EC_{50} values of 0.002 to 0.025 μM [256, 265]. This compound induced mitotic arrest and apoptosis in HL-460 tumors at an IV dose of 60 mpk in mice. At 40 mpk IV once or twice weekly for 4 weeks, **103** inhibited tumor growth in a HCT-116 colon cancer xenograft model. The compound was also active against BxPC-3 pancreas cancer and A549 non-small cell lung cancer xenograft models [266]. In a Phase I dose escalation trial in patients with advanced or metastatic disease, the maximum tolerated dose for **103** has been set at 200 mg as a single 1-h IV infusion. The plasma elimination half-life was determined to be 18 h. Dose-limiting toxicity was neuropenic infection in two of six patients at the 250 mg dose [267].

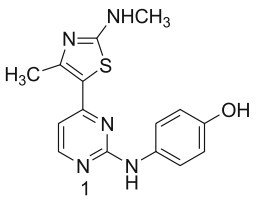


103: BI 2536

Structure 103

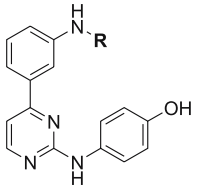
4.3.3
Pyrimidines

Cyclacel described thiazolo-pyrimidine analogs, exemplified by **104** [268, 269], and a related series of 2-aminophenyl-4-phenylpyrimidines, exemplified by **105a,b** [270] with activity against PLK-1. These compounds were derived



104

Structure 104



105a: R = CH_3
105b: R = $C(O)CH_3$

Structure 105

from a previously reported series of 2-anilino-4-heteroaryl-pyrimidine CDK inhibitors and had IC_{50} values between 5–50 μM for PLK-1 and < 0.60 μM for CDK2. All analogs had a *para*-hydroxy group in the aniline portion of the core and this moiety was clearly linked to the observed PLK-1 inhibitory activity. Docking experiments with a homology model suggested that these inhibitors make the essential hinge region H-bonds through the aniline N – H group and the N-1 of the pyrimidine scaffold while the *para*-hydroxyl group of the aniline interacts with Arg135 located on the solvent accessible cleft of the ATP binding pocket (Fig. 16).

Kyowa Hakko Kogyu Pharmaceuticals reported a series of 2,4,5-trisubstituted pyrimidine compounds as PLK-1 inhibitors [271]. Two of the most potent analogs were **106** (PLK-1 IC_{50} = 0.33 μM) and **107** (PLK-1 IC_{50} = 0.55 μM). Active compounds contained either a tetrazole or a nitrile group at the 5-position of the pyrimidine while 2-heteroalkylamine or 2-arylalkylamine substituents were required. Substituents at the 4-position appeared to have less of an impact on PLK-1 inhibition activity. No preferred stereochemistry was exemplified for the 1-phenylethylamine in **107**. GlaxoSmithKline has also claimed a related series of 2,4,5-trisubstituted pyrimidines as PLK-1 inhibitors [272]. The most potent compound from this series was **108** with submicromolar activity against the enzyme as well as submicromolar

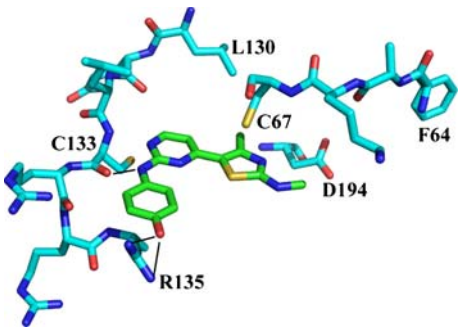
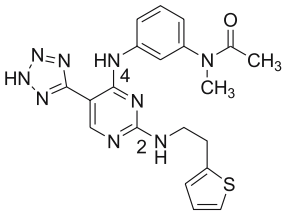
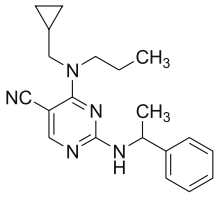


Fig. 16 Compound **104** in a model of PLK-1



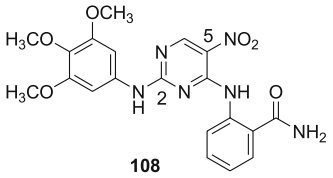
106

Structure 106



107

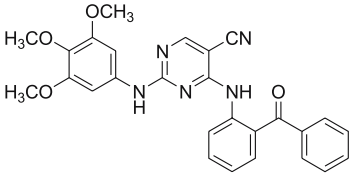
Structure 107



108

Structure 108

anti-proliferative activity against a wide range of tumor cell lines. The preferred substituents at the 2-position were 3,4,5 trimethoxyaniline and *N*-(4-aminophenyl)acetamide. A variety of 4-arylamine substituents were tolerated while the presence of a 5-nitro was important for cell activity. Replacing the 5-nitro functionality with a 5-nitrile yielded **109**, a compound with submicromolar activity against PLK-1 enzyme but substantially lower tumor cell anti-proliferative activity.

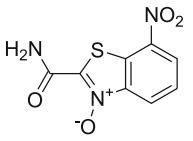


109

Structure 109

4.3.4
Benzothiazoles

Cyclacel screened a large collection of commercially available compounds in a homology model of the PLK-1 active site using a high-throughput docking program. The top-ranking candidates were evaluated in an assay against the human recombinant enzyme. This approach resulted in the identification of a novel series of benzothiazole *N*-oxide PLK-1 inhibitors [273]. These compounds were reported to be highly selective for PLK-1, however, no supporting data has been presented. The most potent compound was **110** (PLK-1 IC_{50} = 0.06 μM) with anti-proliferative activity against tumor cells in the low micromolar range (A475 IC_{50} = 4.0 μM; HeLa IC_{50} = 6.0 μM; MCF-7 IC_{50} = 2.5 μM; U2OS IC_{50} = 8.2 μM).

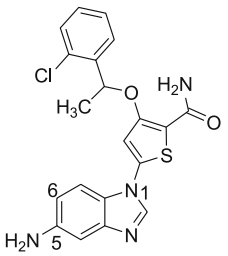


110

Structure 110

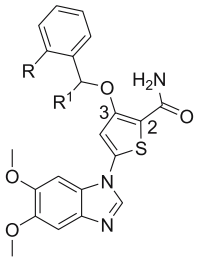
4.3.5
5-(Benzimidazol-1-yl)thiophene and 2-(benzimidazol-1-yl)thiazole

GlaxoSmithKline has filed patent applications covering 5-(benzimidazol-1-yl)thiophenes **111** and **112** and 2-(benzimidazol-1-yl)thioazoles **113** as inhibitors of PLK-1 [274, 275]. Many potent compounds were claimed for 5-(benzimidazol-1-yl)thiophene series, including analog **111** with submicromolar inhibition against PLK-1 and anti-proliferative activity in five out of the six tumor cell lines tested. According to the data disclosed in the filing, a variety of substituents were accommodated on the 5- and 6-positions of the ben-



111

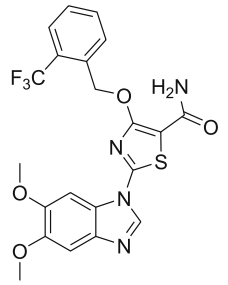
Structure 111



112: R = Cl, CF_3, CH_3, NO_2
R^1 = H, CH_3

Structure 112

zimidazole ring with the 5,6-dimethoxy- (**112**) and 6-aminobenzimidazole (**111**) most preferred. Substitution at the 2-position of the benzimidazole was not tolerated. A series of analogs with *ortho*-substituted benzyl ethers on the 3-position of the thiophene **112** were active compounds. Preferred *ortho* substituents, – *R* in **112**, were chloro-, trifluoromethyl-, methyl- and nitro. Substitution of the benzylic methylene with a methyl group, – R^1 in **112**, was also tolerated. No stereochemical preference was reported for this substitution. The thiophene-2-carboxamide in **111** and **112** was an absolute requirement for activity. Substitution of the amide nitrogen with an alkyl group or replacing the amide with an ester, acid, thioamide, nitrile, or tetrazole resulted in the loss of enzyme and cell activity. The SAR of the 2-(benzimidazol-1-yl)thiazoles parallels the relationships reported for the corresponding 5-(benzimidazol-1-yl)thiophenes. Although several of the thiazole analogs had good activity against PLK-1, none of these compounds exhibited comparable cellular potency. The most potent compound from this series was **113**. It had submicromolar potency against PLK-1 and tumor cell anti-proliferative activity with IC_{50} values in the range 1–10 μM.

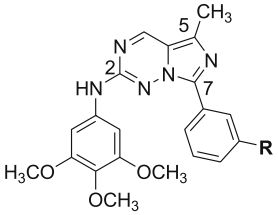


113

Structure 113

4.3.6
Imidazotriazines

A GlaxoSmithKline patent reported a series of imidazo[5,1-*f*] [1, 2, 4]triazines to have activity against PLK-1 [276]. The most potent analogs, exemplified by compounds **114a,b**, were active against PLK-1 in the submicromolar range and had anti-proliferative activity with IC_{50} values of 1 to 10 μM. The preferred 2-substituents on the imidazotriazine core were the 3,4,5 trimethoxyaniline and *N*(4-amino-phenyl)acetamide, while the preferred 7-substituents were phenyl and *meta*-trifluoromethylphenyl.

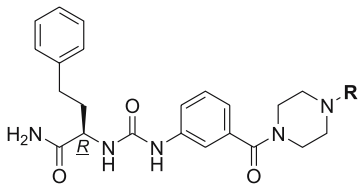


114a: R = H
114b: R = -CF_3

Structure 114

4.3.7
Phenylureas

In a recently published patent application GlaxoSmithKline described a series of phenylureas as PLK-1 inhibitors [277]. The most active compound was **115a** (R = Cbz) which was reported to have an IC_{50} value against PLK-1 in the range 0.1–10 μM. No cell data were supplied for these analogs. The *R*-stereoisomer was the preferred one for this series while the free piperazine analog **115b** (R = H) was inactive.

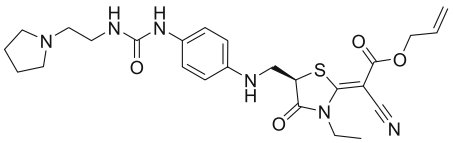


115a: R = Cbz
115b: R = H

Structure 115

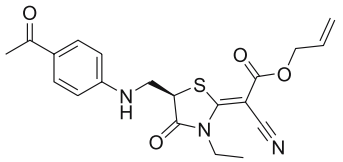
4.3.8
3-Ethylthiazolidinone

Schering published two patent applications describing a series of ethylthiazolidinones as PLK-1 inhibitors [278, 279]. The most potent compounds were **116** (PLK-1 IC_{50} = 0.023 μM) and **117** (PLK-1 IC_{50} = 0.034 μM). Compounds **116** (MaTu IC_{50} = 1.1 μM) and **117** (MaTu IC_{50} = 1.4 μM) also demonstrated anti-proliferative effects in MaTu cells.



116

Structure 116



117

Structure 117

5
Conclusion

Flavopiridol (**1**), the first CDK inhibitor to enter clinical trials and the prototype for the general class of cell cycle therapeutics, still garners considerable attention in the clinic with some 20 Phase I and II clinical trials currently enrolling patients. While **1** may not be an optimal drug, this work is extremely important for defining the dosing sequence for combination studies, the dosing schedule to manage drug exposure and toxicity, and the pharmacodynamic markers for future studies [40]. Most importantly, although objective clinical responses with flavopiridol have been modest, there is good reason to believe that compounds with improved efficacy and selectivity will be more effective clinically. Responding to this challenge, the pharmaceutical industry has entered into early human clinical trials with improved inhibitors of the CDK, Aurora, and PLK kinases. As of this writing there have been few reports on the clinical studies of this "second wave" of clinical compounds but

the data are much anticipated. These compounds will provide the ultimate test of the hypothesis that rapidly cycling tumor cells can be selectively killed by inhibition of cell cycle dynamics.

References

1. Manning G, Whyte DB, Martinez R, Hunter T, Sudarsanam S (2002) Science 298:1912
2. Noble MEM, Endicott JA, Johnson LN (2004) Science 303:1800
3. Morgan DO (1997) Annu Rev Cell Dev Biol 13:261
4. An HX, Beckmann MW, Reifenberger G, Bender HG, Niederacher D (1999) J Am Pathol 154:113
5. Dobashi Y, Goto A, Fukayama M, Abe A, Ooi A (2004) Int J Cancer 110:532
6. Al-Aynati MM, Radulovich N, Ho J, Tsao MS (2004) Clin Cancer Res 10:6598
7. Liang B, Wang S, Yang X, Ye Y, Yu Y, Cui Z (2003) Chin Med J (Engl) 116:20
8. Sui L, Dong Y, Ohno M, Sugimoto K, Tai Y, Hando T, Tokuda M (2001) Gynecol Oncol 83:56
9. Kohzato N, Dong Y, Sui L, Masaki T, Nagahata S, Nishioka M, Konishi R, Tokuda M (2001) Hepatol Res 21:27
10. Depowski PL, Brien TP, Sheehan CE, Stylos S, Johnson RL, Ross JS (1999) J Am Clin Pathol 112:459
11. Chang JT, Wang HM, Chang KW, Chen WH, Wen MC, Hsu YM, Yung BY, Chen IH, Liao CT, Hsieh LL, Cheng AJ (2005) Int J Cancer 114:942
12. Sandhu C, Slingerland J (2000) Cancer Detect Prev 24:107
13. Knighton DR, Zheng JH, Ten KLF, Ashford VA, Xuong NH, Taylor SS, Sowadski JM (1991) Science 253:404
14. Russo AA, Jeffrey PD, Pavletich NP (1996) Nat Struct Biol 3:696
15. Russo AA, Tong L, Lee JO, Jeffrey PD, Pavletich NP (1998) Nature 395:237
16. Pavletich NP (1999) J Mol Biol 287:821
17. Schulze-Gahmen U, De Bondt HL, Kim SH (1996) J Med Chem 33:4540
18. Brown NR, Noble ME, Lawrie AM, Morris MC, Tunnah P, Divita G, Johnson LN, Endicott JA (1999) J Biol Chem 274:8746
19. Brown NR, Noble ME, Endicott JA, Johnson LN (1999) Nat Cell Biol 1:438
20. Lowe E, Tews I, Cheng K, Brown N, Gul S, Noble M, Gamblin S, Johnson L (2002) Biochemistry 41:15625
21. Jeffrey PD, Russo AA, Polyak K, Gibbs E, Hurwitz J, Massague J, Pavletich NP (1995) Nature 376:313
22. Russo AA, Jeffrey PD, Patten AK, Massague J, Pavletich NP (1996) Nature 382:325
23. Song H, Hanlon N, Brown NR, Noble ME, Johnson LN, Barford D (2001) Mol Cell 7:615
24. Cook A, Lowe ED, Chrysina ED, Skamnaki VT, Oikonomakos NG, Johnson LN (2002) Biochemistry 41:7301
25. Honda R, Lowe ED, Dubinina E, Skamnaki V, Cook A, Brown N, Johnson LN (2005) EMBO J 24:452
26. Dreyer MK, Borcherding DR, Dumont JA, Peet NP, Tsay JT, Wright PS, Bitonti AJ, Shen J, Kim SH (2001) J Med Chem 44:524
27. Beattie JF, Breault GA, Ellston RPA, Green S, Jewsbury PJ, Midgley CJ, Naven RT, Minshull CA, Pauptit RA, Tucker JA, Pease JE (2003) Bioorg Med Chem Lett 13:2955
28. Russo AA, Tong L, Lee JO, Jeffrey PD, Pavletich NP (1998) Nature 395:237

29. Brotherton DH, Dhanaraj V, Wick S, Brizuela L, Domaille PJ, Volyanik E, Xu X, Parisini E, Smith BO, Archer SJ, Serrano M, Brenner SL, Blundell TL, Laue ED (1998) Nature 395:244
30. Jeffrey PD, Tong L, Pavletich NP (2000) Genes Dev 14:3115
31. Schulze-Gahmen U, Kim SH (2002) Nat Struct Biol 9:177
32. Lu HS, Chang DJ, Baratte B, Meijer L, Schulze-Gahmen U (2005) J Med Chem 48:737
33. Lolli G, Lowe ED, Brown NR, Johnson LN (2004) Structure 12:2067
34. Ikuta M, Kamata K, Fukasawa K, Honma T, Machida T, Hirai H, Suzuki-Takahashi I, Hayama T, Nishimura S (2001) J Biol Chem 276:27548
35. Cavalli A, Dezi C, Folkers G, Scapozza L, Recanatini M (2001) Prot Struct Funct Genet 45:478
36. Verma S, Nagarathnam D, Shao J, Zhang L, Zhao J, Wang Y, Li T, Mull E, Enyedy I, Wang C, Zhu Q, Altieri M, Jordan J, Dang TTA, Reddy S (2005) Bioorg Med Chem Lett 15:1973
37. Senderowicz AM (1999) Invest New Drugs 17:313
38. Zhai S, Senderowicz AM, Sausville EA, Figg WD (2002) Ann Pharmacother 36:905
39. Kelland LR (2000) Expert Opin Investig Drugs 9:2903
40. Shapiro GI (2004) Clin Cancer Res 10(suppl):4270
41. Collins I, Garrett MD (2005) Curr Opin Pharmacol 5:366
42. Pevarello P, Villa M (2005) Exp Opin Therap Pat 15:675
43. Deshpande A, Sicinski P, Hinds PW (2005) Oncogene 24:2909
44. Kong N, Fotouhi N, Wovkulich PM, Roberts J (2003) Drug Future 28:881
45. Huwe A, Mazitschek R, Giannis A (2003) Angew Chem Int Ed 42:2122
46. Fischer PM, Gianella-Borradori A (2003) Exp Opin Inv Drugs 2:955
47. Fischer PM (2005) Drug Future 30:911
48. Misra RN (2006) Drug Future 31:43
49. Vesely J, Havlicek L, Strand M, Blow JJ, Donella-Deana A, Pinna L, Letham DS, Kato JY, De'tivaud L, Leclerc S, Meijer L (1994) Eur J Biochem 224:771
50. Shum PW, Peet NP, Weintraub PM, Le TB, Zhao Z, Barbone F, Cashman B, Tsay J, Dwyer S, Loos PC, Powers EA, Kropp K, Wright PS, Bitonti A, Dumont J, Borcherding DR (2001) Nucleosides Nucleotides Nucl 20:1067
51. O'Connor DS, Wall NR, Porter AC, Altieri DC (2002) Cancer Cell 2:43
52. McClue SJ, Blake D, Clarke R, Cowan A, Cummings L, Fischer PM, MacKenzie M, Melvil le J, Stewart K, Wang S, Zhelev N, Zheleva D, Lane DP (2002) Int J Cancer 102:463
53. Meijer L, Raymond E (2003) Acc Chem Res 36:417
54. Haesslein J-L, Jullian N (2002) Curr Top Med Chem 2:1037 (Hilversum, Netherlands)
55. Fischer PM, Gianella-Borradori A (2005) Exp Opin Inv Drugs 14:457
56. Hardcastle IR, Arris CE, Bentley J, Boyle FT, Chen Y, Curtin NJ, Endicott JA, Gibson AE, Golding BT, Griffin RJ, Jewsbury P, Menyerol J, Mesguiche V, Newell DR, Noble MEM, Pratt DJ, Wang L-Z, Whitfield HJ (2004) J Med Chem 47:3710
57. Sayle KL, Bentley J, Boyle FT, Calvert AH, Cheng Y, Curtin NJ, Endicott JA, Golding BT, Hardcastle IR, Jewsbury P, Mesguiche V, Newell DR, Noble MEM, Parsons RJ, Pratt DJ, Wang L-Z, Griffin RJ (2003) Bioorg Med Chem Lett 13:3079
58. Davies TG, Bentley J, Arris CE, Boyle FT, Curtin NJ, Endicott JA, Gibson AE, Golding BT, Griffin RJ, Hardcastle IR, Jewsbury P, Johnson LN, Mesquiche V, Newell DR, Noble MEM, Tucker JA, Wang L, Whitfield HJ (2002) Nat Struct Biol 9:7
59. Zhu G, Conner SE, Zhou X, Shih C, Li T, Anderson BD, Brooks HB, Campbell RM, Considine E, Dempsey JA, Faul MM, Ogg C, Patel B, Schultz RM, Spencer CD, Teicher B, Watkins S (2003) J Med Chem 46:2030

60. Sanchez-Martinez C, Shih C, Zhu G, Li T, Brooks HB, Patel BKR, Schultz RM, DeHahn TB, Spencer CD, Watkins SA, Ogg CA, Considine E, Dempsey JA, Zhang F (2003) Bioorg Med Chem Lett 13:3841
61. Zhu G, Conner S, Zhou X, Shih C, Brooks HB, Considine E, Dempsey JA, Ogg C, Patel B, Schultz RM, Spencer CD, Teicher B, Watkins SA (2003) Bioorg Med Chem Lett 13:1231
62. Sanchez-Martinez C, Shih C, Faul MM, Zhu G, Paal M, Somoza C, Li T, Kumrich CA, Winneroski LL, Xun Z, Brooks HB, Patel BKR, Schultz RM, DeHahn TB, Spencer CD, Watkins SA, Considine E, Dempsey JA, Ogg CA, Campbell RM, Anderson BA, Wagner J (2003) Bioorg Med Chem Lett 13:3835
63. Engler TA, Furness K, Malhotra S, Sanchez-Martinez C, Shih C, Xie W, Zhu G, Zhou X, Conner S, Faul MM, Sullivan KA, Kolis SP, Brooks HB, Patel B, Schultz RM, DeHahn TB, Kirmani K, Spencer CD, Watkins SA, Considine EL, Dempsey JA, Ogg CA, Stamm NB, Anderson BD, Campbell RM, Vasudevan V, Lytle ML (2003) Bioorg Med Chem Lett 13:2261
64. Zaharevitz DW, Gussio R, Leost M, Senderowicz AM, Lahuen T, Kunick C, Meijer L, Sausville EA (1999) Cancer Res 59:2566
65. Schultz C, Link A, Leost M, Zharevitz DW, Gussio R, Sausville E, Meijer L, Kunick C (1999) J Med Chem 42:2909
66. Kunick C, Schultz C, Lemcke T, Zaharevitz D, Gussio R, Jalluri RK, Sausville, Leost M, Meijer L (2000) Bioorg Med Chem Lett 10:567
67. Kunick C, Zeng Z, Gussio R, Zaharevitz D, Leost M, Totzke F, Schachtele C, Kubbutat MHG, Meijer L, Lemcke T (2005) Chem Bio Chem 6:541
68. Wieking K, Knockaert M, Leost M, Zaharevitz DW, Meijer L, Kunick C (2002) Arch Pharm Pharm Med Chem 7:311
69. Gussio R, Zaharevitz DW, McGrath CF, Pattabirman N, Kellogg GE, Schultz C, Link A, Kunick C, Leost M, Meijer L, Sausville EA (2000) Cancer Drug Design 15:53
70. Pies T, Schaper K-J, Leost M, Zaharevitz DW, Gussio R, Meijer L, Kunick C (2004) Arch Pharm Pharm Med Chem 337:486
71. Kunick C, Lauenroth K, Wieking K, Xie X, Schultz C, Gussio R, Zaharevits D, Leost M, Meijer L, Weber A, Jorgensen FS, Lemcke T (2004) J Med Chem 47:22
72. Misra RN, Xiao H-Y, Rawlins DB, Shan W, Kellar KA, Mulherony JG, Sack JS, Tokarski JS, Kimball SD, Webster KR (2003) Bioorg Med Chem Lett 13:2405
73. Misra RN, Rawlins DB, Xiao H-Y, Shan W, Bursuker I, Kellar KA, Mulheron JG, Sack JS, Tokarski JS, Kimball SD, Webster KR (2003) Bioorg Med Chem Lett 13:1133
74. Misra RN, Kimball SD, Rawlins DB, Webster KR, Bursuker I (1999) WO 9930710 A1
75. Kephart SE, McAlpine IJ, Reich SH (2005) WO 2005009997 A1
76. McAlpine IJ, Deal JG, Johnson MC, Kephart SE, Park JY, Romines WH, Tikhe JG (2005) US Patent 2 005 090 529 A1
77. Zhang CC, Troche G, Yan Z, Arango ME, Higgins J, Romero D, Kephart S, McAlpine I, Koudriakova T, Skaptason J, Nonomiya J, Knighton D, Ferre RA, Tikhe J, Verkhivker G, Xu M, Romines W, Palmer C, Park J, Reich S, Los G, Lewis C (2005) Proceeds 96th AACR Annual Meeting, Orlando, FL, USA, Abstract 4413
78. Nugiel DA, Etzkorn AM, Vidwans A, Benfield PA, Boisclair M, Burton CR, Cox S, Czerniak PM, Doleniak D, Seitz SP (2001) J Med Chem 44:1334
79. Nugiel DA, Vidwans A, Etzkorn A-M, Rossi KA, Benfield PA, Burton CR, Cox S, Doleniak D, Seitz SP (2002) J Med Chem 45:5224
80. Yue EW, Higley CA, DiMeo SV, Carini DJ, Nugiel DA, Benware C, Benfield PA, Burton CR, Cox S, Grafstrom RH, Sharp DM, Sisk LM, Boylan JF, Muckelbauer JK, Smallwood AM, Chen H, Chang C-H, Seitz SP, Trainor GL (2002) J Med Chem 45:5233

81. Yue EW, DiMeo SV, Higley CA, Markwalder JA, Burton CR, Benfield PA, Grafstrom RH, Cox S, Muckelbauer JK, Smallwood AM, Chen H, Chang C-H, Trainor GL, Seitz SP (2004) Bioorg Med Chem Lett 14:343
82. Pevarello P, Brasca MG, Amici R, Orsini P, Traquandi G, Corti L, Piutti C, Sansonna P, Villa M, Pierce BS, Pulici M, Giordano P, Martina K, Fritzen EL, Nugent RA, Casale E, Cameron A, Ciomei M, Roletto F, Isacchi A, Fogliatto GP, Pesenti E, Pastori W, Marsiglio A, Leach KL, Clare PM, Fiorentini F, Varasi M, Vulpetti A, Warpehoski MA (2004) J Med Chem 47:3367
83. Pevarello P, Brasca MG, Orsini P, Traquandi G, Longo A, Nesi M, Orzi F, Piutti C, Sansonna P, Varasi M, Cameron A, Vulpetti A, Roletto F, Alzani R, Ciomei M, Albanese C, Pastori W, Marsiglio A, Pesenti E, Fiorentini F, Bischoff JR, Mercurio C (2005) J Med Chem 48:2944
84. Brasca MG, Amici R, Fancelli D, Nesi M, Orsini P, Orzi F, Roussel P, Vulpetti A, Pevarello P (2004) WO 2004056827 A2
85. Pevarello P, Fancelli D, Vulpetti A, Amici R, Villa M, Pittala V, Vianello P, Cameron A, Ciomei M, Mercurio C, Bischoff JR, Roletto F, Varasi M, Brasca MG (2006) Bioorg Med Chem Lett 16:1270
86. Tang J, Shewchuk LM, Sato H, Hasegawa M, Washio Y, Nishigaki N (2003) Bioorg Med Chem Lett 13:2985
87. Barvian M, Boschelli D, Cossrow J, Dobrusin E, Fattaey A, Fritsch A, Fry D, Harvey P, Keller P, Garrett M, La F, Leopold W, McNamara D, Quin M, Trumpp-Kallmeyer S, Toogood P, Wu Z, Zhang E (2000) J Med Chem 43:4606
88. Booth RJ, Chatterjee A, Malone TC (2001) WO 2001055148 A1
89. Barvian MR, Booth RJ, Quin J, Repine JT, Sheehan DJ, Toogood PL, Vanderwel SN, Zhou H (2003) WO 2003062236 A1
90. Toogood PL, Harvey PJ, Repine JT, Sheehan DJ, VanderWel SN, Zhou H, Keller PR, McNamara DJ, Sherry D, Zhu T, Brodfuehrer J, Choi C, Barvian MR, Fry DW (2005) J Med Chem 48:2388
91. VanderWel SN, Harvey PJ, McNamara DJ, Repine JT, Keller PR, Quin J, Booth RJ, Elliott WL, Dobrusin EM, Fry DW, Toogood PL (2005) J Med Chem 48:2371
92. Barvian MR, Toogood PL, Vanderwel SN (2005) US Patent 2 005 182 078 A1
93. Denny WA, Dobrusin EM, Kramer JB, Mc Namara DJ, Rewcastle GW, Showalter HDH, Toogood PL (2001) WO 2001019825 A1
94. Barvian MR, Bathini Y, Dobrusin EM, Kaltenbronn JS, Micetich RG, Sidhu IS, SinghR, Toogood PL, Winters RT (2001) WO 2001038315 A1
95. Sielecki TM, Johnson TL, Liu J, Muckelbauer JK, Grafstrom RH, Cox S, Boylan J, Burton CR, Chen H, Smallwood A, Chang C-H, Boisclair M, Benfield PA, Trainor GL, Seitz SP (2001) Bioorg Med Chem Lett 11:1157
96. Hatayama S, Hayashi K, Honma M, Takahashi I (2002) Jpn Kokai Tokkyo Koho JP 2002220338 A2
97. Hayama T, Hayashi K, Honma M, Takahashi I (2001) WO 2001007411 A1
98. Honma T, Yoshizumi T, Hashimoto N, Hayashi K, Kawanishi N, Fukasawa K, Takaki T, Ikeura C, Ikuta M, Suzuki-Takahashi I, Hayama T, Nishimura S, Morishima H (2001) J Med Chem 44:4628
99. Honma T, Hayashi K, Aoyama T, Hashimoto N, Machida T, Fukasawa K, Iwama T, Ikeura C, Ikuta M, Suzuki-Takahashi I, Iwasawa Y, Hayama T, Nishimura S, Morishima H (2001) J Med Chem 44:4615
100. Ikuta M, Kamata K, Fukasawa K, Honma T, Machida T, Hirai H, Suzuki-Takahashi I, Hayama T, Nishimura S (2001) J Biol Chem 276:27548
101. Hayama T, Kawanishi N, Takaki T (2002) WO 2002002550 A1

102. Hirai H, Kawanishi N, Hirose M, Sugimoto T, Kamijyo K, Shibata J, Masutani K (2004) WO 2004039809 A1
103. Chu X-J, Lovey A, DePinto W, Bartkovitz D, So S-S, Vu B, Ding O, Packman K, Yin X, Jiang N, Moliterni J, Kaplan G, Mullin J, Lukacs C, Graves B, Smith M, Chen Y (2005) Proceedings 96th AACR Annual Meeting, Orlando, FL, USA, Abstract 1221
104. Beattie JF, Breault GA, Ellston RPA, Green S, Jewsbury PJ, Midgley CJ, Naven RT, Minshull CA, Pauptit RA, Tucker JA, Pease JE (2003) Bioorg Med Chem Lett 13:2955
105. Breault GA, Ellston RPA, Green S, James SR, Jewsbury PJ, Midgley CJ, Pauptit RA, Minshull CA, Tucker JA, Pease J (2003) Bioorg Med Chem Lett 13:2961
106. Anderson M, Beattie JF, Breault GA, Breed J, Byth KF, Culshaw JD, Ellston RPA, Green S, Minshull CA, Norman RA, Pauptit RA, Stanway J, Thomas AP, Jewsbury PJ (2003) Bioorg Med Chem Lett 13:3021
107. Byth KF, Culshaw JD, Green S, Oakes SE, Thomas AP (2004) Bioorg Med Chem Lett 14:2245
108. Byth KF, Cooper N, Culshaw JD, Heaton DW, Oakes SE, Minshull CA, Norman RA, Pauptit RA, Tucker JA, Breed J, Pannifer A, Rowsell S, Stanway JJ, Valentine AL, Thomas AP (2004) Bioorg Med Chem Lett 14:2249
109. Hamdouchi C, Keyser H, Collins E, Jaramillo C, De Diego JE, Spencer CD, Dempsey JA, Anderson BD, Leggett T, Stamm NB, Schultz RM, Watkins SA, Cocke K, Lemke S, Burke TF, Beckmann RP, Dixon JT, Gurganus TM, Rank NB, Houck KA, Zhang F, Vieth M, Espinosa J, Timm DE, Campbell RM, Patel BKR, Brooks HB (2004) Mol Cancer Ther 3:1
110. Jaramillo C, De Diego JE, Hamdouchi C, Collins E, Keyser H, Sanchez-Martinez C, Del Prado M, Norman B, Brooks HB, Watkins SA, Spencer CD, Dempsey JA, Anderson BD, Campbell RM, Leggett T, Patel B, Schultz RM, Espinosa J, Vieth M, Zhang F, Timm DE (2004) Bioorg Med Chem Lett 14:6095
111. Hamdouchi C, Zhong B, Mendoza J, Collins E, Jaramillo C, De Diego JE, Robertson D, Spencer CD, Anderson BD, Watkins SA, Zhang F, Brooks HB (2005) Bioorg Med Chem Lett 15:1943
112. Paruch K, Guzi TJ, Dwyer MP, Doll RJ, Girijavallabhan VM, Mallams AK (2004) WO 2004026877 A1
113. Dwyer MP, Guzi TJ, Paruch K, Doll RJ, Keertikar KM, Girijavallabhan VM (2004) WO 2004026867 A2
114. Dwyer MP, Guzi TJ, Paruch K, Doll RJ, Keertikar KM, Girijavallabhan VM (2004) WO 2004026872 A1
115. Guzi TJ, Paruch K, Dwyer MP, Doll RJ, Girijavallabhan VM, Mallams A, Alvarez CS, Keertikar KM, Rivera J, Chan T-Y, Madison V, Fischmann TO, Dillard LW, Tran VD, He ZM, James RA, Park H, Paradkar VM, Hobbs DW (2004) US Patent 2 004 209 878 A1
116. Guzi TJ, Paruch K, Dwyer MP, Doll RJ, Girijavallabhan VM, Alvarez CS, Chan T-Y, Knutson C, Madison V, Fischmann TO, Dillard LW, Tran VD, He ZM, James RA, Park H (2004) WO 2004026229 A2
117. Tang PC, Miller TA, Li X, Zhang R, Cui J, Huang P, Wei CC (2001) WO 2001064681 A2
118. Li X, Huang P, Cui JJ, Zhang J, Tang C (2003) Bioorg Med Chem Lett 13:1939
119. Luk K-C, Simcox ME, Schutt A, Rowan K, Thompson T, Chen Y, Kammlott U, DePinto W, Dunten P, Dermatakis A (2004) Bioorg Med Chem Lett 14:913
120. Dermatakis A, Luk K-C, DePinto W (2003) Bioorg Med Chem 11:1873
121. Kley J, Heckel A, Hilberg F, Roth GJ, Lehmann-Lintz T, Lotz RRH, Tontsch-Grunt U, Van Meel JCA (2004) WO 2004026829 A2

122. Bramson HN, Corona J, Davis ST, Dickerson SH, Edelstein M, Frye SV, Gampe RT Jr, Harris PA, Hassell A, Holmes WD, Hunter RN, Lackey KE, Lovejoy B, Luzzio MJ, Montana V, Rocque WJ, Rusnak D, Shewchuk L, Veal JM, Walker DH, Kuyper LF (2001) J Med Chem 44:4339
123. Dickerson SH, Drewry DH (2002) WO 2002020524 A1
124. Lin R, Connolly PJ, Wetter S, Huang S, Emanuel S, Guninger RH, Middleton S (2002) WO 2002057240 A1
125. Lin R, Connolly PJ, Huang S, Wetter SK, Lu Y, Murray WV, Emanuel SL, Gruninger RH, Fuentes-Pesquera AR, Rugg CA, Middleton SA, Jolliffe LK (2005) J Med Chem 48:4208
126. Emanuel S, Rugg CA, Gruninger RH, Lin R, Fuentes-Pesquera A, Connolly PJ, Wetter SK, Hollister B, Kruger WW, Napier C, Jolliffe L, Middleton SA (2005) Cancer Res 65:9038
127. Kim KS, Kimball SD, Misra RN, Rawlins DB, Hunt JT, Xiao H-Y, Lu S, Qian L, Han W-C, Shan W, Mitt T, Cai Z-W, Poss MA, Zhu H, Sack JS, Tokarski JS, Chang C-Y, Pavletich N, Kamath A, Humphreys WG, Marathe P, Bursuker I, Kellar KA, Roongta U, Batorsky R, Mulheron JG, Bol D, Fairchild CR, Lee FY, Webster KR (2002) J Med Chem 45:3905
128. Misra RN, Xiao HY, Kim KS, Lu S, Han WC, Barbosa SA, Hunt JT, Rawlins DB, Shan W, Ahmed SZ, Qian L, Chen BC, Zhao R, Bednarz MS, Kellar KA, Mulheron JG, Barorsky R, Roongta U, Kamath A, Marathe P, Ranadive SA, Sack JS, Tokarski JS, Pavletich NP, Lee FY, Webster KR, Kimball SDJ (2004) J Med Chem 47:1719
129. Misra RN, Xiao H, Williams DK, Kim KS, Lu S, Keller KA, Mulheron JG, Batorsky R, Tokarski JS, Sack JS, Kimball SD, Lee FY, Webster KR (2004) Bioorg Med Chem Lett 14:2973
130. Chong WKM, Chu S, Duvadie RK, Li L, Na J, Schaffer L, Yang Y (2004) PCT Int Appl WO 2004072070 A1
131. Chu SS, Alegria LA, Bender SL, Pritchett-Benedict S, Borchardt AJ, Kania RS, Nambu MD, Tempczyk-Russell AM, Sarshar S (2000) WO 2000075120 A1
132. Chen L, Ding Q, Gillespie P, Kim K, Lovey AJ, McComas WW, Mullin JG Jr, Perrotta A (2002) WO 2002057261 A2
133. Chu X, Ding Q, Jiang N, Kim K, Lovey AJ, McComas WW, Mullin JG Jr, Tilley JW (2003) WO 2003097048 A1
134. Ding Q, Jiang N, Kim K (2005) US Patent 2 005 239 843 A1
135. Andrews PD, Knato E, Moore WJ, Swedlow JR (2003) Curr Opin Cell Biol 15:672
136. Jeng YM, Peng SY, Lin CY, Hsu HC (2004) Clin Cancer Res 10:2065
137. Dicioccio RA, Song H, Waterfall C, Kimura MT, Nagase H, McGuire V, Hogdall E, Shah MN, Luben RN, Easton DF, Jacobs IJ, Ponder BA, Whittemore AS, Gayther SA, Pharoah PD, Kruger-Kjaer S (2004) Cancer Epidemiol Biomarkers Prev 13:1589
138. Chung CM, Man C, Jin Y, Jin C, Guan XY, Wang Q, Wan TS, Cheung AL, Tsao SW (2005) Mol Carcinog 43:165
139. Fukushima N, Sato N, Prasad N, Leach SD, Hruban RH, Goggins M (2004) Oncogene 23:9042
140. Araki K, Nozaki K, Ueba T, Tatsuka M, Hashimoto N (2004) J Neurooncol 67:53
141. Sorrentino R, Libertini S, Pallante PL, Troncone G, Palombini L, Bavetsias V, Spalletti-Cernia D, Laccetti P, Linardopoulos S, Chieffi P, Fusco A, Portella G (2005) J Clin Endocrinol Metab 90:928
142. Ewart-Toland A, Briassouli P, de Koning JP, Mao JH, Yuan J, Chan F, MacCarthy-Morrogh L, Ponder BA, Nagase H, Burn J, Ball S, Almeida M, Linardopoulos S, Balmain A (2003) Nat Genet 34:403

143. Sun T, Miao X, Wang J, Tan W, Zhou Y, Yu C, Lin D (2004) Carcinogenesis 25:2225
144. Miao X, Sun T, Wang Y, Zhang X, Tan W, Lin D (2004) Cancer Res 64:2680
145. Kramer A, Lukas J, Bartek J (2004) Cell Cycle 3:1390
146. Cazales M, Schmitt E, Montembault E, Dozier C, Prigent C, Ducommun B (2005) Cell Cycle 4:1233
147. Katayama H, Sasai K, Kawai H, Yuan ZM, Bondaruk J, Suzuki F, Fujii S, Arlinghaus RB, Czerniak BA, Sen S (2004) Nat Genet 36:55
148. Kufer TA, Sillje HH, Korner R, Gruss OJ, Meraldi P, Nigg EA (2002) J Cell Biol 158:617
149. Bayliss R, Sardon T, Vernos I, Conti E (2003) Mol Cell 12:851
150. Honda R, Korner R, Nigg EA (2003) Mol Biol Cell 14:3325
151. Chen J, Jin S, Tahir SK, Zhang H, Liu X, Sarthy AV, McGonigal TP, Liu Z, Rosenberg SH, Ng SC (2003) J Biol Chem 278:486
152. Bolton MA, Lan W, Powers SE, McCleland ML, Kuang J, Stukenberg PT (2002) Mol Biol Cell 13:3064
153. Yang H, Burke T, Dempsey J, Diaz B, Collins E, Toth J, Beckmann R, Ye X (2005) FEBS Lett 579:3385
154. Brown JR, Koretke KK, Birkeland ML, Sanseau P, Patrick DR (2004) BMC Evol Biol 4:39
155. Heron NM, Anderson M, Blowers DP, Breed J, Eden JM, Green S, Hill GB, Johnson T, Jung FH, McMiken HHJ, Mortlock AA, Pannifer AD, Pauptit RA, Pink J, Roberts NJ, Rowsell S (2006) Bioorg Med Chem Lett 16:1320
156. Anderson M, Keen NJ, Pannifer ADB, Pauptit RA, Rowsell S (2003) WO 2003031606 A2
157. Fancelli D, Berta D, Bindi S, Cameron A, Cappella P, Carpinelli P, Catana C, Forte B, Giordano P, Giorgini ML, Mantegani S, Marsiglio A, Meroni M, Moll J, Pittala V, Roletto F, Severino D, Soncini C, Storici P, Tonani R, Varasi M, Vulpetti A, Vianello P (2005) J Med Chem 48:3080
158. Cheetham G, Knegtel R, Swenson L, Coll JT, Renwick S, Weber P (2003) WO 2003092607 A2
159. Cheetham GM, Knegtel RM, Coll JT, Renwick SB, Swenson L, Weber P, Lippke JA, Austen DA (2002) J Biol Chem 277:42419
160. Nowakowski J, Cronin CN, McRee DE, Knuth MW, Nelson CG, Pavletich NP, Rogers J, Sang BC, Scheibe DN, Swanson RV, Thompson DA (2002) Structure (Camb) 10:1659
161. Johnson LN, Lowe ED, Noble ME, Owen DJ (1998) FEBS Lett 430:1
162. Adams JA (2001) Chem Rev 101:2271
163. Wang Z, Canagarajah BJ, Boehm JC, Kassisa S, Cobb MH, Young PR, Abdel-Meguid S, Adams JL, Goldsmith EJ (1998) Structure 6:1117
164. Sicheri F, Moarefi I, Kuriyan J (1997) Nature 385:602
165. Xu W, Harrison SC, Eck MJ (1997) Nature 385:595
166. Hubbard SR (1997) Embo J 16:5572
167. Parang K, Till JH, Ablooglu AJ, Kohanski RA, Hubbard SR, Cole PA (2001) Nat Struct Biol 8:37
168. Russo AA, Jeffrey PD, Pavletich NP (1996) Nat Struct Biol 3:696
169. Brown NR, Noble ME, Endicott JA, Johnson LN (1999) Nat Cell Biol 1:438
170. Sessa F, Mapelli M, Ciferri C, Tarricone C, Areces LB, Schneider TR, Stukenberg PT, Musacchio A (2005) Mol Cell 18:379
171. Mortlock AA, Keen NJ, Jung FH, Heron NM, Foote KM, Wilkinson RW, Green S (2005) Curr Topics Med Chem 5:807
172. Harrington EA, Bebbington D, Moore J, Rasmussen RK, Ajose-Adeogun AO, Gladwell S, Dawson DA, Furey B, Ma J, Firestone B, Nakayama T, Graham JA, Demur C,

Hercend T, Diu-Hercend A, Yao YM, Su M, Golec JMC, Miller KM (2004) 95th Annual Meeting of the AACR, Orlando, FL, USA
173. Harrington EA, Bebbington D, Moore J, Rasmussen RK, Ajose-Adeogun AO, Nakayama T, Graham JA, Demur C, Hercend T, Diu-Hercend A, Su M, Golec JM, Miller KM (2004) Nat Med 10:262
174. Bebbington D, Charrier J-D, Golec JMC, Miller A, Knegtel R (2002) WO 2002062789 A1
175. Bebbington D, Charrier J-D, Davies R, Golec JMC, Kay D, Knegtel R, Patel S (2002) WO 2002059111 A2
176. Davies R, Bebbington D, Binch H, Knegtel R, Golec JMC, Patel S, Charrier J-D, Kay D, Davies R (2002) WO 2002022604 A1
177. Bebbington D, Charrier J-D, Davies R, Everitt S, Kay D, Knegtel R, Patel S (2002) WO 2002050065 A2
178. Bebbington D, Charrier J-D, Golec JMC, Miller A, Knegtel R (2002) WO 2002062789 A1
179. Bebbington D, Charrier J-D (2002) WO 2002059112 A2
180. Golec JMC, Pierard F, Charrier J-D, Bebbington D (2002) WO 2002050066 A2
181. Bebbington D, Binch H, Knegtel R, Golec JMC, Patel S, Charrier J-D, Kay D, Davies R, Li P, Wannamaker M, Forster C, Pierce A (2002) WO 2002022606 A1
182. Bebbington D, Knegtel R, Golec JMC, Li P, Davies R, Charrier J-D (2002) WO 2002022608 A1
183. Davies R, Bebbington D, Knegtel R, Wannamaker M, Li P, Forester C, Pierce A, Kay D (2002) WO 2002022607 A1
184. Davies R, Li P, Golec JMC, Bebbington D (2002) WO 2002022603 A1
185. Golec JMC, Charrier J-D, Knegtel R, Bebbington D, Davies R, Li P (2002) WO 2002022605 A1
186. Knegtel R, Bebbington D, Binch H, Golec JMC, Patel S, Charrier J-D, Kay D, Davies R, Li P, Wannamaker M, Forster C, Pierce A (2002) WO 2002022601 A1
187. Bebbington D, Knegtel R, Binch H, Golec JMC, Li P, Charrier J-D (2002) WO 2002022602 A2
188. Bebbington D, Binch H, Charrier JD, Everitt S, Golec JMC, Kay D, Knegtel R, Miller A, Pierard F (2003) WO 2003078427 A1
189. Bebbington D, Binch H, Charrier JD, Everitt S, Golec JMC, Kay D, Knegtel R, Miller A, Pierard F (2003) WO 2003077921 A1
190. Binch H, Charrier JD, Everitt S, Golec JMC, Kay D, Knegtel R, Miller A, Pierard F, Bebbington D (2003) WO 2003078426 A1
191. Aronov A, Lauffer DJ, Li HQ, Tomlinson RC, Li P (2004) WO 2004037814 A1
192. Green J, Arnost MJ, Pierce A (2003) WO 2003011287 A1
193. Fancelli D, Pittala V, Varasi M (2002) WO 2002012242 A2
194. Fancelli D, Berta D, Bindi S, Cameron A, Forte B, Giordano P, Moll J, Pittala V, Severino D, Varasi M, Vianello P (2004) 95th AACR Annual Meeting, Orlando, FL, USA, Abstract #2481
195. Tonani R, Bindi S, Fancelli D, Pittala V, Varasi M (2004) WO 2004013146 A1
196. Tonani R, Bindi S, Fancelli D, Pittala V, Danello M (2004) WO 2004007504 A1
197. Damour D, Terrier C, Nemecek P (2003) FR 2836914 A1
198. Maltais F, Aronov A, Hale MR, Moon Y (2004) WO 2004083203 A1
199. Cochran JN S, Nanthakumar S, Harrington E, Wang J(2002) WO 2002096905 A1
200. Wang S, Wood G, Duncan KW, Meades C, Gibson D, McLachlan JC, Perry A, Blake D, Zheleva DI, Fischer PM (2005) WO 2005042525 A1
201. Wang S, Meades C, Gibson D, Fischer PM (2005) WO 2005012298 A1

202. Wang S, McLachlan J, Gibson D, Causton A, Turner N, Fischer PM (2005) WO 2005012262 A1
203. Ledeboer M, Wang J, Moon YC (2004) WO 2004005283 A1
204. Mortlock AA, Keen NJ (2001) WO 2001021595 A1
205. Mortlock AA, Keen NJ (2001) WO 2001021594 A1
206. Mortlock AA, Keen NJ, Jung FH, Brewster AG (2001) WO 2001021596 A1
207. Mortlock AA (2004) WO 2004058752 A1
208. Mortlock AA, Jung FH (2002) WO 2002000649 A1
209. Mortlock AA (2004) WO 2004058782 A1
210. Jung FH, Pasquet GR (2003) WO 2003055491 A1
211. Mortlock AA, Keen NJ, Jung FH, Pasquet GR, Lohman JJ, Heron NM, Green S, Renaud F, Boutron P, Johnson T, Roberts NJ (2004) 95th AACR Annual Meeting, Orlando, FL, USA, Abstract #2480
212. Wilkinson RW, Keen NJ, Wedge SR, Odedra R, Heaton SP, Brown E, Brightwell S, Jung FH, Heron NM, Mortlock AA, Allen J, Kearney S, Foster JR, Green S (2004) 95th AACR Annual Meeting, Orlando, FL, USA, Abstract #842
213. Jung FH, Pasquet G, Lambert-van der Brempt C, Lohmann J-JM, Warin N, Renaud F, Germain H, De Savi C, Roberts N, Johnson T, Dousson C, Hill GB, Mortlock AA, Heron N, Wilkinson RW, Wedge SR, Heaton SP, Odedra R, Keen NJ, Green S, Brown E, Thompson K, Brightwell S (2006) J Med Chem 49:995
214. Wilkinson RW, Keen N, Odedra R, Heaton SP, Wedge SR, Foote KM, Mortlock AA, Jung FH, Heron NM, Brady MC, Walker M, Khatri L, Barrass C, FosterJJ, Green S (2006) Proc Am Assoc Cancer Res 97A (Abs 5673)
215. Mortlock AA, Foote KM, Heron NM, Jung FH, Keen N, Wilkinson RW, Wedge SR, Brady MC, Green S, Khatri L, McKillop D (2006) Proc Am Assoc Cancer Res 97A, Abstract 5712
216. Walsby E, Walsh V, Pepper C, Mills C, Burnett AK (2005) Blood 106(11):2759
217. Wilkinson RW, Odedra R, Heaton SP, Keen N, Wedge SR, Brown E, Crafter C, Foote KM, Mortlock AA, Jung FH, Heron NM, Brady MC, Khatri L, Foster JJ, Green S (2005) Clin Cancer Res 11(23 suppl A):B214
218. Keen N, Brown E, Crafter C, Wilkinson R, Wedge S, Foote KM, Mortlock AA, Jung FH, Heron NM, Green S (2005) Clin Cancer Res 11(23 suppl A):B220
219. Mortlock AA, Foote KM, Heron NM, Jung FH, Keen N, Wilkinson RW, Wedge SR, Brady MC, Green S, Khatri L, McKillop D (2006) Proceedings 97th AACR Annual Meeting, Washington DC, USA, Abstract 5712
220. Mortlock AA, Heron NM, Jung FH (2004) WO 2004113324 A1
221. Mortlock AA, Heron NM, Jung FH, Pasquet GR (2004) WO 2004105764 A1
222. Heron NM, Jung FH, Pasquet GR, Mortlock AA (2004) WO 2004058781 A1
223. Heron NM, Pasquet GR, Mortlock AA, Jung FH (2004) WO 2004094410 A1
224. Mortlock AA, Jung FH (2001) WO 2001055116 A2
225. Hurley LH, Mahadevan D, Han H, Bearss DJ, Vankayalapati H, Bashyam S, MunozRM, Warner SL, Della CK, Von Hoff DD, Grand CL (2005) WO 2005037825 A2
226. Hauf S, Cole RW, LaTerra S, Zimmer C, Schnapp G, Walter R, Heckel A, van Meel J, Rieder CL, Peters JM (2003) J Cell Biol 161:281
227. Walter R, Heckel A, Roth GJ, Kley J, Schnapp G, Lenter M, Van Meel JCA, Spevak W, Weyer-Czernilofsky U (2002) WO 2002036564 A1
228. Manfredi M, Escedy J, Meetze K, Balani S, Burenkova O, Chen W, Hoar K, Huck J, LeRoy P, Sells T, Stroud S, Vos T, Weatherhead G, Wysong D, Zhang M, Clairborne C (2006) Proceedings 97th AACR Annual Meeting, Washington DC, USA, Abstract 4724

229. Escedy J, Hoar K, Wysong DR, Rabino C, Bowman DS, Charkravarty A, Roy N (2006) Proceedings 97th AACR Annual Meeting, Washington DC, USA, Abstract 2066
230. Huck J, Zhang M, Burenkova O, Connolly K, Manfredi M, Meetze K (2006) Proceedings 97th AACR Annual Meeting, Washington DC, USA, Abstract 4698
231. Glover DM, Hagan IM, Tavares AA (1998) Genes Dev 12:3777
232. Lowery DM, Lim D, Yaffe MB (2005) Oncogene 24:248
233. Lee KS, Grenfell TZ, Yarm FR, Erikson RL (1998) Proc Natl Acad Sci USA 95:9301
234. van Vugt MA, Medema RH (2005) Oncogene 24:2844
235. Toyoshima-Morimoto F, Taniguchi E, Shinya N, Iwamatsu A, Nishida E (2001) Nature 410:215
236. Yuan J, Eckerdt F, Bereiter-Hahn J, Kurunci-Csacsko E, Kaufmann M, Strebhardt K (2002) Oncogene 21:8282
237. Toyoshima-Morimoto F, Taniguchi E, Nishida E (2002) EMBO Rep 3:341
238. Smits VA, Klompmaker R, Arnaud L, Rijksen G, Nigg EA, Medema RH (2000) Nat Cell Biol 2:672
239. Hamanaka R, Maloid S, Smith MR, O'Connell CD, Longo DL, Ferris DK (1994) Cell Growth Differ 5:249
240. Smith MR, Wilson ML, Hamanaka R, Chase D, Kung H, Longo DL, Ferris DK (1997) Biochem Biophys Res Commun 234:397
241. Liu X, Erikson RL (2003) Proc Natl Acad Sci USA 100:5789
242. Weichert W, Schmidt M, Jacob J, Gekeler V, Langrehr J, Neuhaus P, Bahra M, Denkert C, Dietel M, Kristiansen G (2005) Pancreatology 5:259
243. Weichert W, Kristiansen G, Winzer KJ, Schmidt M, Gekeler V, Noske A, Muller BM, Niesporek S, Dietel M, Denkert C (2005) Virchows Arch 446:442
244. Weichert W, Schmidt M, Gekeler V, Denkert C, Stephan C, Jung K, Loening S, Dietel M, Kristiansen G (2004) Prostate 60:240
245. Weichert W, Denkert C, Schmidt M, Gekeler V, Wolf G, Kobel M, Dietel M, Hauptmann S (2004) Br J Cancer 90:815
246. Takahashi T, Sano B, Nagata T, Kato H, Sugiyama Y, Kunieda K, Kimura M, Okano Y, Saji S (2003) Cancer Sci 94:148
247. Kneisel L, Strebhardt K, Bernd A, Wolter M, Binder A, Kaufmann R (2002) J Cutan Pathol 29:354
248. Dai W, Li Y, Ouyang B, Pan H, Reissmann P, Li J, Wiest J, Stambrook P, Gluckman JL, Noffsinger A, Bejarano P (2000) Genes Chromosomes Cancer 27:332
249. Wiest J, Clark AM, Dai W (2001) Genes Chromosomes Cancer 32:384
250. Ko MA, Rosario CO, Hudson JW, Kulkarni S, Pollett A, Dennis JW, Swallow CJ (2005) Nat Genet 37:883
251. Elia AEH, Rellos P, Haire LF, Chao JW, Ivins FJ, Hoepker K, Mohammed D, Cantley LC, Smerdon SJ, Yaffe MB (2003) Cell 115:83
252. McInnes C, Mezna M, Fischer PM (2005) Curr Top Med Chem 5:181
253. McInnes C, McLachan J, Mezna M, Fischer PM (2005) WO 2005/047526 A2
254. Fischer PM (2004) Curr Med Chem 11:1563
255. Singh J, Dobrusin EM, Fry DW, Haske T, Whitty A, McNamara DJ (1997) J Med Chem 40:1130
256. Strebhardt K, Ullrich A (2006) Nature Rev (Cancer) 6:321
257. Stevenson CS, Capper EA, Roshak AK, Marquez B, Eichman C, Jackson JR, Mattern M, Gerwick WH, Jacobs RS, Marshall LA (2002) J Pharmacol Exp Ther 303:858
258. Stevenson CS, Capper EA, Roshak AK, Marquez B, Grace K, Gerwick WH, Jacobs RS, Marshall LA (2002) Inflamm Res 51:112
259. Jacobs RS, Stevenson CS, Gerwick WH, Marshall LA (2001) WO 2001/62900 A1

260. Gray NS, Wodicka L, Thunnissen AM, Norman TC, Kwon S, Espinoza FH, Morgan DO, Barnes G, LeClerc S, Meijer L, Kim SH, Lockhart DJ, Schultz PG (1998) Science 281:533
261. Ruetz S, Fabbro D, Zimmermann J, Meyer T, Gray N (2003) Curr Med Chem Anti-Cancer Agents 3:1
262. Vlahos CJ, Matter WF, Hui KY, Brown RF (1994) J Biol Chem 269:5241
263. Agullo G, Gamet-Payrastre L, Manenti S, Viala C, Remesy C, Chap H, Payrastre B (1997) Biochem Pharmacol 53:1649
264. Gamet-Payrastre L, Manenti S, Gratacap MP, Tulliez J, Chap H, Payrastre B (1999) Gen Pharmacol 32:279
265. Steegmaier M (2005) Clin Cancer Res 11(23 suppl):9147
266. Baum A, Garin-Chesa P, Quant J, Colbatzky F, Munzert G, Grauert M, Hoffmann M, Steegmaier M (2005) Clin Cancer Res 11(23 suppl):C191
267. Mross K, Steinbild S, Frost A, Hedborn S, Rentschler J, Kaiser R, Trommeshauer D, Stehle G, Munzert G (2005) Clin Cancer Res 11(23 suppl):B219
268. Wang S, Meades C, Wood G, Osnowski A, Anderson S, Yuill R, Thomas M, Mezna M, Jackson W, Midgley C, Griffiths G, Fleming I, Green S, McNae I, Wu SY, McInnes C, Zheleva D, Walkinshaw MD, Fischer PM (2004) J Med Chem 47:1662
269. Wu SY, McNae I, Kontopidis G, McClue SJ, McInnes C, Stewart KJ, Wang S, Zheleva DI, Marriage H, Lane DP, Taylor P, Fischer PM, Walkinshaw MD (2003) Structure (Camb) 11:399
270. Wang S, McLachlan J, Gibson D, Causton A, Turner N, Fischer PM (2005), UK WO 2005/012262 A1, WO 2005012262
271. Umehara H, Yamashita Y, Tsujita T, Arai H, Hagihara K, Machii D (2004) WO 2004/043936
272. Davis-Ward D, Mook RAJ, Neeb MJ, Salovich JM (2004) WO 2004/074244
273. McInnes C, Meades C, Mezna M, Fischer PM (2004) WO 2004/067000
274. Andrews CWI, Cheng M, Davis-Ward RG, Drewry DH, Emmitte KA, Hubbard RD, Kunts KW, Linn JA, Mook RA, Smith GK, Veal JM (2004) WO 2004/014899
275. Emmitte KA (2005) WO 2005/075470
276. Cheng MK, King NP, Kunts KW, Mook RA, Pobanz MA, Salovich JM, Wilson BJ (2004) WO 2004/087652
277. Drewry DHM, Mook RA Jr, Salovich JM, Schoenen FJ, Wagner DS, Wagner RW (2005) WO 2005/019193
278. Schulze V, Eis K, Wortmann L, Schwede W, Siemeister G, Briem H, Schneider H, Eberspacher U, Hess-Stumpp H (2005) WO 2005/042505 A1
279. Siemeister G, Briem H, Schulze V, Eis K, Wortmann L, Schwede W, Schneider H, Eberspächer U, Hess-Stumpp H (2005) DE 103 51 744 A1

Top Med Chem (2007) 1: 293–331
DOI 10.1007/7355_2006_007

Published online: 13 January 2007

HDAC Inhibition in Cancer Therapy: An Increasingly Intriguing Tale of Chemistry, Biology and Clinical Benefit

P. ten Holte[1] (✉) · K. Van Emelen[1] · M. Janicot[2] · P. C. Fong[3] · J. S. de Bono[3] · J. Arts[2]

[1]Dept. of Medicinal Chemistry,
Johnson & Johnson Pharmaceutical Research & Development,
Division of Janssen Pharmaceutica NV, 2340 Beerse, Belgium
ptenholt@prdbe.jnj.com

[2]Dept. of Oncology,
Johnson & Johnson Pharmaceutical Research & Development,
Division of Janssen Pharmaceutica NV, 2340 Beerse, Belgium

[3]Drug Development Unit, Royal Marsden Hospital, Sutton UK

1 Introduction 295

2 Biochemistry of the Histone Deacetylases and Histone Acetyl Transferases 296
2.1 HDACs and their Link to Cancer 296
2.2 The HDAC Family of Enzymes 297

3 HDAC Inhibitors 300
3.1 Historic Overview 300
3.2 Recent Medicinal Chemistry Efforts—An Overview 303
3.2.1 The Cyclic Peptides 303
3.2.2 Hydroxamic Acid Replacements—the Holy Grail? 305
3.2.3 Is the Type of Spacer Really all that Important? 309
3.2.4 The Capping Group under Scrutiny 311
3.2.5 Connecting the Spacer with the Capping Group 313
3.2.6 The Quest for Selective HDAC Inhibitors 314

4 Clinical Experience with HDAC Inhibitors 317
4.1 Hydroxamates 318
4.1.1 Vorinostat (SAHA) 319
4.2 NonHydroxamates 320
4.2.1 Depsipeptide (FR901228 or FK-228) 322
4.2.2 MS-275 322
4.2.3 CI-994 (Tacedinaline) 323
4.3 Summary and Future Development 323

5 Perspectives and Conclusion 324

References 326

Abstract This review presents a wide-ranging selection of key literature examples in the histone deacetylase (HDAC) field. The review starts off with the biological background

of HDACs and their link to cancer and cancer treatment. The body of the work consists of a categorized and chronological medicinal chemistry overview. This part describes key medicinal chemistry contributions ranging from the very early HDAC inhibitors to compounds currently in the clinic. The result of all these medicinal chemistry and biology efforts have been captured in the last section that gives an overview of the current status of HDAC inhibitors in the clinic.

Keywords Histone deacetylase · HDAC inhibitor · Hydroxamic acid · Clinical development · Isoform selective

Abbreviations

AML	acute myeloid leukemia
APC	adenomatosis polyposis coli
APHA	aroyl pyrrolyl hydroxamic acids
APL	acute promyelocytic leukemia
CBHA	*m*-carboxycinnamic acid bishydroxamide
CDK	cyclin dependent kinase
CHAP	cyclic hydroxamic acid-containing peptide
CR	complete response
CTCL	cutaneous T cell lymphoma
DLT	dose limiting toxicity
DTT	dithiothreitol
EKG	electrocardiograph
HAT	histone acetyl transferase
HDAC	histone deacetylase
HDACi	HDAC inhibitor
HDLP	histone deacetylase-like protein
hERG	human ether-a-go-go related gene
HIF	hypoxia-inducible factor
MEF	myocyte enhancer factor
MEL	murine erythroleukemia
MITR	MEF2-interacting transcription repressor
MTD	maximum tolerated dose
n.d.	not determined
PBMNC	peripheral blood mononuclear cells
PD	pharmacodynamic
PK	pharmacokinetic
PR	partial response
PTCL	peripheral T-cell lymphoma
RAR	retinoic acid receptor
RR	response rate
SAHA	suberoylanilide hydroxamic acid
SCC	squamous cell carcinoma
SCOP	sulfur-containing cyclic peptides
TPX	trapoxin
TRAIL	tumor necrosis factor related apoptosis inducing ligand
TSA	trichostatin A
uPR	unconfirmed PR
VEGF	vascular endothelial growth factor

1 Introduction

During the past decade, epigenetic phenomena have been proven to be involved in the onset and promotion of carcinogenesis. Aberrations in the complex chromatin control of gene expression result in silencing of tumor-suppressor genes, decreased DNA repair and inactivation of apoptotic pathways. Chromatin plays a central role in these processes, since it represents a key component in the compact structure of the mammalian genome to allow for the nucleus to accommodate the full DNA sequence. The fundamental repeating unit of chromatin, the nucleosome, consists of an octamer of core histones, which have long been shown to play an essential role in the assembly of chromatin into higher-order structures of the DNA, required for efficient condensation. Furthermore, it has become clear that post-translational modifications of the histones by various chromatin-associated proteins regulate the gene-expression profile. These modifications target mainly the N-terminal tails of highly conserved lysine residues within the histones and include acetylation, methylation, phosphorylation, ubiquitinylation and glycosylation.

Historically, histone deacetylases (HDACs) were considered as promising drug targets in anticancer therapy due to their regulating role in the histone acetylation status implicated in the epigenetic chromatin control. As a consequence, the potential of HDAC inhibitors was initially attributed to their capacity as chromatin-modulating drugs.

An increasing amount of data has recently led to better insight into the pleiotropic effects of HDAC inhibitors, demonstrating that HDACs also act as regulators of cellular processes such as proliferation, apoptosis and angiogenesis through deacetylation of other protein substrates.

The identification of the first small molecule HDAC inhibitors in the late seventies triggered an exponential growth in medicinal chemistry activity. Three decades and many thousand compounds later, the availability of diverse HDAC inhibitors such as short-chain fatty acids, hydroxamic acids, benzamides and tetracyclic peptides, has not only enabled the elucidation of the catalytic mechanism underlying the deacetylating capacity of HDACs, but has also assisted in the investigation of the biological role of the various HDAC subtypes. Furthermore, HDAC inhibitors are currently being evaluated in the clinic and have shown therapeutic potential in the treatment of cancer.

In this work, we will discuss in detail the role of HDACs as regulators of critical cellular processes, the implication of disregulated HDAC activity in carcinogenesis, as well as a retrospective account of the continued medicinal chemistry efforts in the field and an overview of HDAC inhibitors currently undergoing clinical evaluation.

2
Biochemistry of the Histone Deacetylases and Histone Acetyl Transferases

2.1
HDACs and their Link to Cancer

The family of HDAC enzymes has been named after their first substrate identified, i.e., the nuclear histone proteins. Histone proteins (H2A, H2B, H3 and H4) form an octamer complex, around which the DNA helix is wrapped in order to establish a condensed chromatin structure. The acetylation status of histones is in a dynamic equilibrium governed by histone acetyl transferases (HATs), which acetylate and HDACs which are responsible for the deacetylation of histone tails (Fig. 1). Inhibition of the HDAC enzyme promotes the acetylation of nucleosome histone tails, favoring a more transcriptionally competent chromatin structure, which in turn leads to altered expression of genes involved in cellular processes such as cell proliferation, apoptosis and differentiation. Inhibition of HDAC activity results in the activation of only a limited set of pre-programmed genes; microarray experiments have shown that ~ 2% of all genes are activated by structurally different HDAC inhibitors [1–5]. In recent years, a growing number of additional nonhistone HDAC substrates have been identified, which will be discussed in more detail below.

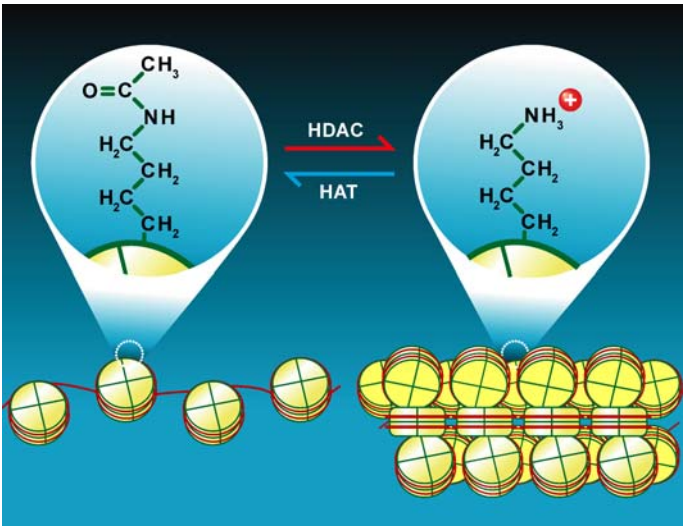


Fig. 1 The dynamic equilibrium between acetylation and deacetylation of lysine residues of the histones is controlled by the opposing enzymatic activities of HATs and HDACs. The acetylation status determines whether a lysine residue is either neutral (acetylated) or positively charged (deacetylated). The consequent changes in the internucleosomal interactions and condensation status of chromosomal domains govern the transcriptional competence of DNA (© Diane Bruyninckx)

Disruption of HAT or histone deacetylase (HDAC) activity is associated with the development of cancer [6]. HAT mutations or translocations are frequently observed in tumors from both hematological and epithelial origin, e.g., acute myeloid leukemia (AML), colorectal, breast and gastric tumors, and glioblastomas. A HAT mutation also lies at the root of the Rubinstein–Taybi syndrome, a developmental disorder associated with an increased risk of cancer. Disregulated and constant HDAC recruitment in conjunction with oncogenic transcription factors to the chromatin is observed in specific forms of leukemia and lymphoma, such as acute promyelocytic leukemia (APL), non-Hodgkin's lymphoma and AML M2 subtype 2,3 [6, 7]. Upregulation of HDAC1 at the protein level was observed in prostate cancer cells, as the disease progresses from pre-malignant lesions and well-differentiated androgen-responsive prostate adenocarcinoma towards the phenotypically de-differentiated androgen-insensitive prostate cancer [8]. In addition, increased HDAC2 expression is found in the majority of human colon cancer explants which is triggered by the loss of the tumor suppressor adenomatosis polyposis coli (APC) [9].

In agreement with the aberrant HDAC/HAT activity equilibrium in cancer, HDAC inhibitors have been shown to induce cell-cycle arrest, terminal differentiation and/or apoptosis in a broad spectrum of human tumor cell lines in vitro, to inhibit angiogenesis and to exhibit in vivo antitumor activity in human xenograft models in nude mice [10–12]. Several HDAC inhibitors are in advanced stages of development and antitumor activity has been observed in hematological malignancies at doses that were well tolerated (Sect. 3).

2.2 The HDAC Family of Enzymes

The HDAC family of enzymes are commonly divided into three classes: i.e., classes I, II and III [13]. In this review, the focus will be on classes I and II only, since these have been predominantly implied to mediate the effects of HDAC inhibitors currently in clinical development.

The class-I group HDACs, which consists of HDAC family members 1–3 and 8 have been shown to be crucial for tumor cell proliferation. Knock-down of HDAC1 and HDAC3 using siRNA techniques caused inhibition of proliferation and changed the cell's structure into a more flattened morphology with extensive focal contacts [14]. Lagger et al. [15] showed that disruption of HDAC1 in mouse embryonic stem cells resulted in an increase in H3 and H4 acetylation and gene induction, thereby linking histone deacetylation and the subsequent transcriptional modulation to the enzymatic activity of the class-I HDACs. Recently Ropero et al. also reported a truncating mutation in HDAC2 found in human cancers that renders them less sensitive to the HDAC inhibitor trichostatin A (TSA), further emphasizing the key role of class-I HDACs [16].

Among the wide variety of transcription factors that utilize class-I HDACs to silence specific promoters, the best known example is the class of nuclear hormone receptors, which only bind HDAC3 in absence of their ligand, and thus maintain a state of transcriptional silencing. This complex is dissociated in a ligand-dependent manner, e.g. by retinoids, estrogens or androgens, resulting in gene expression and differentiation. Another key example is the HDAC1-dependent silencing of the cyclin-dependent kinase inhibitor $p21^{waf1,cip1}$. The crucial role of $p21^{waf1,cip1}$ induction in the antiproliferative effects of HDAC inhibitors was demonstrated by studies showing a 6-fold increase in resistance to the HDAC inhibitor TSA in $p21^{waf1,cip1}$ deficient cells as compared to the parental HCT-116 cells [17, 18]. In addition, unlike genuine tumor suppressor genes, $p21^{waf1,cip1}$ is ubiquitously present in tumor cells, and induced by HDAC inhibitors.

It should be noted that histones are not the only substrates of the class-I HDACs. For example, HDACs 1–3 deacetylate the tumor suppressor p53, which as a consequence gets ubiquitinated and degraded. Since p53 is a potent tumor suppressor, inducing cell cycle arrest and apoptosis, maintaining low levels of this protein is key for allowing survival and uncontrolled proliferation of tumor cells [19]. A concise overview of the acetylome has recently been published by Minucci and Pelicci [20].

The class-II HDACs can be divided into two subclasses: class-IIa containing HDACs 4, 5, 7, 9 and the HDAC9 splice variant MEF2-interacting transcription repressor (MITR). Class IIb comprises HDAC6 and HDAC10, which both have duplicated HDAC domains. Class-IIa HDACs do not possess intrinsic histone deacetylase enzymatic activity [21] but regulate gene expression by functioning as bridging factors since they associate both with class-I HDAC complexes and with transcription factor/DNA complexes.

So far, inhibition of class-IIa HDAC isotypes has not been shown to affect tumor cell proliferation directly, since inhibition of expression of class-II HDACs 4 and 7 in HeLa cells using siRNA technology did not result in decreased proliferation [14]. Although HDAC4 is not directly involved in cell cycle progression, HDAC4 does interact with p53BP1 to mediate the DNA damage response to agents causing double strand breaks. Silencing of HDAC4 abrogates DNA-damage induced G2 arrest in HeLa cells [22]. Concerning the other class-IIa family members, HDAC5 over-expression was found to induce tumor cell apoptosis, but a role for the endogenous level of this protein in cell cycle progression has not been shown [23]. Attar et al. [24] reported the identification of a novel class-II HDAC9 isoform which is over expressed in breast and prostate tumor tissue and promotes anchorage independent growth, oncogenic transformation and proliferation in NIH3T3 cells.

HDAC6, a member of class IIb, has received attention due to its identification as a Hsp90 deacetylase. This results in degradation of Hsp-90 associated pro-survival and pro-proliferative client proteins. Key examples include

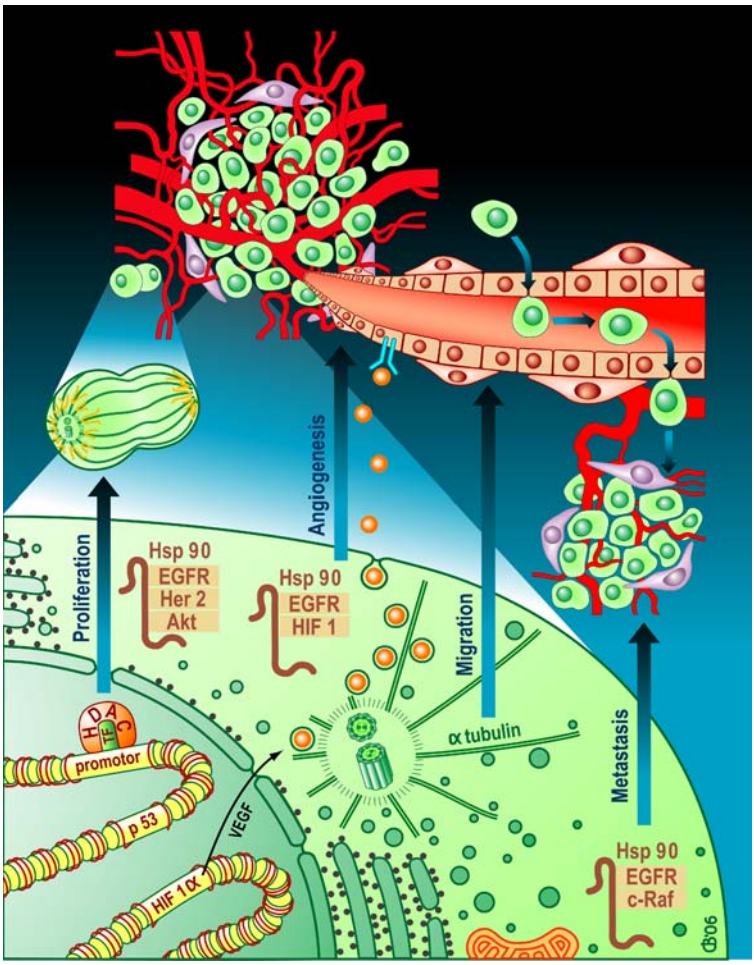


Fig. 2 HDACs deacetylate a panel of protein substrates, resulting in the regulation of several signaling pathways that are key in tumorigenesis. Class-I HDACs, which have been shown to be crucial for tumor cell proliferation, are recruited to the chromatin by transcription factors, and locally deacetylate histone proteins, thereby regulating gene expression. Class-I HDACs also deacetylate the tumor suppressor p53, resulting in its degradation. HDAC6, a member of class IIb, is a Hsp-90 deacetylase, and inhibition of this protein results in degradation of Hsp-90 associated pro-survival and pro-proliferative client proteins. Key examples include Her-2, Bcr-Abl, glucocorticoid receptor, mutant FLT-3, c-Raf and Akt. Hsp90 has also been demonstrated to be key for the stabilization of constitutively activated oncogenic kinases, such as for EGFR (L858R) and B-raf (V600E). In addition to Hsp90, HDAC6 also mediates tubulin deacetylation, which results in microtubule destabilization under stressed conditions, which is key for cell motility. HDAC7 has been shown to activate Hypoxia-inducible factor (HIF)1α, which is also a client protein of Hsp90. HIF1α is activated in tumor cells, and induces the transcription of vascular endothelial growth factor (VEGF), which is a key regulator of angiogenesis (© Diane Bruyninckx)

Her-2, Bcr-Abl, glucocorticoid receptor, mutant FLT-3, c-Raf and Akt [25, 26]. In addition to Hsp90, HDAC6 also mediates tubulin deacetylation, which results in microtubule destabilization under stressed conditions [27]. The biological role of HDAC6 was further confirmed by a recent report showing that a specific small molecule inhibitor of HDAC6, tubacin, caused α-tubulin hyperacetylation and decreased cell motility without affecting cell cycle progression [28]. In agreement, HDAC6 was found to be key for the estradiol-stimulated cell migration of MCF-7 breast carcinoma cells [28]. Finally, HDAC6 plays a crucial role in the cellular management of misfolded protein-induced stress by binding poly-ubiquitinated misfolded proteins and clearing these from the cytoplasm [29].

In summary, due to the large panel of cell cycle regulatory proteins regulated by HDACs at the level of either their expression or activity, the antiproliferative effect of HDAC inhibitors cannot be linked to a single mechanism of action. The relative importance of the different proteins affected by HDACs varies between tumors. In Fig. 2, a visual overview of the role of HDACs in various hallmark processes in the development of cancer is shown.

3 HDAC Inhibitors

In the past decade, the scientific interest in HDAC inhibitors has increased enormously. This growing interest was accompanied by a sudden increase of the number of publications on the subject. The extensive publishing and patenting in the field of HDAC inhibition does not allow us to even consider a full coverage of the literature here. Instead, in this review we will focus on the evolution of HDAC inhibitors, significant medicinal chemistry studies from the literature that have contributed to the understanding of HDAC inhibition and a number of examples from the patent literature. This review is by no means an attempt to cover all the literature on this subject.

3.1 Historic Overview

The impact of small molecules on the acetylation status of histones has attracted the interest of the medicinal chemistry community for almost a decade now. Nevertheless, the fast and reversible increase in cellular histone acetylation in the presence of *n*-butyrate was already recognized in 1977 by Riggs et al. (Fig. 3) [30]. Two years later, it was proven that *n*-butyrate, among some related and less active small linear aliphatic carboxylates, was a non-competitive inhibitor of histone deacetylating enzymes [31–34]. More than ten years after the initial interest in *n*-butyrate, Yoshida et al. showed that trichostatin A (TSA, Fig. 3), originally reported as an antifungal agent [35],

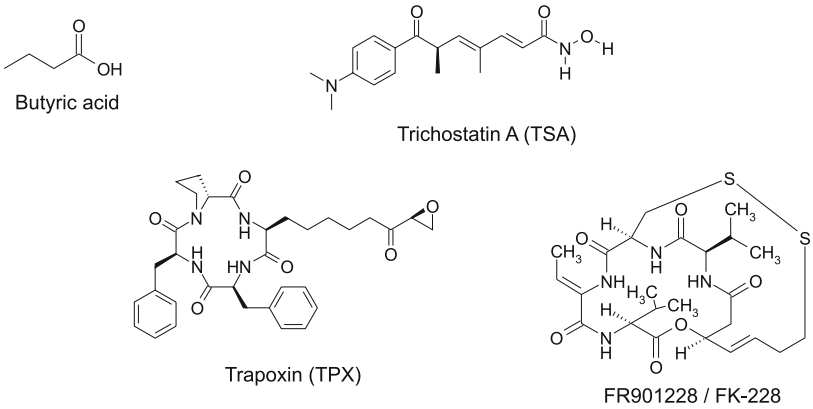


Fig. 3 Structures of the early HDAC inhibitors—identified as HDAC inhibitors in the time frame 1977 to 1998. (*n*-Butyrate: MIT, TSA: Univ. of Tokyo, TPX: Univ. of Tokyo, FR901228/FK-228: Fujisawa Pharmaceutical Co., and Univ. of Tokyo)

also affects histone acetylation and deacetylation processes by specific and reversible inhibition of mammalian histone deacetylase [36]. TSA displays inhibitory activity in the nanomolar range of concentrations in cell-free assays, whereas the IC_{50} of *n*-butyric acid is in the micromolar range. A few years earlier, TSA had already been shown to possess antitumor activity by causing cell differentiation of Friend leukemia cells and inhibition of the cell cycle of rat fibroblasts [37]. The observation that only the *R*-configuration of TSA inhibits histone deacetylase (HDAC) activity at nanomolar concentrations suggested a highly specific interaction of TSA with the enzyme, implying that TSA binds the enzyme at an identifiable binding site.

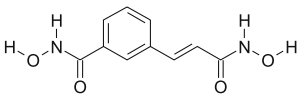
In 1993, Yoshida et al. published trapoxin (TPX, Fig. 3), a fungal product, which, in contrast to TSA, is an irreversible inhibitor of mammalian histone deacetylase [38]. When the epoxide moiety is reduced to the corresponding primary alcohol, HDAC inhibiting activity is completely lost. This observation emphasizes the importance of the oxirane ring, which most likely binds irreversibly via ring opening at the activated 2-position to a nucleophilic active site residue.

The isolation and structural elucidation of yet another natural product with antitumoral activity, FR901228 (FK-228; Fig. 3), was published in 1994 [39–41]. This bicyclic depsipeptide was isolated from the fermentation product of a strain of *Chromobacterium violaceum* and exhibited potent in vitro antiproliferative activity against several human lung, stomach, breast and colon cancer cell lines. FR901228 also showed promising tumor growth inhibition in mice bearing solid tumors. Four years later it was recognized that FR901228 acts as a HDAC inhibitor [42], although details of its molecular mechanism of action were not elucidated until 2002 when it was shown that the disulfide bridge is reduced in cells by glutathione to release the

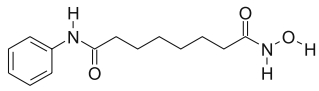
thiol that subsequently interacts with the active-site zinc of primarily class-I HDACs [43]. FR901228/FK-228 is currently in phase II clinical trials.

As outlined before, it is believed that the TPX epoxyketone chain acts as an isosteric substrate mimic for the natural *N*-acetyl lysine. In 1996, Schreiber et al. exploited the irreversible binding nature of TPX in an affinity matrix by immobilizing modified TPX onto an activated agarose support [44]. In this way a mammalian histone deacetylase protein (HDAC1) was isolated and characterized for the first time.

In the same year, *m*-carboxycinnamic acid bishydroxamide (CBHA) and suberoylanilide hydroxamic acid (SAHA) were identified as inducers of terminal differentiation of murine erythroleukemia (MEL) cells (Fig. 4) [45]. It was not until two years later, however, that the HDAC1 and HDAC3 inhibiting capacities of these compounds were recognized [46]. SAHA is currently the leading compound in the clinic, and is undergoing phase III clinical trials for the treatment of cutaneous T cell lymphoma (CTCL).



m-Carboxycinamic acid bishydroxamide (CBHA)



Suberoylanilide hydroxamic acid (SAHA)

Fig. 4 Structures of CBHA and SAHA—identified as HDAC inhibitors in 1998. (Memorial Sloan-Kettering Cancer Center, Picower Institute for Medical Research, Univ. of California SF, and Columbia Univ.)

The presence of metal-chelating hydroxamic acid moieties in some of the most potent HDAC inhibitors triggered the idea that the HDAC enzyme family might consist of metalloproteins [47]. This suggestion was confirmed by Pavletich et al. who found that the in vitro deacetylase activity of purified *A. aeolicus* HDAC homologue or HDLP (histone deacetylase-like protein) was only present after incubation with Zn^{2+} or Co^{2+} [48]. The crystal structure of HDLP in the presence of zinc then revealed that the zinc ion is positioned near the bottom of the tube-like pocket at a depth of approximately 11 Å [48]. Co-crystallization of HDLP with SAHA or TSA clearly shows the similar binding mode of both compounds [48]. The hydroxamic acid moiety is located at the bottom of the hydrophobic tube, chelating the zinc ion, while the aliphatic chains bridge the depth of the pocket to allow the aromatic group to interact with the pocket entrance while capping it.

The disclosure of the HDLP crystal structure in 1999 provided a clear framework and starting point for medicinal chemists for the further development of HDAC inhibitors as antitumor agents. It confirmed the suggested general structural requirements of such inhibitors. These proposed requirements and the resemblance of the inhibitors to the acetylated ε-amino groups of lysine residues are shown in Fig. 5.

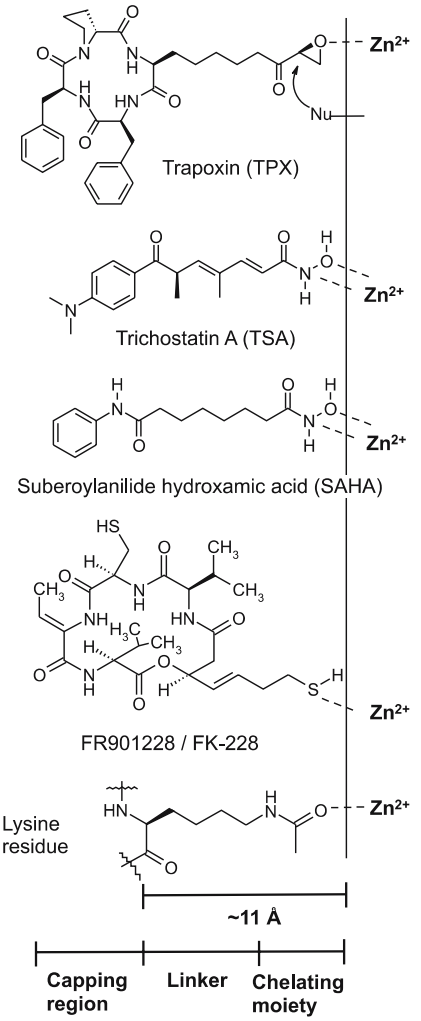


Fig. 5 Generally supposed structural requirements of HDAC inhibitors and some actual examples

3.2 Recent Medicinal Chemistry Efforts—An Overview

3.2.1 The Cyclic Peptides

Since the discovery of trapoxin (TPX), a number of related cyclic peptides have been found to also demonstrate HDAC inhibitory activity, explaining in part the phenotypic effects previously described for these compounds. One

example, closely related to TPX, is chlamydocin, differing from TPX in only one amino acid residue (Fig. 6) [49, 50]. More specific, in chlamydocin one of the two phenylalanine residues of TPX is replaced with a 2-aminoisobutyric acid. The mode of action of both molecules is believed to be identical, and to proceed via covalent and thus irreversible binding to the HDAC enzyme through reaction of an active site nucleophile with the electrophilic oxirane ring of chlamydocin or TPX.

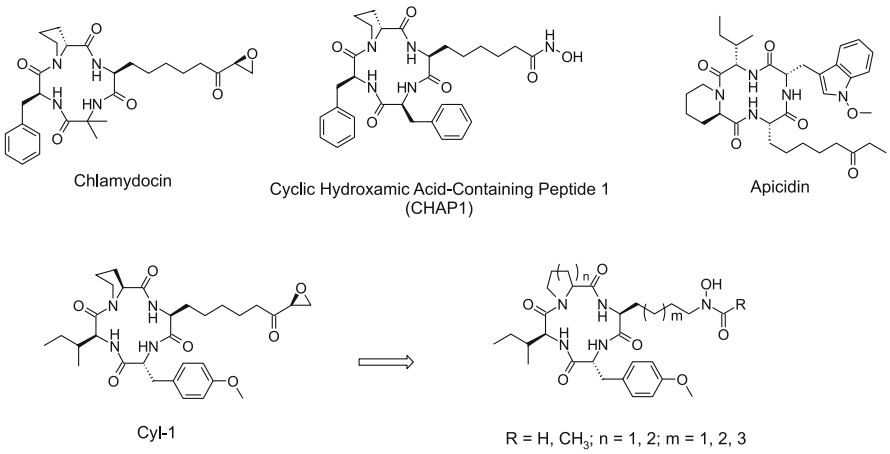


Fig. 6 Cyclic peptides as reversible and irreversible HDAC inhibitors. (Chlamydocin: Sandoz AG; CHAP 1: Univ. of Tokyo; Apidicin: Merck & Co; reversed hydroxamic acid analogs of Cyl-1: Kyushu Institute of Technology and Japan Science and Technology Agency)

Replacing the electrophilic epoxy ketone moiety in TPX by a reversible zinc chelator such as a hydroxamic acid was carried out by Yoshida et al. (Fig. 6) [51]. This modification led to a low nanomolar reversible inhibitor of the HDAC1 enzyme. Several other cyclic tetrapeptides containing the epoxyketone feature, such as chlamydocin, were converted into their hydroxamic acid counterparts as well [52]. Additionally, the introduction of reversed hydroxamic acids (– N(OH)COR, with R = H or Me) onto the structure of Cyl-1 was reported to give potent HDAC inhibitors as illustrated in Fig. 6 [53]. Generally, the most potent inhibitors were the examples with R = H and $m = 2$. Apicidin, a cyclic peptide more remotely related to TPX, exhibits potent antiprotozoal activity via HDAC inhibition in parasites [54].

The prodrug concept of FK-228, outlined in Sect. 3.1, was exploited by Nishino et al. in the development of sulfur-containing cyclic peptide-(SCOP)-based prodrugs [55]. A set of SCOP prodrugs, based on CHAP31, was synthesized and their in vitro HDAC inhibitory activity was evaluated (Fig. 7 and Table 1). The dimer was 4-fold less potent (IC_{50} = 0.142 μM) on HDAC1 than reference FK228, but on HDAC4 it was almost 4-fold more active (IC_{50} 0.145 μM) than FK228. The dimer did not show any activity on HDACs 6 and

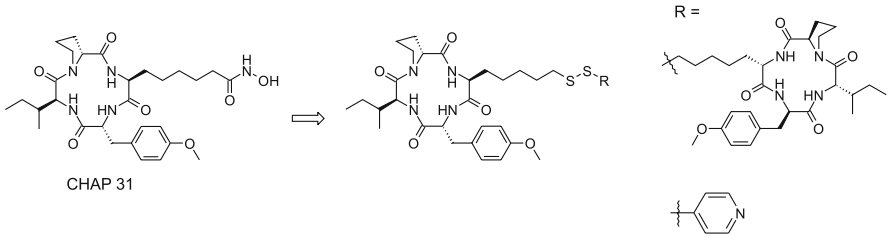


Fig. 7 Sulfur-containing cyclic peptides as prodrugs for HDAC inhibition. (Kyushu Institute of Technology)

Table 1 Enzymatic data on different HDAC isoforms for the structures shown in Figs. 3 and 7

Compound	HDAC1 (μM)		HDAC4 (μM)		HDAC6 (μM)		HDAC8 (μM)	
	No DTT	DTT	No DTT	DTT	No DTT	DTT	No DTT	DTT
FK228	0.036	0.001	0.512	n.d.	> 500	0.624	n.d.	n.d.
Dimer	0.142	0.005	0.145	0.002	> 500	1.4	> 500	1.69
R = 4-pyridyl	0.007	0.0006	0.068	0.001	1.61	2.01	3.14	0.494

8. Addition of the reducing agent dithiothreitol (DTT) to the dimer, however, increased the potency dramatically to low nanomolar levels for HDACs 1 and 4 (IC_{50} 4.6 and 2.1 nM, respectively) and to low micromolar activity on HDACs 6 and 8 (IC_{50} 1.4 and 1.7 μM, respectively). As summarized in Fig. 7 and Table 1 below, the activity of these disulfide prodrugs was further increased by introduction of the 4-pyridyl as the R group.

3.2.2 Hydroxamic Acid Replacements—the Holy Grail?

Although hydroxamic acids are excellent metal chelators and generally make good HDAC inhibitors in vitro, they are not frequently found in the medicinal chemist's wish list of property-improving functionalities. The hydroxamic acid functionality can potentially result in unsatisfactory pharmacokinetic profiles and toxicity issues. Poor pharmacokinetic properties of hydroxamic acids can be the result of fast phase II metabolism to form the N,O-sulfonate and N,O-glucuronide conjugates [56]. In turn, the sulfonate intermediates are highly reactive and can therefore cause toxicity by covalent binding of the parent compound to protein, RNA and DNA [56]. While these hydroxamic acid-related liabilities can often be improved by structural modification of alternative fragments of the molecule, a considerable amount of work has also been dedicated to replacing the hydroxamic acid. The various attempts to replace the hydroxamic acid with alternative zinc-binding functionalities have

seen different degrees of success. A brief overview with a number of examples of some of the approaches pursued is given below.

Replacement of the hydroxamic acid moiety of SAHA by an alternative chelator has been the subject of several studies. Suzuki and Miyata et al. have shown that replacement of the hydroxamic acid of SAHA with a free thiol moiety does not affect the enzymatic HDAC inhibition capability of the compound [57]. Furthermore, replacement of the hydroxamic acid of SAHA by a trifluoromethyl ketone was investigated by Frey et al. (Fig. 8) [58]. The activated ketone is readily hydrated to form the vicinal diol, a structural feature known to bind to zinc-dependent proteases [59]. The in vitro evaluation was done on a partially purified HDAC preparation consisting largely of HDAC1 and HDAC2 [60], exhibiting an IC_{50} of 6.7 μM.

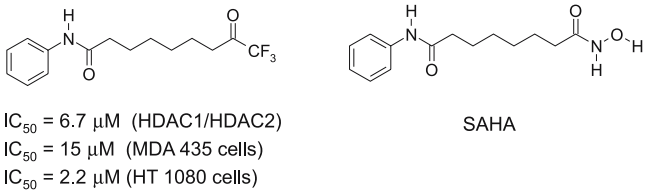


Fig. 8 The hydroxamic acid in SAHA replaced by a trifluoromethyl ketone – IC_{50}s in the micromolar range (reference data for SAHA not given). (Abbott Laboratories)

The analogous methyl ketone and trifluoromethyl alcohol were found to be inactive, clearly showing the importance of the trifluoromethyl ketone for HDAC inhibition. Regrettably, the trifluoromethyl ketone group demonstrated a half-life of only ~ 0.5 h and a low i.v. exposure in mice at 10 mg/kg. Another obstacle faced by these molecules is their poor aqueous solubility.

From the same laboratories, a series of heterocyclic ketones were published as HDAC inhibitors [61]. This work is a continuation and further elaboration of the concept of the use of electrophilic ketones as hydroxamic acid replacements. α-Keto oxazole derivatives appeared to act as the most potent HDAC inhibitors in the HDAC1/HDAC2 enzyme assay [60], displaying low micromolar activity (Fig. 9).

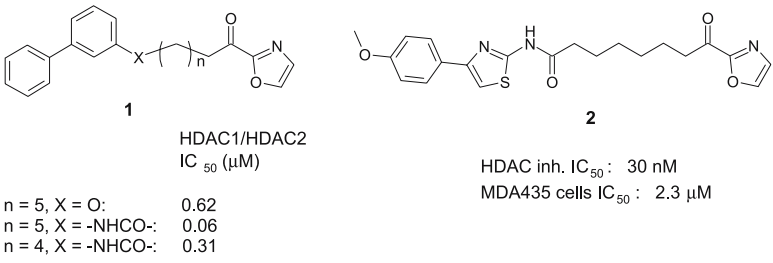
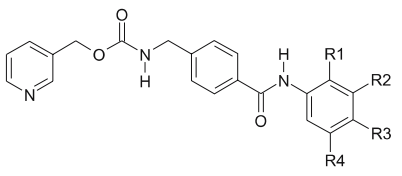


Fig. 9 Keto oxazoles as hydroxamic acid replacements. (Abbott Laboratories)

The potency of these α-keto oxazole derivatives was influenced by both the length of the spacer as well as the mode of connection to the capping group. From all the mono- and bisaromatic moieties tested, the *meta*-substituted bisphenyl α-keto oxazoles **1** (Fig. 9) displayed the most potent inhibition and were thus used to perform the initial comparison studies. A spacer length of $n = 5$, in combination with the presence of an amide connector to the capping region (X = – NHCO –) proved to be the most active combination, displaying an IC_{50} of 60 nM for the HDAC enzyme assay. After further variation of the capping moiety, while keeping the optimal spacer length and amide connector, a *para*-methoxyphenyl substituted thiazole capping group **2** was found to give the most potent HDAC inhibitor (IC_{50} 30 nM) that, in addition, showed antiproliferative activity in MDA435 cells (IC_{50} 2.3 μM, Fig. 9). The authors suggest that the cellular activity, however, was compromised by the instability of these compounds due to rapid reduction of the keto functionality to the inactive alcohol.

A series of benzamides as replacement for the zinc-binding hydroxamic acid was also synthesized and investigated for HDAC inhibitory activity [62, 63]. A clear SAR could be derived from the examples prepared (Fig. 10).



	R1	R2	R3	R4	HDAC1/HDAC2 IC_{50} (μM)
1)	NH_2	H	H	H	4.8
2)	H	NH_2	H	H	>100
3)	H	H	NH_2	H	>100
4)	OH	H	H	H	2.2
5)	NH_2	CH_3	H	H	>100
6)	NH_2	H	CH_3	H	>100
7)	NH_2	H	H	CH_3	2.8
8)	H	H	H	H	>100

Fig. 10 Benzamides as hydroxamic acid substitutes—discovery of MS-275. (Mitsui Pharmaceuticals)

Thus, the 2′-amino benzamide (entry 1, MS-275) showed low micromolar inhibition of HDAC enzyme (IC_{50} = 4.8 μM), whereas the activity was completely abolished by shifting the amino group to the 3′ or 4′ position (entries 2 and 3). Remarkably, when a hydroxyl group was introduced in the 2′ position (entry 4), HDAC inhibitory activity was fully restored (IC_{50} = 2.2 μM) suggesting that the hydrogen-bonding capability of the 2′ group is an important requisite for interaction with the enzyme. Taking MS-275 (entry 1) as a starting point, methyl groups were introduced at the 3′, 4′ and 5′ positions (entries 5, 6 and 7). Only the product with the methyl at the 5′ position showed HDAC inhibition (entry 7, IC_{50} = 2.8 μM). Steric hindrance seems to be the most plausible reason for the inactivity of compounds with methyl substitution at the 3′ and 4′ positions (entries 5 and 6).

An additional example of a benzamide showing HDAC inhibitory activity is *N*-acetyl dinaline, also known as CI-994 or tacedinaline (Fig. 11) [64–66], which is the acetylated derivative of the earlier identified dinaline (GOE 1734, PD 104 208) [67]. Both CI-994 and MS-275 have been in clinical development [68, 69].

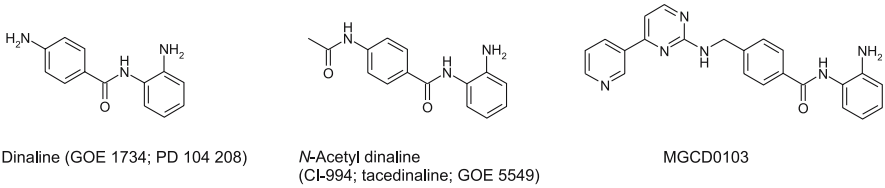


Fig. 11 Other HDAC inhibiting benzamides. (dinaline and *N*-acetyl dinaline (CI-994 or tacedinaline): Erasmus Univ.; MGCD0103: MethylGene Inc.)

Although both MS-275 and CI-994 elicit the classical hallmarks of HDAC inhibitors in tumor cell-based assays—accumulation of histone H3 acetylation, increased expression of cyclin-dependent kinase inhibitor p21$^{WAF1/Cip1}$ and accumulation in the G1 phase—their molecular mechanism of action is still poorly understood and remains somewhat controversial. As compared to the hydroxamic acid-containing HDAC inhibitors (e.g., TSA, SAHA, PXD-101, LBH-589, R306465), benzamide derivatives have been shown in numerous independent studies to be relatively weak inhibitors (10- to 1000-fold lower inhibition) of HDAC activity in classical HeLa cell nuclear extract, and immunoprecipitated or recombinant HDAC enzymes [70–73]. Nevertheless, extensive SAR analyses on benzamide derivatives have been carried out to support HDAC inhibition, coupled to the claim of a high degree of flexibility in the active site-pocket for accommodating groups with different stereoelectronic properties. As outlined in detail before, a structural analogue of MS-275—possessing a 3′-aminophenyl instead of a 2′-aminophenyl group—did not display any inhibition of HDAC activity in biochemical cell-free assays, and binding of the 2′-aminophenyl group to an unidentified but specific site on HDAC enzymes has been hypothesized. The 2′-substituent of benzanilide might act as a hydrogen-bonding site or other electrostatic interaction site and be indispensable to the specific interaction with the enzymes. In addition, steric hindrance may play an important role. Over the years, these consistent observations on weak in vitro potency and the lack of a molecular mechanism of action have supported the question of the identification of these benzamide derivatives as "genuine" HDAC inhibitors. Furthermore, recent studies comparing gene expression profiling of multiple HDAC inhibitors have indicated substantial differences in up- or down-regulation of sets of genes induced by TSA or SAHA, as compared to MS-275 [74]. One may speculate that these distinctly different expression profiles could be related to their differences in potency against the HDAC enzymes, but these observations do not ex-

clude the possibility that benzamide derivatives, or at least MS-275, may act on HDAC-dependent downstream signalling pathways by indirect mechanism(s).

MGCD0103, a compound that is structurally closely related to MS-275, Fig. 11, is currently in clinical trials (Sect. 4.2).

3.2.3 Is the Type of Spacer Really all that Important?

The spacer region of HDAC inhibitors has been the subject of optimization in several medicinal chemistry reports. Examples of a class of compounds having an aromatic moiety present in the spacer region are depicted in Fig. 12 [75–77].

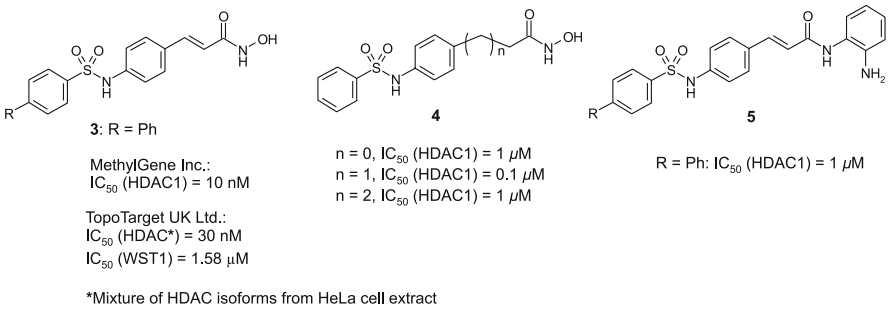


Fig. 12 Hydroxamic acid and benzamide containing HDAC inhibitors with aromatic spacers. (**4** and **5**: MethylGene Inc.; **3**: MethylGene Inc. and TopoTarget UK Ltd.)

Vinyl benzene hydroxamic acid **3** with R = Ph, (IC_{50} = 10 nM for HDAC1) was found after variation of the spacer length as well as modification of the substituent on the capping sulfonyl aryl moiety. Electron-rich groups at the para position of the arylsulfonyl provided the most active inhibitors. For the analogous alkyl benzene hydroxamic acids **4** it was also found that the activity for HDAC could be tuned by changing the length of the spacer. Addition or removal of only one methylene group in the alkyl benzene hydroxamic acid series resulted in a ten-fold decrease in activity in both instances (Fig. 12). Interestingly, the vinyl benzene hydroxamic acids were also compared to a set of analogs containing the 2-amino benzamide group (**5**) instead of the hydroxamic acid, also depicted in Fig. 12, which appeared to be a factor 10–100 less potent on HDAC1 than their hydroxamate counterparts (Fig. 12).

An extensive study focusing on sulfonamide containing hydroxamic acid derivatives as HDAC inhibitors led to the discovery of PXD101 (Fig. 13) [78–80], which is currently in clinical trials. It was shown that for the *meta* substituted sulfonamides, the so-called "reverse" sulfonamides were consistently (2- to 7-fold) more potent HDAC inhibitors than the "forward" sulfonamides.

This trend was not observed for *para* substituted compounds. An interesting comparison in this respect is that of vinyl benzene hydroxamic acid **3** (R = Ph) shown earlier in Fig. 12 with its "reverse" analog **6** (Fig. 13) [78]. These two compounds, having reversed sulfonamide bonds, show similar activities.

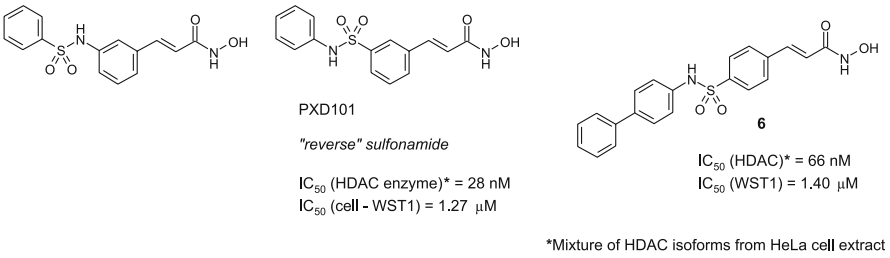


Fig. 13 PXD101 and its reversed analog possess unsaturated spacers. (TopoTarget UK Ltd.)

The role of aromaticity in the spacer region on the potency of hydroxamic acid containing inhibitors of HDAC was further investigated by Uesato et al. [81]. The data in Fig. 14 represent HDAC inhibitory activities against partially purified HDACs from human T cell leukemia Jurkat cells [82]. These data suggest that aromatic hydroxamic acids exhibit a much greater affinity for HDACs than their aliphatic counterparts. It is speculated in the paper that the 1,4-phenylene moiety may interact with aromatic and hydrophobic amino acid residues in the pocket, while the 2-naphthyl group would be engaged in similar interactions with aromatic amino acid residues at the pocket entrance, thereby capping the pocket.

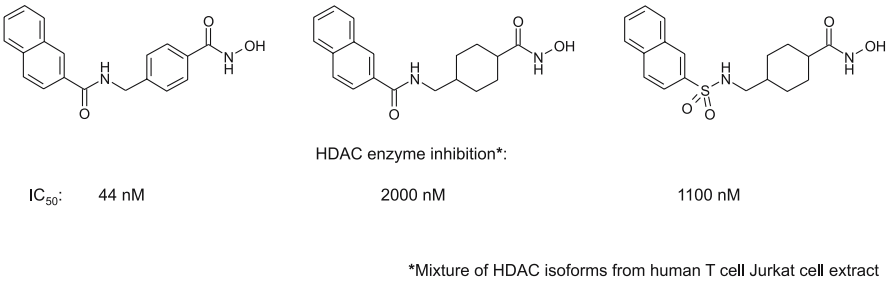


Fig. 14 Saturation of the aromatic spacer deteriorates the potency of a hydroxamic acid-based HDAC inhibitor. (Kansai Univ.)

Introduction of a heteroaromatic group in the spacer proved beneficial in the work by Johnson & Johnson researchers Arts et al. [83] and Angibaud et al. [84]. Optimization of the initial lead, having a 1,4-substituted phenyl present in the spacer region (Fig. 15, entry 1), was done by replacing this R

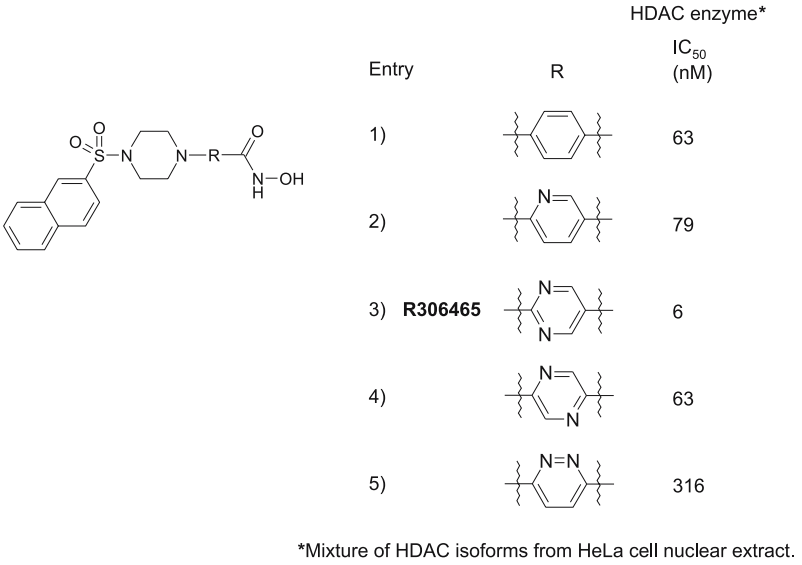


Fig. 15 Fine tuning of the (hetero)aromatic spacer of a hydroxamic acid-based HDAC inhibitor—discovery of R306465. (Johnson & Johnson Pharmaceutical R&D)

group with a series of six-membered heteroaryls. It was found that pyrimidyl (entry 3) was the most efficient R group of the series, demonstrating an IC_{50} of 6 nM for the enzyme assay consisting of a mixture of HDAC isoforms from a HeLa cell nuclear extract[1]. This increase of activity is likely to be the result of both steric and electronic factors. Finally, introduction of a functional group (such as dimethylamino or ethoxy) at the 4-position of the pyridyl causes complete loss of activity. These optimization studies of the linker led to the discovery of R306465, which is currently in Phase I clinical trials.

3.2.4 The Capping Group under Scrutiny

The importance of the structure of the capping group of HDAC inhibitors was underlined by a study in which Dai et al. carried out systematic modifications in this capping region [85]. SAHA analogues with reversed amides were functionalized with heteroaromatic residues as in the general structure (**7**) shown in Fig. 16. The indole capping group (e.g., see compound **8**) proved the most effective, demonstrating nanomolar inhibitory activity on HDAC1/2 enzyme.

[1] HeLa cell nuclear extracts were incubated at different concentrations with a radiolabeled acetylated Histone 4 peptide fragment as substrate. HDAC activity was assessed measuring release of free acetyl groups.

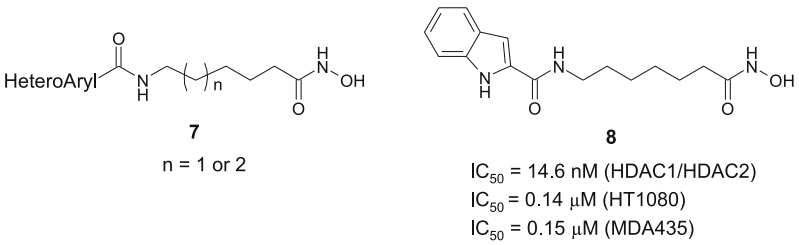


Fig. 16 Introduction of heteroaromatic groups in the capping region of hydroxamic acid containing HDAC inhibitors. (Abbott Laboratories)

The indole functionality was also used as a capping group by researchers from Novartis [86]. The initial hit from high-throughput screening (NVP-LAK974, Table 2 and Fig. 17), bearing a phenyl propylamine capping group, demonstrated acceptable in vitro activity but had poor efficacy in the HCT116 human colon tumor xenograft model. Replacement of this phenyl propylamine moiety with a tryptamine (**9**) triggered an overall increase of in vitro potency up to a factor 2 compared to the original hit. Introduction of a methyl group at either the chain carbon α to the benzylamine (**10**) or the benzylic carbon (**11**) provided a 2-fold increase in enzymatic HDAC inhibitory activity and an approximate 2-fold increase in cellular potency on the HCT116 cell line while keeping the same antiproliferative activity on H1299 cells. Cellular potency was further improved by a factor 2 via methylation of the indole *N* (**12**). Finally, nonpolar aliphatic substituents on the benzylic amine generally improved cellular potency as is illustrated by the introduction of an isopropyl group (**13**), giving a HDAC inhibitor with IC_{50}s of 6 and 30 nM on HCT116 and H1299 cells, respectively.

The *N*-hydroxyethyl analogue NVP-LAQ824 (Table 2, Fig. 17) also showed good overall potency in vitro, but excelled in the succeeding in vivo experiments. It demonstrated the highest maximum tolerated dose (MTD;

Table 2 Published enzymatic and cellular data for the structures shown in Fig. 17 [86]

Compound	HDAC enzyme * (IC_{50}, nM)	H1299 cells (IC_{50}, nM)	HCT116 cells (IC_{50}, nM)
NVP-LAK974	150 (±94)	800	50
9	63 (±10)	400	30
10	24 (±5)	410	20
11	23 (±12)	480	15
12	23 (±12)	170	10
13	23 (±6)	30	6
NVP-LAQ824	32 (±18)	150	10

* Mixture of HDAC isoforms from H1299 whole cell extracts

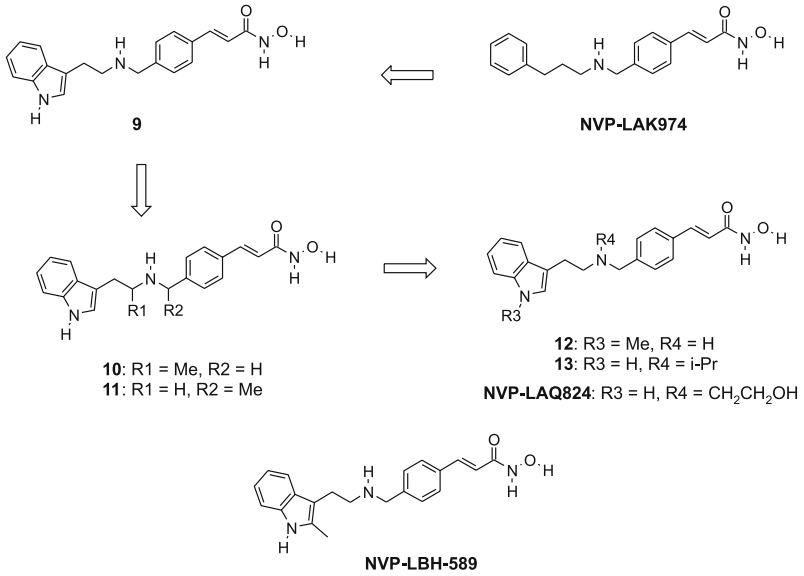


Fig. 17 Optimization of the capping group—LBH589 and the discovery of LAQ824. (Novartis Institute for Biomedical Research)

> 200 mg/kg) of all compounds that were selected for the in vivo study. In the dose-response studies, NVP-LAQ824 caused the least weight loss and was thus best tolerated. Also, it was 2- to 3-fold more potent in tumor growth inhibition than the other selected compounds. NVP-LBH589 has recently joined NVP-LAQ824 in phase I clinical trials. As compared to LAQ824, the new candidate LBH589 has a 2-methyl substitution on the indole and a free benzylic NH (Fig. 17).

3.2.5 Connecting the Spacer with the Capping Group

Having covered the effect of the zinc binding moiety, the importance of the spacer and the role of the capping group, only a limited number of functionalities that connect the spacer with the capping group have so far been described. The most common examples of such a connector are the sulfonamide group or the amide functionality such as in SAHA. The function of this connection unit has been the subject of a comparison study by Dai et al. [87]. In this study, the amide connector of SAHA was replaced by a set of (hetero) aromatic ring systems. Introduction of a phenyl, oxazole or thiazole moiety at the position of the amide in SAHA (entries 2 through 5, Fig. 18) provided compounds with a 2- to 14-fold increase in HDAC inhibiting potency as compared to SAHA, with the oxazole as the most potent (IC_{50} = 10 nM, entry 3, Fig. 18). Reversal of the amide connector in SAHA (entry 6)

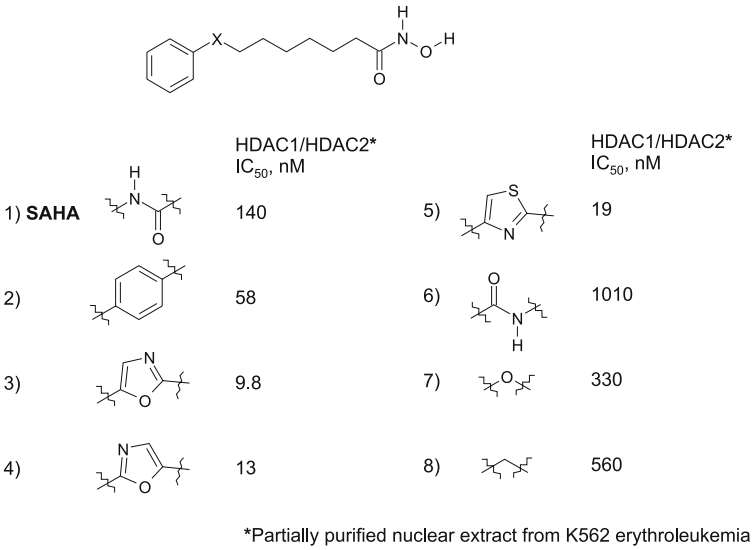


Fig. 18 Extensive variation of the amide connection unit of SAHA leads to a single digit nanomolar HDAC inhibitor. (Abbott Laboratories)

resulted in a much weaker inhibitor that showed only micromolar HDAC inhibition. Replacement with an ether or methylene linkage (entries 7 and 8, respectively) also led to deterioration of HDAC inhibition.

3.2.6 The Quest for Selective HDAC Inhibitors

Over the years, it has become evident that HDACs not only play a key role in carcinogenesis but also in a number of nonmalignant differentiation processes. This is most apparent for the class-IIa HDACs 4, 5, 7, 9. For example, HDAC7 has been suggested to play a critical role in the thymic maturation of T-cells [88], while HDAC4 has been implicated in the regulation of chondrocyte hypertrophy and endochondral bone formation by inhibiting the activity of the Runx2 transcription factor [89]. Most concerns, however, have focused around the role of the class-IIa HDACs in muscle differentiation. HDACs 4, 5, 7 and 9 all suppress the differentiation of myocytes (muscle cells) as a consequence of being transcriptional co-repressors of myocyte enhancer factor 2 (MEF2) [90]. Deletion of the MEF2 binding domain of the most abundant myocyte class-II HDAC, HDAC9, results in development of cardiac hypertrophy in 9-months old mice due to hypersensitivity to hypertrophic signaling [91].

The observations above have led to speculation that HDAC inhibitors may cause cardiac hypertrophy. Surprisingly however, it was observed that HDAC inhibitors may actually be beneficial in treating cardiac hypertrophy. TSA has

been shown to block the fetal gene program associated with cardiomyocyte hypertrophy in response to hypertrophic agents. It was therefore proposed that inhibition of other HDACs (e.g. class I) may counteract the expression of the hypertrophic genes associated to the class-II HDACs [92].

Even though it is currently unclear whether any of the side effects observed in the clinic with the current pan-HDAC inhibitors are linked to inhibition of the class-II HDACs, these observations, nevertheless, triggered the quest for HDAC isotype specific inhibitors, which will be further discussed below.

The HDAC inhibitors TSA and TPX (Fig. 2) have been utilized as structural leads in the early stages of the quest for new and more selective small molecule inhibitors of the HDAC enzyme family. In order to investigate the function of the individual HDAC members, Schreiber et al. synthesized a library of 7200 potential HDAC inhibitors based on the structural features of TSA and TPX [93]. The members of this library were prepared on solid support by means of split pool methods. The key characteristics of these compounds consist of a dioxane-containing capping region and a zinc binding motive, connected via an aliphatic chain. Three different zinc binders, i.e., carboxylic acid, *o*-aminoanilide and hydroxamic acid were used.

Assuming that equal purities were obtained for the different classes of metal chelators, it was found in both the AcTubulin and the AcLysine cytoblot assays that the hydroxamic acids were the most active inhibitors of both α-tubulin and histone deacetylation [94]. On the other hand, the *o*-aminoanilides demonstrated the weakest inhibition of HDAC enzyme. Analysis of the compounds using principal component analysis followed by resynthesis disclosed the structures of two examples of selective inhibitors. The first, tubacin, is a selective inhibitor of α-tubulin deacetylation with no effect on the histone acetylation status. The function of HDAC6 as an α-tubulin deacetylase enzyme [95] and its role in mediating cell cycle progression, microtubule stability, and cell motility has been studied using tubacin as the selective inhibitor [28]. The second, histacin, is a selective inhibitor of histone deacetylation (Fig. 19).

Mai et al. have carried out a comprehensive study of the aroyl pyrrolyl hydroxamic acids (APHAs) as HDAC inhibitors [96–98] and succeeded in obtaining class-II selectivity (Fig. 20) [99].

The APHA with a fluorine atom at the 3-position of the aryl exhibits a class-II/class-I HDAC selectivity of 176, whereas substitution at the 2- or 4-position gives respectively much lower selectivity ranging from 34 to a value less than 2. It is interesting to note that this "meta-effect" is much less pronounced when the substituent is a chloro atom and the effect is completely lost when a bromo-atom is introduced (data not shown).

A rational approach toward the design of class-I isoform selective HDAC inhibitors was reported recently by Wiest et al. [100]. In order to understand the difference between these class-I isoforms, three-dimensional models of HDAC1, HDAC2, HDAC3, and HDAC8 were built using homology modeling.

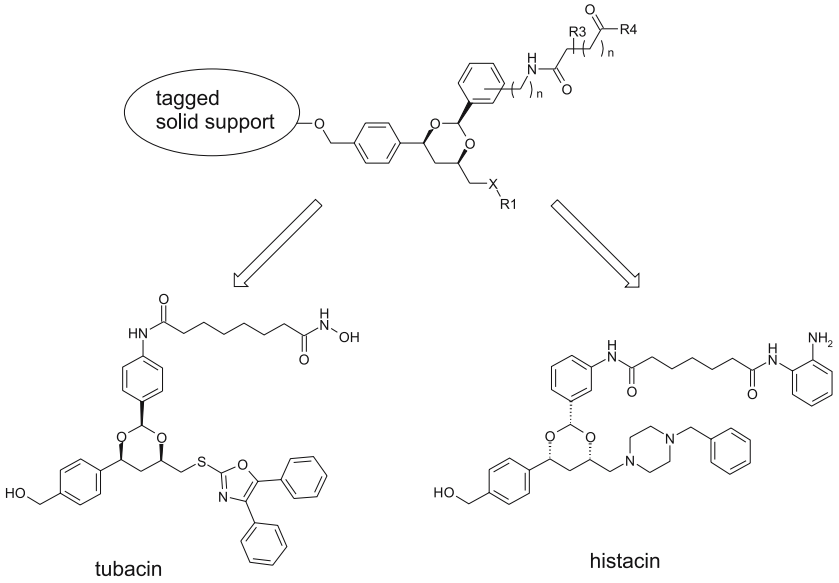
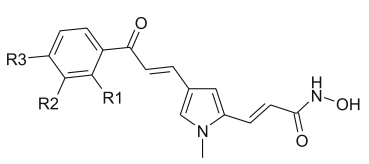


Fig. 19 Discovery of tubacin and histacin—two selective deacetylation inhibitors. (Harvard University)



R1	R2	R3	HDAC Class II / Class I ratio
F	H	H	34
H	F	H	**176.4**
H	H	F	1.59
Cl	H	H	32
H	Cl	H	**71.4**
H	H	Cl	1.58

Fig. 20 Class-II selectivity has been accomplished for some APHAs. (Università degli Studi di Roma "La Sapienza")

The high homology of the active site region of the different class-I HDACs as well as the considerable similarity of their 11 Å channel do not leave enough room for a selectivity prediction based on these parts of the enzyme. The models show, however, that electronic and steric dissimilarity around the opening of the active sites holds potential for differentiating between HDAC1, HDAC3, and HDAC8. Differentiation between HDAC1 and HDAC2, though, is predicted to be more difficult. The design of novel HDAC inhibitors using these models is currently in progress.

It is evident that the quest for selective HDAC inhibitors has just begun and that the optimal HDAC subtype selectivity profile for an anticancer drug based on HDAC inhibition is still far from being established. Never-

theless, the first important steps toward the rational design and synthesis of isoform selective HDAC inhibitors have been taken. The first clinical trials with MGCD0103 (Fig. 11), an isotype selective inhibitor of human HDACs are ongoing [101]. Moreover, the understanding of the biological and clinical consequences of different HDAC inhibitory profiles is increasing steadily as more and more of the biology becomes known while a growing number of compounds are being evaluated in the clinic.

4 Clinical Experience with HDAC Inhibitors

Histone deacetylases are linked to the pathogenesis of malignancy from a mechanistic perspective. The capacity of HDAC inhibitors (HDACi) to interfere with the enzyme function has led to the observed preclinical and clinical activity in cancer therapy. Although the exact mechanism of anti-tumor activity is not fully elucidated, various cellular pathways have been shown to be involved. From the first clinical trials involving HDACi with short chain fatty acids to the newer generation hydroxamic acid derivatives and cyclic tetrapeptides, a number of structurally diverse compounds have made the transition from the laboratory to the clinical arena. For purposes of this part of the discussion, HDACi are arbitrarily divided into the hydroxamates and nonhydroxamates.

Most of the studies reported are in early phase (Phase I and II) with the exception of Vorinostat (suberoylanilide hydroxamic acid [SAHA]), which has entered Phase III. Some of these studies have only been published in abstract form. Encouragingly, activity has been seen especially in lymphoproliferative diseases, leukemia and some solid tumors, including prostate cancer.

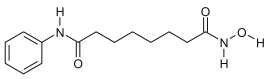
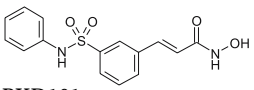
Generally, the impression so far is that HDACi display a somewhat lower toxicity profile compared to conventional cytotoxics. The most common toxicity seen is nausea/vomiting and fatigue, mild myelosuppression and diarrhea and these feature as adverse effects in many clinical trials. The toxicities observed may be due to the individual drug or the consequence of inhibiting HDAC itself (class effect). It is postulated that interference with additional cellular pathways, not just histone acetylation, may be responsible for the differential toxicity seen clinically, especially if these different compounds are used at higher doses.

The relationship between toxicity and pharmacokinetic (PK) and pharmacodynamic (PD) parameters is a difficult one and somewhat poorly characterized. The key targets of HDACi are unknown and predicting which patients will respond to HDACi therapy is difficult. Correlation between surrogate markers (for example, levels of acetylated histones in peripheral blood mononuclear cells [PBMNC] pre- and post-dosing) is not always in keeping with measured PK profiles.

4.1 Hydroxamates

Various agents in this class have gone on to phase I evaluation and beyond. These include: vorinostat (SAHA), LAQ824, LBH589, PXD101 and R306465, some of which are discussed in greater detail below and listed in Table 3.

Table 3 Selected hydroxamates continuing in clinical development in solid tumors. CR: complete response, PR: partial response, uPR: unconfirmed PR, SCC: squamous cell carcinoma

Drug/Phase	Schedule	Tumor type	Response/ no. of patients	Main toxicities
 SAHA Phase I [102]	2 hours i.v. days 1–5, 8–12, 15–19 every 4 weeks	Solid tumors	$n = 37$	Fatigue, dehydration, anorexia
See above for structure SAHA Phase I [103]	Various oral, 200–600 mg qd to bd	Solid tumors and hematological	1CR, 3 PR, 2 uPR, $n = 73$	Fatigue, gastrointestinal, hematological (thrombocytopenia)
See above for structure SAHA Phase I [114]	Oral, bid or tid d1–14 every 21 days	Leukemia, myelodysplastic syndrome	3 CR, 1 PR $n = 41$	Nausea, vomiting, diarrhea, fatigue
See above for structure SAHA Phase II [115]	Oral, 400 mg qd	Head & neck SCC	1 minor response $n = 13$	Anorexia, anemia, fatigue, hematological
See above for structure SAHA Phase II [116]	Various oral, 400 mg qd, 300 mg bid, 200 mg bid	Cutaneous T-cell lymphoma	10 PR $n = 37$	Fatigue, rash, hematological (thrombocytopenia)
 PXD101 Phase I [117]	30 minutes i.v. days 1–15 every 21 days	Solid tumors	$n = 42$ Accrual continues	Fatigue, nausea, vomiting, diarrhea, phlebitis

4.1.1 Vorinostat (SAHA)

SAHA is the HDACi that has advanced farthest in clinical trials. Both intravenous and oral Phase I trials involving 110 patients have been reported [102, 103]. Briefly from these studies, the mean intravenous half life ($t_{1/2}$) is between 92 to 127 minutes, whereas the oral half life is longer. There is demonstration of linear pharmacokinetics, oral bioavailability of more than 40%, and increased duration of acetylated histone H3 (AcH3) was seen with increasing dose and prolonged dosing. However, acetylation effects, although rapid, are transient and return to near baseline levels by 8 hours except at higher dose cohorts. The maximum tolerated dose (MTD) was 200 mg twice daily or 400 mg daily continuously or 600 mg twice daily 3 times per week.

The most common drug related Grade 3/4 toxicities are fatigue, nausea, vomiting, diarrhea, anorexia, anemia, thrombocytopenia, hyperglycemia and hypocalcemia. No clinically significant electrocardiograph (EKG) changes or cardiac toxicities, including arrhythmias attributable to the drug, were seen. In fact, SAHA is probably the only HDACi that has not resulted in EKG changes. Toxicities including myelosuppression were rapidly reversible upon discontinuation of drug. It has been postulated that the thrombocytopenia is due to impairment of megakaryocyte differentiation [104].

A significant proportion (30%) of patients on SAHA remained on the drug between 4 to >37 months, with chronic dosing demonstrating prolonged disease stabilization, maintained biological effect and drug tolerability. Responses were seen in lymphoma, laryngeal carcinoma, thyroid cancer and mesothelioma.

Phase I studies have also been conducted in various hematological malignancies including myeloma [105]. Other Phase I studies looking at SAHA in combination with retinoic acid and gemcitabine, Phase II studies in tumor specific areas of head and neck squamous cell carcinoma, T-cell lymphoma, melanoma and glioma, and a Phase III study in mesothelioma have been completed or are in progress.

Phase I studies of intravenous LAQ824 and LBH589 [106–109], novel cinnamyl hydroxamates, have also been completed, with 112 patients dosed in trials of both agents. LBH589 has also been administered orally. LAQ824 is a potent HDACi given intravenously and has been shown to also inhibit Hsp90 [110]. In these trials cardiac toxicity, including prolonged QT interval (QTc) effects, nonspecific ST segment and T wave changes on EKG and arrhythmia were reported at high doses when administered intravenously. Overall, both LAQ824 and LBH-589 were found to induce dose-related increases in QTcF (Fridericia correction) of 20 msec or less at doses up to 200 mg/m^2 and 20 mg/m^2, respectively [111, 112]. Cardiac repolarization changes were often delayed until day 3, and may not be due to a direct effect of the agents on the hERG (human ether-a-go-go related gene) channel [113].

Currently, orally administered LBH589 is in Phase II clinical trials and at lower doses the electrocardiographic change can be abrogated. A Phase II study for LBH-589 in solid and liquid tumors is ongoing.

Enrolment is ongoing for PXD101, for which another Phase I study in hematological malignancies is in progress, exploring the possibility for oral dosing. A Phase II study in multiple myeloma is also currently ongoing.

4.2 NonHydroxamates

Various classes of short-chain fatty acids, cyclic tetrapeptides and benzamides have also been in clinical trials (Table 4).

The short chain fatty acids include butyrate derivatives like phenylbutyrate, AN-9 (pivaloyloxymethyl butyrate) and valproate. Unfortunately, these compounds have poor potency and pharmacokinetic properties, including short half-life. Numerous Phase I studies with phenylbutyrate, in various oral and intravenous schedules [118–120] have been performed, with neurological toxicity at higher doses being reported. AN-9 showed initial promise in a Phase I study, where the MTD was not reached [121]. The subsequent Phase II study in nonsmall cell lung cancer in 47 patients resulted in fatigue, nausea and dysgeusia as common toxicities. Three partial responses (PR)

Table 4 Selected nonhydroxamates continuing in clinical development. MTD: maximum tolerated dose, PR: partial response

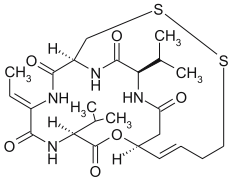
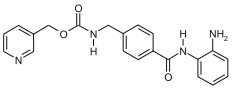
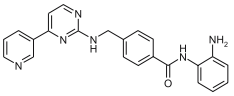
Drug/Phase	Schedule	Tumor type	Response/ *n* = no. of patients	Main toxicities	Comment
 Depsipeptide (FK-228) Phase I [124]	4 hr i.v. days 1 and 5 every 21 days	Solid tumors	1 PR *n* = 37	Nausea, vomiting, thrombocytopenia, atrial fibrillation	MTD 17.8 mg/m^2
See above for structure Depsipeptide (FK-228) Phase I [125]	4 hr i.v. days 1, 8 and 15 every 28 days	Solid tumors	*n* = 33	Fatigue, thrombocytopenia	MTD 13.3 mg/m^2
See above for structure Depsipeptide (FK-228) Phase I [137]	4 hr i.v. days 1, 8 and 15 every 28 days	Hematological	*n* = 20	Nausea, fatigue, anorexia	dosed at 13 mg/m^2

Table 4 (continued)

Drug/Phase	Schedule	Tumor type	Response/ *n* = no. of patients	Main toxicities	Comment
See above for structure Depsipeptide (FK-228) Phase II [129]	13 mg/m^2 4 hr i.v. days 1, 8 and 15 every 28 days	Castration refractory prostrate cancer	2 PR *n* = 16	Fatigue, nausea, anorexia	
See above for structure Depsipeptide (FK-228) Phase II [128]	13 mg/m^2 4 hr i.v. days 1, 8 and 15 every 28 days	Renal cancer	1 PR *n* = 30	Fatigue, nausea, vomiting, anemia, anorexia	
 MS-275 Phase I [133]	Oral fortnightly	Solid tumors	*n* = 30	Anorexia, nausea, vomiting, fatigue	MTD 10 mg/m^2
See above for structure MS-275 Phase I [135]	Oral various	Solid tumors	1 PR *n* = 24	Fatigue, neutropenia, hypophos-photaemia	
See above for structure MS-275 Phase I [134]	Oral weekly X4 every 6 weeks	Solid tumors, lymphoma	*n* = 13	Neutropenia, nausea/ vomiting	Accrual continues
 MGCD0103 Phase I [123]	3 times/wk for 2 wk every 3 wk	Solid tumors	*n* = 24	Fatigue, nausea, vomiting, anorexia	Accrual continues

were reported [122]. However, safety concerns regarding its combination with cytotoxics has led to interruption of its development.

Depsipeptide is the leading compound in the cyclic peptide class, and is currently in Phase II trials in CTCL, with a response rate (RR) of 38% in this disease. MS-275 and tacedinaline (CI-994) have undergone Phase I trials and are now in Phase II trials. Other drugs like MGCD0103, a class-I isotype selective HDACi are in Phase I trials [123].

4.2.1
Depsipeptide (FR901228 or FK-228)

Preclinical studies have shown improved tolerability and antitumor activity, with an intermittent dosing schedule as the result of the ability to administer higher doses, with shorter infusions found to induce less toxicity [124]. In the initial study, dose limiting toxicities (DLTs) included nausea and vomiting, thrombocytopenia and cardiac arrhythmia with atrial fibrillation. Because cardiac toxicity had been predicted from preclinical studies (myocardial hemorrhage and ischemia) patients were treated under continuous cardiac monitoring. This and a further study [125] showed no clinically significant cardiac adverse effects were observed, although subtle EKG changes were reported (QTc interval prolongation, ST segment and T wave changes). Toxicities observed included nausea/vomiting, thrombocytopenia, fatigue and hypophosphotemia.

Studies in patients with T-cell lymphoma have used a schedule of depsipeptide administered on days 1, 8, and 15 of a 28-day cycle at a dose of 14 mg/m^2 [126]. This study involved intensive cardiac monitoring, cardiac biochemistry markers and functional imaging monitoring. No definitive or clinically significant changes have been seen so far. In the updated multiple cohort Phase II study of cutaneous T-cell lymphoma (CTCL) and peripheral T-cell lymphoma (PTCL), 66 patients have been treated with responses in 10 CTCL and 6 PTCL patients [127], which is very encouraging in this heavily pre-treated group. A Phase II study in renal cancer [128] showed 1 response in 30 patients. An ongoing Phase II study in castration refractory prostate cancer showed 2 partial responses in 16 evaluable patients [129]. Further Phase II studies involving tumor types such as myeloma, acute myeloid leukemia (AML) and colorectal cancer have been conducted or are ongoing [130–132].

4.2.2
MS-275

This synthetic benzamide was studied in two different schedules, with the daily schedule exceeding MTD at first dose-level of 2 mg/m^2, unpredicted, possibly due to long $t_{1/2}$ from possible enterohepatic recirculation. The fortnightly schedule was found to be feasible, and an MTD of 10 mg/m^2 has been established from 28 patients [133]. There were no clinically significant cardiac toxicities either from a rhythm perspective or from assessment of left ventricular ejection fraction. Toxicities seen include anorexia, nausea, vomiting, diarrhea, fatigue, myelosuppression, hypoalbuminemia and hypophosphotemia.

Clinical studies with a weekly dosing schedule are reported to be ongoing [134]. Another study exploring three different schedules of biweekly,

twice weekly and weekly for 3 out of 4 weeks has evaluated 24 patients [135]. Fatigue, hypophosphotemia and neutropenia were some of the significant toxicities.

4.2.3 CI-994 (Tacedinaline)

The mechanism of action of this benzamide compound is not entirely understood but it has been shown to inhibit HDAC and cellular proliferation. It displays linear kinetics and is rapidly absorbed after oral administration. The main dose limiting toxicity (DLT) reported was thrombocytopenia with the MTD at 8 mg/m^2/day, although other toxicities like nausea, vomiting, diarrhea and fatigue were seen [136]. One partial response was seen in the 53 patients evaluated.

4.3 Summary and Future Development

The early and prolonged responses reported in clinical trials with HDACi involving patients with cutaneous T-cell lymphoma (CTCL), acute myeloid leukemia and other solid tumors have been encouraging. A submission to the FDA of vorinostat (SAHA) for CTCL was filed in the second quarter of 2006. The potential for HDACi therapy however probably goes beyond single agent use. The wide ranging molecular pathways affected by HDACi's make it a promising candidate for the exploration of combinatorial studies in the clinical setting.

In vitro studies have evaluated the additive and synergistic antitumor activity of HDACi with many agents including cytotoxics, targeted molecules, and radiation. Considerable interest has been focused on combinations with DNA methyltransferase inhibitors like 5 aza-2′deoxycytidine (decitabine) and retinoic acid receptor (RAR)-targeted drugs. Furthermore, enhancement of apoptosis has been shown with traditional cytotoxics like the topoisomerase II agents and taxanes, TRAIL (tumor necrosis factor related apoptosis inducing ligand), CDK (cyclin dependent kinase) inhibitors, Hsp-90 antagonists like 17-AAG (17-allylamino-17-demethoxygeldanamycin), proteosome inhibitors and enhanced radio sensitivity to ionizing radiation [11, 138]. Combination clinical studies of HDACi with retinoic acid [139] and conventional cytotoxics like carboplatin, paclitaxel, capecitabine and gemcitabine [140–143] have already been shown to be feasible in the clinic. Although improved response rates have yet to be demonstrated, trial characteristics make it difficult to draw definitive conclusions at such an early stage.

Experience so far from clinical trials has shown these agents can be well tolerated at biologically active doses. However, cardiac toxicity, mainly QTC prolongation, and cardiac arrhythmias, including atrial fibrillation and tor-

sades de pointes, appears to be a recurrent theme with both the hydroxamates and nonhydroxamates. The clinical significance of these findings, if at all, will become more apparent with later phase studies. Dose-limiting toxicity from the various agents generally involves constitutional symptoms, in particular fatigue and nausea.

The improved PD effect seen with more frequent dosing in the SAHA study favors the development of oral agents in the effort to sustain HDAC inhibition via more continuous exposure [103]. The next generation hydroxamate agents with prolonged PD responses have entered clinical trials.

With novel and newer generation HDACi emerging, the importance of validating drug effect lies mainly in determining acetylation of histones (H3 and H4), from surrogate tissue/cells such as from PBMNC. Validating drug effect in tumor tissue, although not always practical, is critical in establishing "proof of concept" of biological modulation. However, robust data of correlation of degree of acetylation with tumor response is not available at present. Furthermore, it is unknown if inhibition of histone deacetylation, acetylation of nonhistone proteins or effects on other cellular pathways is responsible for the clinical benefits seen. As the knowledge expands rapidly on nonhistone substrates of HDACs, development of new biomarkers, as well as quick, simple and easily reproducible methods of quantifying the degree of acetylation of HDACi, will be crucial to the future of these drugs.

5 Perspectives and Conclusion

Chromatin has evolved into an established therapeutic target. Accumulating evidence suggests that chromatin-modulating drugs are on the verge of becoming a new drug class on their own with significant medical potential.

HDAC inhibition holds particular promise in anticancer therapy, where the concerted effects on multiple pathways involved in growth inhibition, differentiation and apoptosis may prove to be advantageous in the treatment of a heterogeneous pathology such as tumor formation and growth.

It remains to be seen whether pan-HDAC inhibition is a prerequisite for clinical efficacy, or whether more subtype-specific HDAC inhibition offers clinical advantages in relation to efficacy and/or toxicity. It should be understood however that the current state of the art suggests that chromatin remodeling is not the only way in which HDACi exert their antitumor effects. As more and more evidence indicates that HDACs not only play a central role in the epigenetic status of chromatin, but are also involved in other levels of enzymatic control, their ability to act on multiple molecular pathways only adds to their multi-targeting properties.

From a chemogenomics perspective, the past and present generations of HDAC inhibitors, while providing much insight into the molecular mechan-

isms and resulting biology of HDAC inhibition, remain to some extent limited in diversity.

With the exception of the benzamide class, represented by MS-275, and to a lesser degree natural products such as depsipeptide, the majority of HDAC inhibitors feature the hydroxamic acid functionality, attached to a cap group via a spacer. The fact that this privileged structure appears in many of the recently disclosed HDAC inhibitors may be a consequence of the very specific topology and the resulting restrictive molecular recognition at the catalytic site of the HDAC metallo-enzyme family. The catalytic site has all the appearances of a pocket located inwards of the enzyme and containing the catalytic Zn-cation, which is only moderately accessible to ion chelating functionalities. This restricted access, attributed to the presence of two hydrophobic phenylalanine residues constituting a narrow tube-like bottleneck towards the catalytic site, is reflected in the nature of the spacer moiety, which is invariably of (hetero)aromatic or aliphatic nature, and not amenable to extensive variation. As a result, many of the recently disclosed HDAC inhibitors can be considered as variations on one and the same theme.

Accordingly, pharmaceutical companies are rapidly covering the intellectual property space with generous patent scopes, leaving increasingly less room for maneuvering when in search of novel enzymatic HDAC inhibitors. In addition, all efforts to replace the hydroxamic acid moiety in an established HDAC inhibitor bearing that same moiety have not led to a major breakthrough. Although modifications such as electrophilic ketones, thiols, mercaptoamides and N-formyl hydroxylamines have been reported, some of them showing significant antiproliferative activity and HDAC isoenzyme selectivity, none have improved the often poor pharmacokinetic properties of the hydroxamic acid counterparts.

Many questions and opportunities remain to be investigated before HDAC inhibitors can take the center stage as chromatin-modulating drugs.

Further development of this emerging class of drugs demands greater understanding of the molecular events mediating the observed biological effects and their selectivity for cancer cells in order to design compounds with improved efficacy while minimizing toxicity. Newer HDAC inhibitors are being developed with higher specificity for different classes of HDAC, hopefully enabling correlation of anti-tumor effects with particular patterns of HDAC expression.

Much remains to be done to improve the physicochemical properties and the pharmacokinetic characteristics of the established compound classes. A critical observer cannot help but wonder about the PK/PD profiles of many of the compounds currently undergoing clinical development: with limited oral bioavailability, often necessitating intravenous administration, and rather short half lives in combination with often transient acetylation effects, the need for HDAC inhibitors with a more beneficial pharmacokinetic profile seems key.

This leaves the medicinal chemistry community with the challenge of having to operate in a relatively small chemistry space, while targeting not only pharmacologically relevant HDAC inhibition but also, and perhaps more importantly, improved pharmokinetic properties.

Acknowledgements The authors thank Dr. Karen Vermuyten and Dr. Patrick Angibaud for critically proof-reading the manuscript.

References

1. Suzuki H, Gabrielson E, Chen W, Anbazhagan R, van Engeland M, Weijenberg MP, Herman JG, Baylin SB (2002) Nat Genet 31:141
2. Van Lint L, Emiliani S, Verdin E (1996) Gen Express 5:245
3. Mitsiades CS, Mitsiades NS, McMullan CJ, Poulaki V, Shringarpure R, Hideshima T, Akiyama M, Chauhan D, Munshi N, Gu X, Bailey C, Joseph M, Libermann TA, Richon VM, Marks PA, Anderson KC (2004) Proc Natl Acad Sci 101:540
4. Glaser KB, Staver MJ, Waring JF, Stender J, Ulrich RG, Davidsen SK (2003) Mol Cancer Ther 2:151
5. McLaughlin F, La Thangue NB (2004) Biochem Pharmacol 68:1139
6. Johnstone RW (2002) Nat Rev 1:287
7. Dokmanovic M, Marks PA (2005) J Cell Biochem 96:293
8. Halkidou K, Gaughan L, Cook S, Leung HY, Neal DE, Robson CN (2004) Prostate 59:177
9. Zhu P, Martin E, Mengwasser J, Schlag P, Janssen K-P, Gottlicher M (2004) Cancer Cell 5:455
10. Marks PA, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK (2001) Nat Rev 1:194
11. Drummond DC, Noble CO, Kirpotin DB, Guo Z, Scott GK, Benz CC (2005) Annu Rev Pharmacol Toxicol 45:495
12. Kim MS, Kwon HJ, Lee YM, Baek JH, Jang J-E, Lee S-W, Moon E-J, Kim H-S, Lee S-K, Chung HY, Kim CW, Kim K-W (2001) Nat Med 7:437
13. de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB (2003) Biochem J 370:737
14. Glaser KB, Li J, Staver MJ, Wei RQ, Albert DH, Davidsen SK (2003) Biochem Biophys Res Commun 310:529
15. Lagger G, O'Carroll D, Rembold M, Khier H, Tischler J, Weitzer G, Schuettengruber B, Hauser C, Brunmeir R, Jenuwein T, Seiser C (2002) EMBO J 21:2672
16. Ropero S, Fraga MF, Ballestar E, Hamelin R, Yamamoto H, Boix-Chornet M, Caballero R, Alaminos M, Setien F, Paz MF, Herranz M, Palacios J, Arango D, Orntoft TF, Aaltonen LA, Schwartz S, Esteller M (2006) Nat Genet 38:566
17. Archer SY, Meng S, Shei A, Hodin RA (1998) Proc Natl Acad Sci 95:6791
18. Kim YB, Ki SW, Yoshida M, Horinouchi S (2000) J Antibiot 53:1191
19. Juan L-J, Shia W-J, Chen M-H, Yang W-M, Seto E, Lin Y-S, Wu C-W (2000) J Biol Chem 275:20436
20. Minucci S, Pelicci PG (2006) Nat Rev Cancer 6:38
21. Fischle W, Dequiedt F, Hendzel MJ, Guenther MG, Lazar MA, Voelter W, Verdin E (2002) Mol Cell 9:45

22. Kao GD, McKenna WG, Guenther MG, Muschel RJ, Lazar MA, Yen TJ (2003) J Cell Biol 160:1017
23. Huang Y, Tan M, Gosink M, Wang KK, Sun Y (2002) Cancer Res 62:2913
24. Attar RM, Spires T, Jackson D, Feder J, You D, Vivat-Hannah V, Gottardis MM, Lorenzi MV (2003) American Association for Cancer Research 94th Annual Meeting, April 5th–9th, Toronto, Canada, abstract 72
25. Bali P, Pranpat M, Bradner J, Balasis M, Fiskus W, Guo F, Rocha K, Kumaraswamy S, Boyapalle S, Atadja P, Seto E, Bhalla K (2005) J Biol Chem 280:26729
26. Murphy PJM, Morishima Y, Kovacs JJ, Pao T-P, Pratt WB (2005) J Biol Chem 280:33792
27. Zhang Y, Li N, Caron C, Matthias G, Hess D, Khochbin S, Matthias P (2003) EMBO J 22:1168
28. Haggarty SJ, Koeller KM, Wong JC, Grozinger CM, Schreiber SL (2003) Proc Natl Acad Sci USA 100:4389
29. Kawaguchi Y, Kovacs JJ, McLaurin A, Vance JM, Ito A, Yao TP (2003) Cell 115:727
30. Riggs MG, Whittaker RG, Neumann JR, Ingram VM (1977) Nature 268:462
31. Cousens LS, Gallwitz D, Alberts BM (1979) J Biol Chem 254:1716
32. Boffa LC, Vidali G, Mann RS, Allfrey VG (1978) J Biol Chem 253:3364
33. Sealy L, Chalkley R (1978) Cell 14:115
34. Candido EPM, Reeves R, Davie JR (1978) Cell 14:105
35. Tsuji N, Kobayashi M, Nagashima K, Wakisaka Y, Koizumi K (1976) J Antibiot 29:1
36. Yoshida M, Kijima M, Akita M, Beppu T (1990) J Biol Chem 265:17174
37. Yoshida M, Nomura S, Beppu T (1987) Cancer Res 47:3688
38. Kijima M, Yoshida M, Sugita K, Horinouchi S, Beppu T (1993) J Biol Chem 268:22429
39. Ueda H, Nakajima H, Hori Y, Fujita T, Nishimura M, Goto T, Okuhara M (1994) J Antibiot 47:301
40. Shigematsu N, Ueda H, Takase S, Tanaka H (1994) J Antibiot 47:311
41. Ueda H, Manda T, Matsumoto S, Mukumoto S, Nishigaki F, Kawamura I, Shimomura K (1994) J Antibiot 47:315
42. Nakajima H, Kim YB, Terano H, Yoshida M, Horinouchi S (1998) Exp Cell Res 241:126
43. Furumai R, Matsuyama A, Kobashi N, Lee K-H, Nishiyama M, Nakajima H, Tanaka A, Komatsu Y, Nishino N, Yoshida M, Horinouchi S (2002) Cancer Res 62:4916
44. Taunton J, Hassig CA, Schreiber SL (1996) Science 272:408
45. Richon VM, Webb Y, Merger R, Sheppard T, Jursic B, Ngo L, Civoli F, Breslow R, Rifkind RA, Marks PA (1996) Proc Natl Acad Sci USA 93:5705
46. Richon VM, Emiliani S, Verdin E, Webb Y, Breslow R, Rifkind RA, Marks PA (1998) Proc Natl Acad Sci USA 95:3003
47. Hassig CA, Tong JK, Fleischer TC, Owa T, Grable PG, Ayer DE, Schreiber SL (1998) Proc Natl Acad Sci USA 95:3519
48. Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, Marks PA, Breslow R, Pavletich NP (1999) Nature 401:188
49. Closse A, Huguenin R (1974) Helv Chim Acta 57:533
50. De Schepper S, Bruwiere H, Verhulst T, Steller U, Andries L, Wouters W, Janicot M, Arts J, Van Heusden J (2003) J Pharm Exp Ther 304:881
51. Furumai R, Komatsu Y, Nishino N, Khochbin S, Yoshida M, Horinouchi S (2001) Proc Natl Acad Sci USA 98:87
52. Nishino N, Jose B, Shinta R, Kato T, Komatsu Y, Yoshida M (2004) Bioorg Med Chem 12:5777

53. Nishino N, Yoshikawa D, Watanabe LA, Kato T, Jose B, Komatsu Y, Sumida Y, Yoshida M (2004) Bioorg Med Chem Lett 14:2427
54. Darkin-Rattray SJ, Gurnett AM, Myers RW, Dulski PM, Crumley KM, Allocco JJ, Cannova C, Meinke PT, Colletti SL, Bednarek MA, Singh SB, Goetz MA, Dombrowski AW, Polishook JD, Schimatz DM (1996) Proc Natl Acad Sci USA 93:13143
55. Nishino N, Jose B, Okamura S, Ebisusaki S, Kato T, Sumida Y, Yoshida M (2003) Org Lett 5:5079
56. Mulder GJ, Meerman JH (1983) Environ Health Perspect 49:27
57. Suzuki T, Kouketsu A, Matsuura A, Kohara A, Nimomiya S-I, Kohda K, Miyata N (2004) Bioorg Med Chem Lett 14:3313
58. Frey RR, Wada CK, Garland RB, Curtin ML, Michaelides MR, Li J, Pease LJ, Glaser KB, Marcotte PA, Bouska JJ, Murphy SS, Davidsen SK (2002) Bioorg Med Chem Lett 12:3443
59. Walter MW, Felici A, Galleni M, Soto RP, Adlington RM, Baldwin JE, Frère J-M, Golobov M, Schofield CJ (1996) Bioorg Med Chem Lett 6:2455
60. Yoshida M, Kijima M, Akita M, Beppu T (1990) J Biol Chem 265:17174
61. Vasudevan A, Ji Z, Frey RR, Wada CK, Steinman D, Heyman HR, Guo Y, Curtin ML, Guo J, Li J, Pease L, Glaser KB, Marcotte PA, Bouska JJ, Davidsen SK, Michaelides MR (2003) Bioorg Med Chem Lett 13:3909
62. Saito A, Yamashita T, Mariko Y, Nosaka Y, Tsuchiya K, Ando T, Suzuki T, Tsuruo T, Nakanishi O (1999) Proc Natl Acad Sci USA 96:4592
63. Suzuki T, Ando T, Tsuchiya K, Fukazawa N, Saito A, Mariko Y, Yamashita T, Nakanishi O (1999) J Med Chem 42:3001
64. el-Beltagi HM, Martens AC, Lelieveld P, Haroun EA, Hagenbeek A (1993) Cancer Res 53:3008
65. Kraker AJ, Mizzen CA, Hartl BG, Miin J, Allis CD, Merriman RL (2003) Mol Cancer Ther 2:401
66. Loprevite M, Tiseo M, Grossi F, Scolaro T, Semino C, Pandolfi A, Favoni R, Ardizzoni A (2005) Oncol Res 15:39
67. Lelieveld P, Middeldorp RJF, van Putten LM (1985) Cancer Chemother Pharmacol 15:88
68. Acharya MR, Sparreboom A, Venitz J, Figg WD (2005) Mol Pharmacol 68:917
69. Kouraklis G, Theocharis S (2006) Oncol Rep 15:489
70. Curtin M, Glaser K (2003) Curr Med Chem 10:2373
71. Hu E, Dul E, Sung C-M, Chen Z, Kirkpatrick R, Zhang G-F, Johanson K, Liu R, Lago A, Hofmann G, Macarron R, de los Frailes M, Perez P, Krawiec J, Winkler J, Jaye M (2003) J Pharmacol Exp Ther 307:720
72. Glaser KB, Li J, Pease LJ, Staver MJ, Marcotte PA, Guo J, Frey RR, Garland RB, Heyman HR, Wada CK, Vasudevan A, Michaelides MR, Davidsen SK, Curtin ML (2004) Biochem Biophys Res Comm 325:683
73. Dokmanovic M, Marks PA (2005) J Cell Biochem 96:293
74. Glaser KB, Staver MJ, Waring JF, Stender J, Ulrich RG, Davidsen SK (2003) Mol Cancer Ther 2:151
75. Delorme D, Ruel R, Lavoie R, Thibault C, Abou-Khalil E WO 01/38322 A1 (2001) Chem Abstr 135:5455
76. Lavoie R, Bouchain G, Frechette S, Woo SH, Khalil EA, Leit S, Fournel M, Yan PT, Trachy-Bourget M-C, Beaulieu C, Li Z, Besterman J, Delorme D (2001) Bioorg Med Chem Lett 11:2847

77. Bouchain G, Leit S, Frechette S, Abou Khalil E, Lavoie R, Moradei O, Hyung Woo S, Fournel M, Yan PT, Kalita A, Trachy-Bourget M-C, Beaulieu C, Li Z, Robert M-F, Robert MacLeod A, Besterman JM, Delorme D (2003) J Med Chem 46:820
78. Finn PW, Bandara M, Butcher C, Finn A, Hollinshead R, Khan N, Law N, Murthy S, Romero R, Watkins C, Andrianov V, Bokaldere RM, Dikovska K, Gailite V, Loza E, Piskunova I, Starchenkov I, Vorona M, Kalvinch I (2005) Helv Chim Acta 88:1630
79. Plumb JA, Finn PW, Williams RJ, Bandara MJ, Romero MR, Watkins CJ, La Thangue NB, Brown R (2003) Mol Cancer Ther 2:721
80. Plumb JA, Steele N, Evans TRJ, Finn PW, Jensen PB, Kristeleit R, de Bono JS, Brown R (2004) EORTC-NCI-AACR Int Conf on Molecular Targets and Cancer Therapeutics, September 28–October 1, Geneva
81. Uesato S, Kitagawa M, Nagaoka Y, Maeda T, Kuwajima H, Yamori T (2002) Bioorg Med Chem Lett 12:1347
82. Mori H, Sakamoto K, Tsurumi Y, Takase S, Hino M (2000) WO 00/21979
83. Arts J, de Schepper S, Van Emelen K (2003) Curr Med Chem 10:2343
84. Angibaud P, Arts J, Van Emelen K, Poncelet V, Pilatte I, Roux B, Van Brandt S, Verdonck M, De Winter H, Ten Holte P, Marien A, Floren W, Janssens B, Van Dun J, Aerts A, Van Gompel J, Gaurrand S, Queguiner L, Argoullon J-M, Van Hijfte L, Freyne E, Janicot M (2005) Eur J Med Chem 40:597
85. Dai Y, Guo Y, Guo J, Pease LJ, Li J, Marcotte PA, Glaser KB, Tapang P, Albert DH, Richardson PL, Davidsen SK, Michaelides MR (2003) Bioorg Med Chem Lett 13:1897
86. Remiszewski SW, Sambucetti LC, Bair KW, Bontempo J, Cesarz D, Chandramouli N, Chen R, Cheung M, Cornell-Kennon S, Dean K, Diamantidis G, France D, Green MA, Lulu Howell K, Kashi R, Kwon P, Lassota P, Martin MS, Mou Y, Perez LB, Sharma S, Smith T, Sorensen E, Taplin F, Trogani N, Versace R, Walker H, Weltchek-Engler S, Wood A, Wu A, Atadja P (2003) J Med Chem 46:4609
87. Dai Y, Guo Y, Curting ML, Li J, Pease LJ, Guo J, Marcotte PA, Glaser Kb, Davidsen SK, Michaelides MR (2003) Bioorg Med Chem Lett 13:3817
88. Verdin E, Dequiedt F, Kasler H (2003) Trends Genet 19:286
89. Vega RB, Matsuda K, Oh J, Barbosa AC, Yang X, Meadows E, McAnally J, Pomajzl C, Shelton JM, Richardson JA, Karsenty G, Olson EN (2004) Cell 119:555
90. Lu J, McKinsey TA, Zhang CL, Olson EN (2000) Mol Cell 6:233
91. Zhang CL, McKinsey TA, Chang S, Antos CL, Hill JA, Olson EN (2002) Cell 110:479
92. Antos CL, McKinsey TA, Dreitz M, Hollingsworth LM, Zhang CL, Schreiber K, Rindt H, Gorczynski RJ, Olson EN (2003) J Biol Chem 278:28930
93. Sternson SM, Wong JC, Grozinger CM, Schreiber SL (2001) Org Lett 3:4239
94. Haggarty SJ, Koeller KM, Wong JC, Butcher RA, Schreiber SL (2003) Chem Biol 10:383
95. Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, Yoshida M, Wang XF, Yao TP (2002) Nature 417:455
96. Mai A, Massa S, Cerbara I, Valente S, Ragno R, Bottoni P, Scatena R, Loidl P, Brosch G (2004) J Med Chem 47:1098
97. Mai A, Massa S, Ragno R, Cerbara I, Jesacher F, Loidl P, Brosch G (2003) J Med Chem 46:512
98. Mai A, Massa S, Valente S, Simeoni S, Ragno R, Bottoni P, Scatena R, Brosch G (2006) ChemMedChem 1:225
99. Mai A, Massa S, Pezzi R, Simeoni S, Rotili D, Nebbioso A, Scognamiglio A, Altucci L, Loidl P, Brosch G (2005) J Med Chem 48:3344
100. Wang D-F, Helquist P, Wiech NL, Wiest O (2005) J Med Chem 48:6936

101. Carducci M, Siu LL, Sullivan R, Maclean M, Kalita A, Chen EX, Pili R, Martell RE, Besterman J, Reid GK (2006) J Clin Oncol ASCO Annual Meeting Proc Part I. Vol 24, No. 18S (June 20 Supplement):3007
102. Kelly WK, Richon VM, O'Connor O, Curley T, MacGregor-Curtelli B, Tong W, Klang M, Schwartz L, Richardson S, Rosa E, Drobnjak M, Cordon-Dordo C, Chiao HJ, Rifkind R, Marks PA, Scher H (2003) Clin Cancer Res 9:3578
103. Kelly WK, O'Connor O, Krug LM, Chiao JH, Heaney M, Curley T, MacGregore-Cortelli B, Tong W, Secrist JP, Schwartz L, Richardson S, Chu E, Olgac S, Marks PA, Scher H, Richon VM (2005) J Clin Oncol 23:3923
104. O'Connor OA, Heaney ML, Schwartz L, Richardson S, Willim R, MacGregor-Cortelli B, Curly T, Moskowitz C, Portlock C, Horwitz S, Zelenetz AD, Frankel S, Richon V, Marks P, Kelly WK (2006) J Clin Oncol 24:166
105. Heaney M, O'Connor O, Richon V, et al. (2003) Proc Am Soc Clin Oncol 577:2321
106. Rowinsky EK, Pacey S, Patnaik A, et al. (2004) Proc Am Soc Clin Oncol 22:200s
107. Ottmann OG, Deangelo DJ, Stone RM, et al. (2004) Proc Am Soc Clin Oncol 22:201s
108. Beck J, Fischer T, Rowinsky E, et al. (2004) Proc Am Soc Clin Oncol 22:201s
109. Beck J, Fischer T, George D, Huber C, Calvo E, Atadja P, Peng B, Kwong C, Sharma S, Patnaik A (2005) J Clin Oncol (Meeting Abstracts) 23:3148
110. Kristeleit RS, Tandy D, Atadja P, et al. (2004) Proc Am Soc Clin Oncol 22:200s
111. Rowinsky EK, de Bono JS, Deangelo DJ, van Oosterom A, Morganroth J, Laird GH, Dugan M, Scott JW, Ottmann OG (2005) J Clin Oncol (Meeting Abstracts) 23: 3131
112. Fischer T, Patnaik A, Bhalla K, Beck J, Morganroth J, Laird GH, Sharma S, Scott JW, Dugan M, Giles F (2005) J Clin Oncol (Meeting Abstracts) 23:3106
113. Fischer T, Patnaik A, Bhalla K, Beck J, Morganroth J, Laird GH, Sharma S, Scott JW, Dugan M, Giles F (2005) J Clin Oncol, 2005 ASCO Annual Meeting Proc. Vol 23, No 16S (June 1 Supplement):3106
114. Garcia-Manero G, Yang H, Sanchez-Gonzalez B, Verstovsek S, Ferrajoli A, Keating M, Andreeff M, O'Brien S, Cortes J, Wierda W, Faderl S, Koller C, Davis J, Morris G, Issa J-P, Frankel SR, Richon V, Fine B, Kantarjian H (2005) Blood (ASH Annual Meeting Abstracts)106:2801
115. Blumenschein G, Lu C, Kies M, Glisson B, Papadimitrakopoulou V, Zinner R, Kim E, Gillenwater A, Chiao J, Hong W (2004) J Clin Oncol (Meeting Abstracts) 22:5578
116. Duvic M, Tallpur R, Zhang C, Goy A, Richon V, Frankel SR (2005) J Clin Oncol (Meeting Abstracts) 23:577s (suppl, abstr 6571)
117. de Bono JS, Steele N, Vidal L et al. (2005) AACR-EORTC-NCI Int Conf on Molecular Targets and Cancer Therapeutics: Discovery, Biology, and Clinical Applications, Philadelphia, November 14–18, 220 (Abstract C88)
118. Gilbert J, Baker SD, Bowling MK, Grochow L, Figg WD, Zabelina Y, Donehower RC, Carducci MA (2001) Clin Cancer Res 7:2292
119. Carducci MA, Gilbert J, Bowling MK, Noe D, Eisenberger MA, Sinibaldi V, Zabelina Y, Chen T, Grochow LB, Donehower RC (2001) Clin Cancer Res 7:3047
120. Gore SD, Weng LJ, Figg WD, Zhai S, Donehower RC, Dover G, Grever MR, Griffin C, Grochow LB, Hawkins A, Burks K, Zabelena Y, Miller CB (2002) Clin Cancer Res 8:963
121. Patnaik A, Rowinsky EK, Villalona MA, Hammond LA, Britten CD, Siu LL, Goetz A, Felton SA, Burton S, Valone FH, Eckhardt SG (2002) Clin Cancer Res 8:2142
122. Reid T, Valone F, Lipera W, Irwin D, Paroly W, Natale R, Sreedharan S, Keer H, Lum B, Scappaticci F, Bhatnagar A (2004) Lung Cancer 45:381

123. Siu L, Carducci M, Pearce L, et al. (2005) AACR-EORTC-NCI Int Conf on Molecular Targets and Cancer Therapeutics: Discovery, Biology, and Clinical Applications, Philadelphia, November 14–18, 217 (Abstract C77)
124. Sandor V, Bakke S, Robey RW, Kang MH, Blagosklonny MV, Bender J, Brooks R, Piekarz RL, Tucker E, Figg WD, Chan KK, Goldspiel B, Fojo AT, Balcerzak SP, Bates SE (2002) Clin Cancer Res 8:718
125. Marshall JL, Rizvi R, Kauh J, Dahut W, Figuera M, Kang MH, Figg WD, Wainer I, Chaissang C, Li MZ, Hawkins MJ (2002) J Exp Ther Oncol 2:325
126. Piekarz RL, Frye AR, Wright JJ, Steinberg SM, Liewehr DJ, Rosing DR, Sachdev V, Fojo T, Bates SE (2006) Clin Cancer Res 12:3762
127. Piekarz RL, Frye R, Turner M, Wright J, Leonard J, Allen S, Smith S, Kischbaum M, Zain J, Bates SE for all collaborators (2005) J Clin Oncol (Meeting Abstracts) 23:3061
128. Stadler WM, Swerdloff JN, Margolin K, McCulloch B, Thompson J (2005) J Clin Oncol (Meeting Abstracts) 23:420s (suppl, abstr 4669)
129. Molife R, Patterson S, Riggs C, et al. (2006) Proc Am Soc Clin Oncol Prostate Cancer Symposium:217a
130. Whitehead R, McCoy S, Wollner I et al. (2006) Proc Am Soc Clin Oncol Gastrointestinal Cancers Symposium:255a
131. Odenike OM, Alkan S, Sher D, Godwin JE, Huo D, Myers M, Brandt SJ, Zhang Y, Vesole DH, Larson RA, Stock W (2004) Blood (ASH Annual Meeting Abstracts) 104:264
132. Niesvizky R, Ely S, DiLiberto M, Cho HJ, Gelbshtein UY, Jayabalan DS, Aggarwal S, Gabrilove JL, Pearse RN, Pekle K, Zafar F, Goldberg Z, Leonard JP, Wright JJ, Chen-Kiang S, Coleman M (2005) Blood (ASH Annual Meeting Abstracts) 106:2574
133. Ryan QC, Headlee D, Acharya M, Sparreboom A, Trepel JB, Ye J, Figg WD, Hwang K, Chung EJ, Murgo A, Melillo G, Elsayed Y, Monga M, Kalnitskiy M, Zwiebel J, Sausville EA (2005) J Clin Oncol 23:3912
134. Donovan EA, Ryan Q, Acharya M, Cheng E, Trepel J, Maynard K, Sausville E, Murgo A, Melillo G, Conley B (2005) J Clin Oncol (Meeting Abstracts) 23:3094
135. Gore L, Holden SD, Basche M, et al. (2004) Proc Am Soc Clin Oncol 22:201s
136. Prakash S, Foster BJ, Meyer M, Wozniak A, Heilbrun LK, Flaherty L, Zalupski M, Radulovic L, Valdivieso M, LoRusso PM (2001) Invest New Drugs 19:1
137. Byrd JC, Maarcuccu G, Parthun MR, Xiao JJ, Klisovic RB, Moran M, Lin TS, Liu S, Sklenar AR, Davis ME, Lucas DM, Fischer B, Shank R, Tejaswi SL, Binkley P, Wright J, Chan KK, Grever MR (2005) Blood 105:959
138. Lindemann RK, Gabrielli B, Johnstone R (2004) Cell Cycle 3:779
139. Bug G, Ritter M, Wassmann B, Schoch C, Heinzel T, Schwarz K, Romanski A, Kramer OH, Kampfmann M, Hoelzer D, Neubauer A, Ruthardt M, Ottmann OG (2005) Cancer 104:2717
140. Pauer LR, Olivares J, Cunningham C, Williams A, Grove W, Kraker A, Olson S, Nemunaitis J (2004) Cancer Invest 22:886
141. Undevia SD, Kindler HL, Janisch L, Olson SC, Schilsky RL, Vogelzang NJ, Kimmel KA, Macek TA, Ratain MJ (2004) Ann Oncol 15:1705
142. Nemunaitis JJ, Orr D, Eager R, Cunningham CC, Williams A, Mennel R, Grove W, Olson S (2003) Cancer J 9:58
143. von Pawel J, Shepherd F, Gatzmeier U, et al. (2002) Proc Am Soc Clin Oncol 21:310a

Top Med Chem (2007) 1: 333–382
DOI 10.1007/7355_2006_009

Published online: 13 January 2007

Development of Angiogenesis Inhibitors to Vascular Endothelial Growth Factor Receptor 2 for Cancer Therapy

Keren Paz (✉) · Zhenping Zhu

Departments of Tumor Biology and Antibody Technology,
ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014, USA
keren.paz@imclone.com

1 Introduction . . . 335

2 Vascular Endothelial Growth Factor and Its Receptors . . . 335

3 Vascular Endothelial Growth Factor Receptor-2 . . . 337
3.1 Structure and Function of KDR . . . 337
3.2 KDR as a Target for Antiangiogenesis Therapy . . . 338
3.3 Neutralizing Antibodies Directed Against VEGFR2 . . . 339
3.3.1 DC101 . . . 339
3.3.2 IMC-1C11 . . . 340
3.3.3 IMC-1121B . . . 341
3.3.4 CDP791 . . . 341
3.4 Small Molecular Weight Tyrosine Kinase Inhibitors to KDR . . . 342
3.4.1 Semaxanib (SU5416), SU6668 and Sunitinib (SU11248) . . . 342
3.4.2 Vatalanib (PTK787) . . . 347
3.4.3 ZD4190, Vandetanib (ZD6474) and Cediranib (AZD2171) . . . 349
3.4.4 Neovastat (Ae941) (structure unknown) . . . 354
3.4.5 Pazopanib (GW786034) . . . 355
3.4.6 XL647 and XL999 (structure unavailable) . . . 356
3.4.7 Axitinib (AG13736) . . . 357
3.4.8 AEE788 . . . 359
3.4.9 BIBF1120 (structure unavailable) . . . 360
3.4.10 BAY57-9352 . . . 362
3.4.11 CHIR258 . . . 363
3.4.12 CEP7055 . . . 364
3.4.13 ZK304709 (structure unavailable) . . . 365
3.4.14 BMS582664 . . . 366
3.4.15 L21649 . . . 366
3.5 Additional Approaches . . . 367

4 Future Perspective . . . 368

References . . . 369

Abstract Angiogenesis, the recruitment of new blood vessels, is a crucial mechanism required for both tumor growth and metastasis. Advances in the understanding of the molecular mechanisms underlying the angiogenesis process have led to the discovery of a variety of pharmaceutical agents with antiangiogenic activity. The potential

application of these angiogenesis inhibitors is currently under intense clinical investigation. Decades of investigation suggest that vascular endothelial growth factor (VEGF) and its receptors, particularly VEGF receptor-2 (VEGFR-2, or kinase insert domain-containing receptor, KDR), play a critical role in tumor-associated angiogenesis. VEGFR-2, therefore, represents a good target for therapeutic intervention. A number of agents designed selectively for targeting VEGFR-2 are being evaluated in various phases of clinical trials in cancer patients. This manuscript reviews the biology of the VEGF family of ligands and receptors and of VEGFR-2 in particular. The attempts to develop effective VEGFR-2 antagonists, including small molecules, antibodies and others, for therapeutic purposes are discussed comprehensively with special emphasis on tumor angiogenesis.

Keywords Angiogenesis · Angiogenesis inhibitor · VEGF · VEGFR · KDR · Tyrosine kinase inhibitors · Antibody · Cancer therapy

Abbreviations

AACR	American Association of Cancer Research
AML	Acute myeloid leukemia
ASH	American Society of Hematology
CRT	Concomitant chemo radiotherapy
DLT	Dose-limiting toxicity
EGFR	Epidermal growth factor receptor
EORTC	European Organization for Research and Treatment of Cancer
Flt1	Fms-like tyrosine kinase 1
Flt4	Fms-like tyrosine kinase 4
Flk1	Fetal liver kinase
GIST	Gastrointestinal stromal tumor
HCC	Hepatocellular carcinoma
HUVEC	Human umbilical vein endothelial cells
IC	Induction chemotherapy
KDR	Kinase insert domain receptor
MAA	Marketing authorization application
MM	Multiple myeloma
MMM	Myelofibrosis with myeloid metaplasia
MTD	Maximal tolerated dose
NCI	National Cancer Institute
NDA	New drug application
NSCLC	Non-small-cell lung cancer
PD	Pharmacodynamics
PlGF	Placenta growth factor
PK	Pharmacokinetics
RCC	Renal cell carcinoma
RTK	Receptor tyrosine kinase
TKI	Tyrosine kinase inhibitor
VEGFR	Vascular endothelial growth factor receptor
VPF	Vascular permeability factor
VRAP	VEGFR-associated protein

1 Introduction

Angiogenesis, the recruitment of new blood vessels, is a crucial mechanism required for a number of physiological and pathological conditions. It is a tightly regulated, multiple step process, that results in the formation of blood vessels from pre-existing vasculature [1]. Under normal conditions, angiogenesis occurs during embryonic development, wound healing, and the female menstruation cycle [2–5]. Uncontrolled angiogenesis is observed in various pathological states, such as psoriasis, diabetic retinopathy, rheumatoid arthritis, chronic inflammation, and cancer [6–16]. Tumor cells begin to promote angiogenesis early in tumorigenesis to allow proper nourishment and removal of metabolic wastes from tumor site. In contrast to normal cells, which form a single layer around capillary blood vessels, multiple layers of tumor cells surround the microvasculature, effectively creating a capillary "cuff" [17–19]. Although these in situ tumors may replicate rapidly, their uncontrolled growth and metastatic properties are severely restricted by the absence of adequate blood supply. Tumor cells, therefore, go through a switch from a quiescent to an invasive phenotype. This "switch" is invariably accompanied by the acquisition of angiogenic properties and is considered the hallmark of the malignant process, whereby pro-angiogenic mechanisms overwhelm or circumvent negative regulators of angiogenesis [20]. Indeed, increased tumor vascularization and expression of pro-angiogenic factors has been associated with advanced tumor stage and poor prognosis in a variety of human cancers [18, 21–24]. Decades of investigating the molecular basis of angiogenesis have identified a number of growth factor receptor pathways that contribute to promotion of tumor angiogenesis. One, and probably the major pathway involved in this process, is the vascular endothelial growth factor (VEGF) family [25–29]. This manuscript reviews the biology of VEGF family of ligands and receptors and of VEGF receptor-2 (VEGFR-2, or kinase insert domain-containing receptor, KDR), in particular. The attempts to develop effective KDR antagonists for therapeutic purposes are discussed comprehensively with special emphasis on tumor angiogenesis.

2 Vascular Endothelial Growth Factor and Its Receptors

VEGF is the prototype of the enlarging family of angiogenic and lymphangiogenic growth factors. The family is comprised of six structurally homologous, secreted glycoproteins. These proteins share a great similarity in their primary sequence [30–32]. VEGF-A (also known as VEGF) was first identified in the 1980s as a vascular permeability factor (VPF) secreted by tu-

mor cells [33–37]. Its gene undergoes alternative splicing to yield at least six different mature isoforms of 121, 145, 165, 183, 189, and 206 amino acids [36, 38, 39]. These isoforms vary in their bioavailability, level of expression, affinity to heparin and heparan sulfate, mitogenic strength, and tissue specificity. $VEGF_{121}$ and $VEGF_{165}$ are the most abundant forms [38, 40–42]. Placenta growth factor (PlGF) shares 46% amino acid identity with VEGF and is predominantly expressed in the placenta [43]. VEGF-B is 43% identical to VEGF and is highly expressed in skeletal and cardiac tissue [44, 45]. VEGF-C exhibits approximately 30% identity to VEGF and is a fairly selective growth factor for lymphatic vessels [27, 46]. VEGF-D is most closely related to VEGF-C [47, 48] with 31% identity to VEGF [49–52]. Both VEGF-C and VEGF-D have been shown to be endothelial cells mitogens [47, 48, 53]. Two additional VEGF-related polypeptides were identified in the genome of the *Orf* virus [54]. These polypeptides, NZ-7 VEGF (designated VEGF-E) and ORFV2-VEGF share 25% and 43% amino acid identity with VEGF, respectively [55–58]. The active forms of the VEGF family of ligands appear either as homodimers (40–45 kDa) or as heterodimers with other VEGF family members [32, 59].

VEGF ligands initiate their biological function upon binding to structurally related cell surface receptors [32, 59, 60]. Two receptors were originally identified on endothelial cells, the 180 kDa Fms-like tyrosine kinase (Flt1 or VEGFR1) [58, 61, 62] and the 200 kDa KDR (or VEGFR2), or its murine homolog, fetal liver kinase (Flk1) [63–70]. The overall amino acid sequence identity between Flt1 and KDR is 44%. KDR binds VEGF, VEGF-C, VEGF-D, VEGF-E, and ORFV2-VEGF, whereas Flt1 binds VEGF, VEGF-B, and PlGF. Both Flt1 and KDR are expressed primarily on vascular cells of endothelial lineage [58, 62–64, 71–73]. A third structurally related tyrosine kinase receptor is the 180 kDa Flt4 (or VEGFR3) [74–79]. Flt4 binds VEGF-C and VEGF-D [49, 80]. Similarly to KDR, Flt4 is widely expressed on endothelial cells during early embryonic development. However, Flt4 becomes largely confined to lymphatic endothelial cells in the adult tissues [74, 76–79, 81, 82].

Two additional receptors were recently identified, 130–140 kDa isoforms neuropilin-1 (NRP-1) and NRP-2 [83–85]. NRP-1 binds $VEGF_{165}$, PlGF-2, and ORFV2-VEGF [83, 86, 87]. NRP-2 binds $VEGF_{165}$, $VEGF_{145}$, PlGF, and VEGF-C [88, 89]. NRP-1 and NPR-2 differ greatly from other VEGF receptor family members. Their intracellular domain is short, and does not suffice for independent transduction of biological signals [90–94]. Their activity is likely mediated as a co-receptor for VEGFR-1 and VEGFR-2 by enhancing the binding affinity of ligands to the receptors [90, 94–99].

3
Vascular Endothelial Growth Factor Receptor-2

3.1
Structure and Function of KDR

Kinase insert domain receptor (KDR) is expressed in all adult vascular endothelial cells with perhaps the exception of vascular endothelial cells in the brain [63]. In addition, KDR is detected on circulating endothelial progenitor cells (CEPs) [100–102], pancreatic duct cells [103], retinal progenitor cells [104], and megakaryocytes [105]. Significantly increased levels of KDR are also presented on tumors derived from kidney, bladder, ovaries, and brain [106–108]. KDR-deficient mice have impaired blood island formation and lack mature endothelial cells [109–111]. Similar to Flt1, KDR possesses a characteristic structure consisting of seven extracellular immunoglobulin-like domains, a single transmembrane domain, and a tyrosine kinase domain interrupted by an insert [58, 62–64, 112]. Recent studies have provided direct evidence that two molecules of either KDR or Flt1 bind a single VEGF homodimer [41, 113–118]. Deletion mutant analysis demonstrates that KDR extracellular immunoglobulin-like domains 2 and 3 are sufficient for high affinity binding of VEGF [114, 115]. Detailed analysis of the interaction between VEGF and various KDR immunoglobulin-like domain deletion mutants suggests that the domains 2–4 might be important for VEGF association, and domains 5 and 6 are important for ligand dissociation [117]. The presence of a split kinase domain places both KDR and Flt1 into the same subfamily of class III receptor tyrosine kinases (RTKs), which also includes several 5-immunoglobulin-like domain type receptors such as c-Fms, c-Kit, and the alpha and beta chains of the PDGF receptor. VEGF binding induces conformational changes within KDR, followed by receptor dimerization and autophosphorylation of tyrosine residues in the intracellular kinase domain [119–121]. The use of recombinant KDR cytosolic domain enabled the identification of four tyrosine residues, Tyr-951, Tyr-996, Tyr-1054, and Tyr-1059, as the autophosphorylation sites [122]. Tyrosine phosphorylation forms high-affinity binding sites for a variety of SH2 and PTB domain-containing proteins, including PLCγ, VEGFR-associated protein (VRAP), Ras-GAP, FAK, Sck, Src family of tyrosine kinases, Grb2, PI3-kinase, Akt, PKC, Raf-1, MEK, ERK, p38MAPK, Nck, Crk, Shc, STAT3, and others [113, 119, 122–141]. These proteins either possess an intrinsic enzymatic activity, or serve as docking proteins to position other signaling molecules in close proximity with the receptor, to further propagate the VEGF signal [123–126].

The role of KDR in endothelial cells has been extensively studied [111]. It is suggested that interaction with KDR is a critical requirement to induce VEGF biological responses, which include cell proliferation, migration, differentiation, tube formation, increase of vascular permeability, and maintenance

of vascular integrity [119–121, 142–144]. However, the key molecules involved in VEGF/KDR signaling pathway remain to be completely elucidated. The identification of downstream signaling molecules may provide clues to the biochemical mechanisms used to transmit VEGF activity during angiogenesis and, therefore, guide the rational design of potent antiangiogenesis inhibitors [144].

3.2 KDR as a Target for Antiangiogenesis Therapy

Various angiogenesis inhibitors have been developed to target vascular endothelial cells and block tumor angiogenesis. Compelling evidence suggests that VEGF and its receptors, Flt1 and KDR, provide excellent targets for antiangiogenesis intervention. Although there are many molecules that have been proven to be endothelial growth factors, VEGF is the one most consistently found in a wide variety of conditions associated with angiogenesis. VEGF and its receptors are overexpressed in the great majority of clinically important human cancers. These include carcinomas of the gastrointestinal tract, pancreas, breast, bladder, kidney, endometrium, and Kaposi's sarcoma [24, 106, 145–157]. In addition, overexpression of the VEGF receptors was demonstrated among several intracranial tumors including glioblastoma multiforme [155], as well as in both sporadic and Hippel–Lindau syndrome-associated capillary hemangioblastoma [158]. The mRNA for both KDR and Flt1 is greatly upregulated in tumor-associated endothelial cells, but not in the vasculature surrounding normal tissues [147, 149, 155]. Furthermore, a significant correlation between KDR expression and microvessel density has been observed in several tumors. This increased microvessel density appears to be associated with poor prognosis in patients with a wide spectrum of cancers, including carcinomas of breast, bladder, prostate, ovarian, colorectal, stomach, head and neck, non-small-cell lung, and uterine cervix, as well as melanomas, testicular germ cell, and pediatric brain tumors [159–172]. A retrovirus-mediated expression of a dominant negative Flk1 (mouse KDR analog) mutant inhibited the growth of eight of nine tumor cell lines tested in nude mice, along with significant reduction of vessel density in the tumors [173]. Furthermore, inhibition of endothelial cell mitogenesis in vitro and tumor growth in vivo have also been achieved by using anti-KDR/Flk1 kinase antibodies [174–179] and small molecule KDR/Flk1 inhibitors [180–187]. Additionally, accumulating evidence suggests the existence of a VEGF/KDR autocrine loop in mediating growth and metastasis of several types of tumors [157, 188–190]. Treatment with a neutralizing anti-KDR antibody effectively inhibited VEGF activities both in vitro and in vivo [24, 179, 191–193]. KDR inhibitors also have greater accessibility to their targets since tumor vessel endothelium is in direct contact with the blood. In contrast to conventional therapies that require targeting individual tumor

Table 1 Summary of anti-KDR antibodies and KDR-selective TKI currently in clinical development

Drug	Category	Company	Stage
IMC-1C11	Chimeric antibody	ImClone Systems	Phase I
IMC-1121b	Human antibody	ImClone Systems	Phase I
CDP-791	Antibody fragment	Cerlltech Group	Phase II
Sunitinib (SU11248)	TKI	SUGEN	Launched
Vatalanib (PTK787)	TKI	Novartis	Phase III
Vandetanib (ZD6474)	TKI	AstraZeneca	Phase III
Cediranib (AZD2171)	TKI	AstraZeneca	Phase III
Neovastat (Ae941)	Natural inhibitor	AEterna Zentaris Inc.	Phase III
Pazopanib (GW786034)	TKI	GlaxoSmithKline	Phase III
SU6668	TKI	SUGEN	Phase II
XL647 (EXEL647)	TKI	Exelixis	Phase II
XL999 (EXEL999)	TKI	Exelixis	Phase II
Axitinib (AG13736)	TKI	Pfizer	Phase II
AEE788	TKI	Novartis	Phase II
BIBF1120	TKI	Boehringer	Phase II
Bay57-9352	TKI	Bayer Yakuhin	Phase I
CHIR258 (GFKI258)	TKI	Chiron	Phase I
CEP7055	TKI	Cephalon	Phase I
ZK304709 (ZK-CDK)	TKI	Schering	Phase I
BMS582664	TKI	BMS	Phase I
L000021649	TKI	Merck & Co	Phase I

This list is complied from information obtained via a variety of sources including research articles, reviews, meeting reports, conference proceedings and abstracts, company websites, and press releases

cells, local interruption of tumor vasculature by targeting KDR expressed on endothelial cells may detrimentally affect all tumor cells that are dependent upon the targeted vasculature for nutriment. Taken together, it is not surprising that KDR has become one of the most sought-after antiangiogenesis targets being pursued by various pharmaceutical and biotech companies in the recent years (Table 1).

3.3 Neutralizing Antibodies Directed Against VEGFR2

3.3.1 DC101

A rat anti-mouse VEGFR2 (Flk1) monoclonal antibody (DC101) was developed by ImClone Systems (New York, NY, www.imclone.com) using conventional hybridoma technique [194] to conduct proof-of-concept studies.

DC101 has been studied extensively in mouse models of angiogenesis, mouse tumors, and human tumor xenografts, demonstrating potent antiangiogenic and antitumor activity in these models [195], for review; see [196–198]. In addition, DC101 treatment inhibited the dissemination and growth of metastases in mouse and human tumor metastasis models. Histological examination of DC101-treated tumors showed evidence of decreased microvessel density, reduced tumor cell proliferation along with increased tumor cell apoptosis and extensive tumor necrosis. Further, DC101 showed synergistic or additive antitumor activities when combined with chemotherapeutic drugs or radiation [199–201] and in some cases, led to significant regression of implanted tumors. No overt toxicity has been observed in long-term DC101 treatment experiments of tumor-bearing or non-tumor-bearing mice. These findings are important, since low levels of Flk1 expression are present on the endothelium of some normal tissues and are required for normal angiogenic processes. Indeed, DC101 treatment did have an impact on normal angiogenesis associated with reproduction [202, 203] and bone formation (ImClone Systems, unpublished data). The lack of toxicity observed during DC101 therapy may be due to the limited dependence of resting endothelium for Flk1 stimulation. In contrast, tumor angiogenesis is expected to be more dependent on upregulation and function of Flk1 on tumor vasculature and thus more susceptible to anti-Flk1 blockade. The apparent lack of toxicity associated with anti-Flk1 antibody treatment can also be attributed to the high specificity of an antibody antagonist.

3.3.2 IMC-1C11

As DC101 does not cross-react with human VEGFR2 (KDR), a panel of new mAb directed against the human receptor was generated, using both the traditional hybridoma method and the antibody phage display technique [197, 198, 204]. This effort gave rise to a lead candidate, IMC-1C11 [205], a mouse/human chimeric IgG1 derived from a scFv isolated from a phage display library [206, 207]. Cross-species examination revealed that IMC-1C11 cross-reacts with VEGFR2 expressed on endothelial cells of monkeys and dogs, but not with those on rat and mouse. In a canine retinopathy model, IMC-1C11 significantly inhibited retinal neovascularization in newborn dogs induced by high concentration of oxygen [208]. Furthermore, administration of IMC-1C11 to primate rhesus monkey demonstrated a significant impact on the ovary follicle development during the menstrual cycles, an angiogenesis-related event [202]. Recently, it was shown that certain human leukemia cells, including both primary and cultured cell lines, also express functional VEGFR2 on the cell surface [157, 188, 191]. IMC-1C11 strongly inhibited VEGF-stimulated leukemia cell proliferation and migration, and significantly prolonged the survival of NOD-SCID mice inoculated

with these cells [157, 191]. Since IMC-1C11 does not cross-react with mouse Flk1, the in vivo anti-leukemia effect of the antibody is likely due to a direct inhibition of cell growth via blockade of the VEGF/VEGFR2 autocrine loop in human leukemia cells. ImClone Systems initiated a dose-escalating phase I clinical trial in May 2000 in patients with liver metastatic colorectal cancer. When IMC-1C11 was infused at 0.2, 0.6, 2.0, and 4.0 mg/kg for 4 weeks, no serious toxicities were observed. Five out of a total of 14 enrolled patients had stable disease by week 4 and continued on therapy, with one patient maintaining SD for 6 months [177].

3.3.3 IMC-1121B

ImClone Systems is currently developing a fully human anti-VEGFR2 antibody for the treatment of solid tumors and certain leukemias [175, 176, 179]. This fully human anti-VEGFR2 IgG1 antibody, IMC-1121B, was generated from a Fab fragment originally isolated from a large antibody phage display library [175, 176]. The antibody specifically binds VEGFR2 with high affinity of 50 pM and blocks VEGF/VEGFR2 interaction with an IC50 value of approximately 1 nM. It strongly inhibited VEGF-induced migration of human leukemia cells in vitro, and when administered in vivo, significantly prolonged survival of NOD-SCID mice inoculated with $VEGFR2^+$ human leukemia cells [179]. Phase I clinical trials of IMC-1121B were initiated in January 2005 in patients with advanced malignancies. To date, a total of 14 patients have been enrolled in the study at dose levels ranging from 2 to 10 mg/kg every week. Preliminary results of the study suggest that IMC-1121B is well tolerated. One patient experienced partial response and five patients had disease stabilization.

3.3.4 CDP791

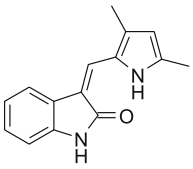
CDP791 is a pegylated F(ab′)2 antibody fragment directed against the KDR under codevelopment by Celltech/UCB (Slough, UK, www.ucb-group.com), and ImClone Systems as a potential treatment for cancer. CDP791 design and construction were based on a pegylation technology developed by Inhale(now Nektar Therapeutics, San Carlos, CA, www.nektar.com). Celltech initiated phase I clinical trials of CDP791 in patients with a variety of advanced solid tumors that had failed to respond to standard therapies in August 2003. The trial was designed to assess the safety of ascending doses of the drug (0.3–30 mg/kg once every 3 weeks) and its pharmacological activity for rapid proof-of-concept for such a regimen. Five disease stabilizations (two renal, one colon, one endometrial, and one melanoma) were achieved. Major toxicities included skin hemangiomatas, hypertension, and hypocal-

cemia. In March 2006, a phase II trial of the drug in combination with standard chemotherapy was initiated in patients with non-small-cell lung cancer (NSCLC).

3.4
Small Molecular Weight Tyrosine Kinase Inhibitors to KDR

An increasing number of small molecule tyrosine kinase inhibitors (TKI) to signal transduction pathways of KDR kinase domain are under various stages of development at several pharmaceutical companies [16, 29, 183, 185, 186, 196, 209–214]. This review summarizes the most advanced compounds in the field, which are currently in different stages of clinical trails. Noticeably, the vast majority of the information on these drugs is not yet available in published manuscripts. Nevertheless, due to the significance of such data for the purpose of further investigation and therapy consideration, information gathered from company web sites, press releases, proceeding of scientific and clinical meetings, and other sources is presented here as unpublished data.

3.4.1
Semaxanib (SU5416), SU6668 and Sunitinib (SU11248)

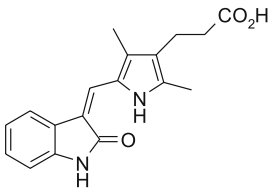


Semaxinib

Semaxanib (SU5416), an antiangiogenic agent developed by SUGEN (Ownership of SUGEN passed to Pfizer www.pfizer.com as part of its acquisition of Pharmacia in April 2003), was one of the most advanced agents in clinical development. Semaxanib is a KDR TK antagonist that exhibits inhibitory activities against PDGFR, Flt1 and Flt4 as well [21, 215]. Biochemical studies indicated that Semaxanib possesses ATP mimetic properties, and exerts its inhibitory effects on the signaling pathway of KDR/Flk1 in an ATP-competitive manner by localizing in the ATP binding pocket of the RTK [21, 216]. Semaxanib blocks VEGF-stimulated mitogenesis and migration of human endothelial cells, and induces apoptosis of endothelial cells. It inhibited the growth of a variety of xenograft tumors in mice, along with reducing tumor vascular density [21, 217–222]. Phase I studies of Semaxanib were carried out in 69 cancer patients with advanced diseases. The drug was given intravenous twice weekly at dose level of 4.4–190 mg/kg/day. Objective responses were

observed in three patients, seven remained on study for more than 6 months, and two for over 18 months [222–229].

Phase II and III trials were carried out, alone and in combination with standard chemotherapy regimens, in patients with cancers of colorectal, breast and lung, malignant mesothelioma, melanoma, acute myeloid leukemia (AML) and Kaposi's sarcoma. Major toxicities associated with Semaxanib have been dose-limiting toxicity (DLT) of projectile vomiting, grade III diarrhea, nausea, fatigue, headache, and pulmonary emboli [230–237]. In February 2002, SUGEN (owned by Pharmacia) made the decision to discontinue the drug based on interim results from phase III trials involving colorectal cancer patients. Analysis of the data showed that the study would not achieve the defined trial endpoints due to lack of clinical benefit. The company closed its phase III trials and discontinued development of Semaxanib for all indications [238].

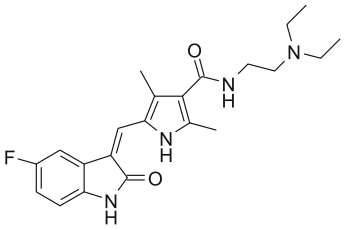


SU6668

SU6668 is a broader RTK inhibitor that targets KDR, PDGFR, and FGFR [215, 239–246]. SU6668 is structurally similar to SU5416 with better toxicity profiles and oral availability. It offers two different mechanisms of action of both antiangiogenic and antitumor effects, by affecting several targets simultaneously. SU6668 blocks recombinant KDR and FGFR kinase activity with IC50 values of 2.4 and 3 nM, respectively. SU6668 induced tumor inhibition or regression following oral administration to mice bearing a variety of tumor xenografts [239, 240, 247–250]. In a metastatic colorectal cancer model, SU6668 increased median survival of tumor-bearing mice by 58% and led to a time-dependent endothelial cell apoptosis and decrease in tumor volume [251]. In addition, pericyte vessel coverage and tumor vascularity were significantly decreased in SU6668-treated mice. Combination of SU6668 with Paclitaxel affects ascites formation and tumor spread in ovarian carcinoma xenograft growing orthotopically [252]. Furthermore, it was suggested that SU6668 sensitizes radiation via targeting survival pathways of vascular endothelium in Lewis lung carcinoma and GL261 xenografts, possibly through reducing the survival of tumor endothelium [246, 253].

In phase I studies, SU6668 was administrated orally once daily to 68 patients with advanced malignancies at dose levels between 100 and 2400 mg/kg/day [254]. No serious drug-related toxicities have been observed

but there were mild–moderate side effects included nausea, diarrhea and fatigue. Median time on study was 13 weeks (range 2–86 weeks), and no maximal tolerated dose (MTD) was reached. In a dose-escalation pharmacological study, SU6668 was administered at 100 or 200 mg/kg to 16 patients with advanced solid tumors. No significant toxicities were observed. SU6668 was extensively bound to plasma proteins. A three-times daily dose regime suggested an MTD of 100 mg/kg when administered with food. Half-life was 3.6 h. A dose of 300 mg/kg administered with food was well tolerated among 35 patients, with adverse effects including fatigue and joint pains. DLT was 400 and 800 mg/kg with grade III thrombocytopenia. Four patients had stable disease for more than 6 months. Phase I data were presented at the 39th ASCO meeting, June 2003. A group of 24 patients with advanced solid tumors were given between 200 and 500 mg/kg/day of SU6668 for 28 days. Grade I and II toxicities were edema, nausea, vomiting, fatigue, anorexia, and abdominal pain. One patient had grade IV pericardial effusion at the 400 mg dose. Plasma concentration was lower on day 28 than day 1. Among this group, ten patients achieved stable disease, but no objective responses were observed [245, 254]. SUGEN (Pharmacia) initiated a US phase II trial and a collaborative (Taiho) Japan phase I/II trial for SU6668 in February 2003 and June 2004, respectively. Data from this trial were presented at the 42nd ASCO meeting, in June 2006. The study involved 15 patients with hepatocellular carcinoma (HCC) who were dosed either 400 or 800 mg/day. The higher dose produced toxicities of grade III abdominal pain and ascites in two patients, while the lower dose did not induce any adverse events. One patient exhibited a partial response and another six had stable disease.



Sunitinib

Sunitinib (SU11248 or Sutent) [255–258] displays selectivity for members of the split kinase domain subgroup, KDR, PDGFR-alpha, PDGFR-beta, c-Kit, and Flt3, with in vitro IC50 values in the nanomolar range (14 nM) [185, 259–262]. In biological and cellular assays, Sunitinib competitively inhibited ligand-dependent KDR and PDGFR-beta autophosphorylation with IC50 values of 10nM [262–265]. In mouse xenograft models, Sunitinib inhibited the phosphorylation of PDGFR-beta, KDR and c-Kit time- and dose-dependently. Sunitinib demonstrated broad and potent antitumor activity,

including regression in murine models of human epidermal (A431), colon (Colo205 and HT-29), lung (NCI-H226 and H460), breast (MDA-MB-435), prostate (PC3-3M-luc), and renal (786-O) cancers, and suppressing or delaying the growth of many others, including the C6 rat and SF763T human glioma xenografts and B16 melanoma lung cancer. Tumor inhibition ranged from 11 to 93%, and was found to be dose-dependent between oral doses of 20 and 40 mg/kg/day. In mice bearing established A431 tumors, administration of 80 mg/kg/day of Sunitinib for 21 days resulted in a complete tumor regression in six of eight mice during the first round of treatment, and in the remaining two mice upon re-treatment. The tumors did not regrow for a duration of 110 days. In Colo205 tumors, Sunitinib treatment induced a dose- and time-dependent, rapid decrease in tumor microvessel density and tumor-cell proliferation, and an associated increase in tumor-cell apoptosis, culminating in tumor regression. In SF763T tumor models, on the other hand, Sunitinib decreased tumor vascularization and proliferation, with no overt tumor tissue destruction, culminating in tumor growth delay [262]. Sunitinib has also demonstrated synergy with both radiation therapy and chemotherapeutic drugs such as Docetaxel, Cisplatin, 5-FU or Doxorubicin in a number of in vitro and in vivo studies [256, 259, 262, 266–268].

Phase I and II clinical data confirm that orally administered Sunitinib is well absorbed and has a half-life of 40 h. The compound was well tolerated during preclinical studies and little toxicity was reported [266]. Sunitinib was shown to be effective as second-line therapy for patients with metastatic renal cell carcinoma (RCC) whose disease had progressed despite standard therapy. All patients were administered repeated cycles of 50 mg/day for 4 weeks followed by a 2-week rest period. Partial responses were observed in 33% patients while 37% had stable disease for over 3 months. At 6 months, 14 out of 63 of the patients were still under treatment with an ongoing partial response. The regimen induced mostly grade I/II toxicities, including fatigue, asthenia, nausea, and diarrhea. Grade III and IV toxicities included lymphopenia and elevated lipase and amylase, but no clinical signs of pancreatitis. Two patients were taken off the study for decreases in left ventricular ejection fraction greater than 20% without clinical symptoms. Data of a similar study were presented at the 41st ASCO meeting, May 2005 and at the fourth International Targeted Therapies for Cancer meeting, September 2005. In a second phase II trial, 106 patients with metastatic RCC were given 50 mg/day as well. An overall response rate was 40%, with 25 to 28% stable disease [257, 269, 270]

Data from a different phase II study in 107 metastatic RCC patients were presented at the 42nd ASCO meeting, June 2006. Patients were randomized to receive 37.5 mg Sunitinib once daily in the morning or the evening. Tumor shrinkage was reported in 33 patients. The continuous dose was well tolerated, with similar tolerability between the morning and evening arms. In 17 patients, the dose was reduced to 25 mg/day following grade II or III adverse

effects after an average of 6 weeks at 37.5 mg/day. Adverse effects, leading to dose reduction, included mucositis, thrombocytopenia, and nausea. Discontinuation was seen in 22 patients due to disease progression, adverse effects, and withdrawal of consent. A phase III study evaluating Sunitinib in patients with metastatic RCC is ongoing.

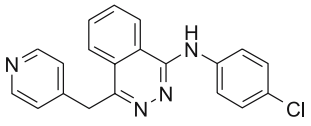
In a different trial, 18 patients with Imatinib (STI571 or Gleevec)-resistant gastrointestinal stromal tumors (GIST) received 25–75 mg/day for a duration of 2 weeks, followed by 2 weeks rest. This regimen resulted in two partial tumor responses and ten stable diseases, the longest being 6 months. Biopsy and imaging studies showed that 10 out of 17 evaluable patients had a reduction in metabolic activity of their tumors. The MTD was determined to be 50 mg, as two patients treated with 75 mg experienced transient DLT including fatigue, nausea, and vomiting during the first cycle. In a similar study, administration of 50 mg/day of Sunitinib for 4 weeks was followed by 2 weeks of rest. Analysis had been conducted in 48 progressing patients, 26 of whom showed a clinical benefit. Data of a subsequent trial were presented at the 42nd ASCO meeting, June 2006. Patients with advanced GIST received 37.5 mg Sunitinib daily in a 4-weeks on/2-weeks off schedule for 1 year. Median cycle number was 4.2. Preliminary efficacy data in 17 patients demonstrated a stable disease in 13 patients and progressive disease among four, with continuous dosing being well tolerated.

A randomized, double blind, placebo-controlled phase III study for the treatment of Imatinib-resistant GIST and RCC patients was initiated in July 2004. Adverse events reported include an 82-year-old man with grade III hypertension, developed the day after receiving a single 200 mg dose of Sunitinib. Another 68-year-old man developed a transient asymptomatic increase in cardiac ectopy with grade I ventricular tachycardia on his ECG the day he received a single 350 mg dose of the drug. Both patients recovered within 24 h [263, 271, 272].

AML patients were treated with repeated doses of 25 to 100 mg/day of Sunitinib for 2 weeks. Grade III fatigue was the DLT in two of 22 patients. In a 50 mg escalating-dose study, 20% patients experienced grade III fatigue. DLT of grade IV fatigue occurred in one patient at 75 mg. Other drug-related adverse events included nausea and vomiting, diarrhea, headache, altered blood counts, and lipase elevations. Most adverse events were rated grade I/II and were considered manageable. Patients with unresectable neuroendocrine tumors and metastatic breast cancer were treated with 50 mg/day Sunitinib for 4 weeks followed by a 2-week rest period. Preliminary results on the first 93 neuroendocrine patients demonstrated grade III/IV toxicities of diarrhea, fatigue, glossodynia, nausea, neutropenia, thrombocytopenia, and vomiting. Nine patients had partial response and 84 patients had stable disease. Among the breast cancer patients grade III toxicities were observed, including neutropenia, thrombocytopenia, and AST increase. Out of 23 evaluable patients, two had a partial response, five had stable disease, and 14 had progressive disease.

In January 2006, the FDA approved Sunitinib for the treatment of GIST and RCC, and it was launched in the USA later that month. In April 2006, the European committee for medical products for human use (CHMP) recommended approval of Sunitinib for second-line RCC and for GIST, and conditional approval in both indications was granted in July 2006. In May 2006, the drug was approved by Health Canada for the treatment of GIST.

3.4.2 Vatalanib (PTK787)



Vatalanib

Vatalanib is a very potent small molecule inhibitor under development by Novartis, (Basel Switzerland www.novartis.com) and Schering (Berlin, Germany www.shering.de) [182, 273–279]. Vatalanib inhibits both KDR and Flt1 with IC50 values of 37 and 77 nM, respectively [280, 281]. It inhibits other class III RTKs, such as PDGFR, Flt4, c-Kit, and c-Fms with a tenfold higher IC50, but is not active against kinases from other receptor families. Vatalanib blocks VEGF-induced KDR phosphorylation, endothelial cell migration and proliferation at nanomolar concentration, but does not have any cytotoxic and anti-proliferative effects on cells that do not express VEGF receptors [280]. Orally, once daily administration of 25 to 100 mg/kg inhibited the growth of several human xenograft tumors, as well as an orthotopic murine syngeneic renal carcinoma in mouse models, along with reduction in microvessel formation in tumors [182, 274, 280]. The compound was rapidly absorbed with exposure time of 1.6 h and average terminal half-life of 5.9 h.

Phase I dose-escalating and PK studies of Vatalanib were performed on a wide spectrum of tumors including colorectal, RCC, NSCLC, AML, glioblastoma, and prostate cancer. Dose ranged up to 2000 mg once daily or 1000 mg twice daily by oral administration. In most studies, results in patients with advanced solid tumors indicated that treatment was well tolerated with no drug-related serious adverse events. Tumor volume reduction was observed in some patients. The MTD was not reached with doses up to 1500 mg/day. The optimal dose was determined as 1250 mg/day. Measurable responses of tumor volume reduction were observed in 19% and 4% of the patients with RCC and glioblastoma, respectively. Over 50% of patients achieved stable disease [282].

Data on phase I trial in 34 patients with unresectable hepatocellular cancer were presented at the 41st ASCO meeting, May 2005. Patients received 750–1250 mg Vatalanib orally, once daily in a 28-day cycle. The most common

adverse effects were minor and included nausea, vomiting, anorexia, fatigue, diarrhea, and dizziness. Five patients had stable disease ranging from 165 to 335 days and ten patients had progressive disease. No complete or partial responses were observed. Studies on Vatalanib in combination with Paclitaxel and Carboplatin were presented at the same meeting. In a phase Ib trial, 19 patients with stage IIC to IV epithelial ovarian cancer received 250–1250 mg Vatalanib orally on days 3–21 of each 21-day chemotherapy cycle, while Paclitaxel and Carboplatin were administered on day 1 of each cycle. Grade III/IV adverse effects were observed, including neutropenia, leukopenia, anemia, constipation, infection, nausea, and weight increase. In June 2006, further data from this trial were presented at the 42nd ASCO meeting. No DLT was observed in all of the evaluated individuals. Of 42 evaluable patients, 67% had complete or partial response.

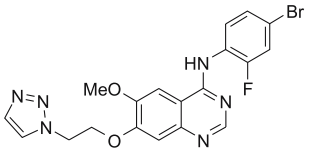
Phase II trials of Vatalanib in combination with 5-FU/Leucovorin/Irinotecan (IFL) in patients with treatment-naive metastatic colorectal cancer were presented at the 39th ASCO meeting, June 2003. A decrease in the extent of Irinotecan bioavailability and its metabolite (SN-38) was detected following co-administration with Vatalanib. The compound exposure decreased by approximately 40% in four of five patients at 1000 mg/day. Of 11 patients evaluable for tumor response, four had partial responses and four achieved stable disease. Common adverse events included nausea, fatigue, vomiting, epistaxis, diarrhea, and dizziness. At 500 mg/day there was one case of DLT of grade III fatigue and at 1000 mg/day there was one case of DLT of grade III hypertension. No other drug-related toxicities were observed. Median time to progression for 11 evaluable patients was 6.7 months. At the median follow-up of 9 months all 16 patients were alive. An additional study on Vatalanib in combination with Oxaliplatin/5-FU/Leucovorin (FOLFOX4) was carried out in patients with metastatic colorectal cancer. Orally administered Vatalanib was well tolerated and no PK interaction between Vatalanib and Oxaliplatin was detected. From 21 patients evaluable for tumor response, nine had partial response. The median time to progression was 11 months. Adverse events included grade III ataxia, grade IV neutropenia, grade III thrombocytopenia, and dizziness. Neurological DLTs were noted in two patients at the 2000 mg/day and grade III expressive dysphasia and intermittent dizziness were DLT at 1500 mg/day.

In a phase II study of Vatalanib in patients with myelofibrosis with myeloid metaplasia (MMM), the first two patients treated with 750 mg/day experienced DLT of grade III dyspepsia and grade II proteinuria. Therefore, the six subsequent patients were treated at 500 mg/day. This dose was well tolerated. Only one of six patients had a grade III dose-limiting thrombocytopenia. Other grade III/IV toxicities at this dose level included elevated liver enzymes and neutropenia, all of which occurred beyond day 28 of therapy and were reversible after drug interruption. Gastrointestinal or CNS toxicities were minimal or absent. In another study, oral, once-daily administration of

Vatalanib was tested in 55 patients with recurrent GBM and the MTD was 1500 mg/day. Median progression-free survival was 11 weeks when the dose of Vatalanib was greater than 1200 mg/day, but only 8.4 weeks with a dose of less than 1000 mg/day. DLTs included liver enzyme elevation, deep vein thrombosis, insomnia, cerebral edema, fatigue, and nausea and vomiting. Of the 47 evaluable patients, two had partial responses, 31 had stable disease and 14 showed disease progression. The median duration of stable disease was 12.1 weeks. Vatalanib in combination with Temozolomide demonstrated the greatest antitumor activity, with a median progression free survival of 16.1 weeks, compared to 12.1 weeks when in combination with Lomustine. Of the 51 patients who were evaluable for response, four had a partial response and 17 had stable disease. The median time to progression was 15.7 and 10.4 weeks for the Temozolomide- and Lomustine-treated groups, respectively. There was one grade III dizziness DLT in the 1500 mg/day group treated with Temozolomide. The MTD was not reached. In March 2005, phase II trials NSCLC patients were initiated at five sites across France and Germany. The trial aimed at assessing the efficacy of Vatalanib as a second-line monotherapy in patients with stage IIIb/IV disease, who had relapsed or were refractory to first-line therapy. Data from this trial were presented at the 42nd ASCO meeting, June 2006. Of 54 patients, administered a dose of 1250 mg once-daily, one exhibited a partial response and 17 had disease stabilization. The most severe grade IV adverse events were hypertension, thromboembolism, and an increase in liver enzymes. Frequent grade III toxicities were dyspnoea and hypertension. Eight patients discontinued treatment due to adverse events, but the agent was regarded as suitable as a second-line monotherapy.

Phase III colorectal cancer trials were initiated in January 2003 in the USA and in Western Europe. Vatalanib was orally administered at a dose of 1250 mg/day. By July 2005, phase III trials were underway in Korea, and in January 2006, Vatalanib was listed as being in phase III for solid tumors in the USA.

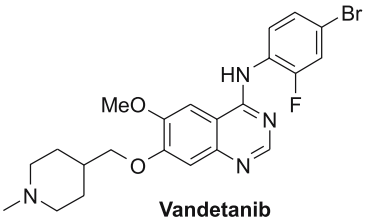
3.4.3
ZD4190, Vandetanib (ZD6474) and Cediranib (AZD2171)



ZD4190

A serial of small TKI under investigation by AstraZeneca (Cheshire, UK, www.astrazeneca.com) inhibits kinase activity of both KDR and Flt1. ZD4190,

the first compound in this serial, blocks VEGF-induced human umbilical vein endothelial cells (HUVEC) proliferation with an IC50 value of 60 μM [283, 284]. Chronic treatment with ZD4190 inhibited the growth of a variety of human tumor xenografts in animal models, including colon, lung, breast, prostate, and ovarian origin [284–286]. However, despite its promising potential, clinical development of ZD4190 was discontinued in 2000 due to intrinsic physiochemical and pharmacokinetic (PK) properties of the compound, which were responsible for its moderate and variable bioavailability in higher animal species and patients. Structural modification of ZD4190, as well as new generation of compounds, aiming at improving its physiochemical properties led to the discovery of two new compounds, ZD6474 and ZD2171 [287, 288].



Vandetanib

Vandetanib (ZD6474, Zactima) is a structural modification of ZD4190 that possesses potent inhibitory characteristics on KDR TK activity [283]. The compound shows selectivity for KDR (IC50, 40 nM) versus other RTK, such as EGFR (IC50, 500 nM), PDGFR (IC50, 1.1 μM), Flt1 (IC50, 1.6 μM), Tie2 (IC50, 2.5 μM), FGFR (IC50, 3.6 μM), IGF-1R and erbB2 (IC50, > 20 μM), and serine/threonine kinases, such as CDK2, Akt and PDK (IC50, > 20 mM) [288–290]. Vandetanib showed a broad spectrum of dose-dependent antitumor activity against lung, prostate, colon, breast, ovarian and vulval cell lines in vitro [181, 291–293]. Vandetanib is approximately 500-fold more soluble than ZD4190 in phosphate buffer at pH 7.4, which led to a significant improvement in oral bioavailability as shown in dogs [283]. Vandetanib has a half-life of 15 h and 8 h in rat and dog, respectively, following intravenous injection [287]. When given orally once-daily, Vandetanib has demonstrated an antitumor activity in a variety of human xenograft models using several different dosing regimens (ranging from 25 to 100 mg/kg/day) including CNS tumors and intestinal adenomas [289, 291]. Dynamic contrast-enhanced MRI assessment indicated that acute Vandetanib treatment significantly reduced vascular permeability in tumor tissue [285, 292]. Chronic administration of Vandetanib was generally well tolerated. However, similar to ZD4190 and other anti-VEGF agents, Vandetanib induced a dose-dependent increase in the femoral epiphyseal growth plate area in young rat [284].

In phase I trials, a total of 49 patients with malignant solid tumors were treated with a single daily oral dose of Vandetanib (50–600 mg/kg) followed

by a seven-day washout period and continuation of daily dosing, until disease progression or dose-limiting toxicity. Drug-related toxicity has been minimal. The MTD was reached at 600 mg in one patient who developed grade III thrombocytopenia. Adverse events were otherwise limited to mild diarrhea and rash increasing with dose. The estimated half-life of the orally administered Vandetanib was 130 h (ranging from 82 to 206 h). Under these conditions, stable disease was observed in two patients. In a follow up study, a total of 18 patients with malignant solid tumors received single oral doses (100 to 400 mg/kg/day) followed by 7 days of rest and then daily dosing at the same dose for a total of 28 days. Partial responses were observed in four of nine patients with NSCLC. MTD was 400 mg, with 100–300 mg doses recommended for phase II studies.

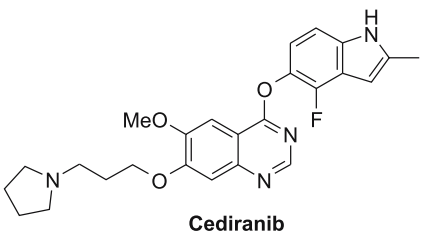
Phase II trials in lung cancer and multiple myeloma (MM) patients were initiated in November 2002. Data were presented at the 40th ASCO meeting, May 2004. In a two-part, open-label, randomized, phase II study, 15 patients, with NSCLC were administered once-daily with 100 and 300 mg/kg of Vandetanib in combination with Docetaxel. Serious adverse events were recorded in eight patients, among which toxic-induced encephalopathy, nail infection, non-Q wave myocardial infraction and bacteremia were considered to be therapy related. Nine patients had dose reduction or interruptions in treatment mainly due to QTc prolongation or grade III rash. Among the four patients that received 100 mg Vandetanib, there were two cases of stable disease for duration greater or equal to 12 weeks and two cases of disease progression. From the 11 patients who received 300 mg of Vandetanib, ten were evaluable for response. Among these, two achieved a partial response, two had stable disease for duration of 6 to 12 weeks and two had stable disease for at least 12 weeks, while three patients experienced disease progression. The median time to progression was 15.1 and 18.6 weeks, respectively, for the 100 and 300 mg groups. Data of a subsequent study were presented at the 41st ASCO meeting, May 2005. Patients with advanced or metastatic NSCLC received 200 or 300 mg Vandetanib daily, in combination with carboplatin (CP) once every 21 days. The median duration of treatment was approximately 4.1 months. The most common adverse effects observed were fatigue, diarrhea, and rash. Of 21 patients, six experienced minor asymptomatic QTc prolongation. Two patients developed a serious adverse effect of rash with desquamation and dehydration, considered to be treatment related. PK data indicated that steady-state concentrations of Vandetanib alone and in combination with CP were similar. Tumor assessments demonstrated a confirmed partial response in seven patients and an unconfirmed partial response in five patients. Two patients had stable disease over 12 weeks while disease progression was observed in three patients. Similar data were presented at the 11th World Conference on Lung Cancer, July 2005. Phase IIa data on Vandetanib were presented at the 42nd ASCO meeting, May 2006. The study assessed the drug's overall response rate in 53 NSCLC patients who received daily Van-

detanib doses of 100–300 mg. Overall response rate was 13%. The median time-to-progression was 12.3 weeks in both the 200- and 300 mg arms but only 8.3 weeks in the 100 mg arm. The adverse events were similar to previous trials and were managed with dose interruption or reduction.

Data from a phase II trial of Vandetanib in MM patients were presented at the 46th ASH meeting, December 2004. A cohort of relapsed MM patients was administered 100 mg once-daily for a mean of 9.8 weeks. Vandetanib was well tolerated. Grade I or II drug-related adverse events included nausea, vomiting, fatigue, rash, pruritis, headache, diarrhea, dizziness, and sensory neuropathy. No serious drug-related adverse events occurred. One patient had grade III anemia, and there were no grade III changes in biochemistry. Data on Vandetanib in patients with medullary thyroid carcinoma were presented at the 17th AACR-NCI-EORTC, November 2005. Fourteen patients received 300 mg Vandetanib orally, once-daily. At 10 months, two partial responses were detected and nine patients were presented with stable disease. Incidences of grade III diarrhea, fatigue, rash, and nausea were reported. In October 2005, FDA had granted Orphan Drug designation to Vandetanib for the treatment of follicular, medullary, anaplastic, and locally advanced and metastatic papillary thyroid cancer.

Phase II trials in breast cancer and head and neck cancer were underway in Japan by November 2005 and in brain cancer by December 2005. By July 2005, a phase III trial in solid tumors had started. In February 2006, the company listed the drug in phase III trials for NSCLC. AstraZeneca plans to file Vandetanib for marketing authorization application (MAA) in Europe and new drug application (NDA) in the USA not earlier then 2007.

As of today, Cediranib (AZD2171) has the potential to be a "best in class" angiogenesis therapy. It is one of a new generation series of orally available, highly potent inhibitors of KDR kinase. The compound inhibited VEGF-stimulated HUVEC cell proliferation with an IC50 value of 0.4 nM and was specific for this type of proliferation.



Cediranib

Cediranib demonstrated selectivity greater than 2000-fold for the inhibition of KDR versus EGFR phosphorylation in cells. Cediranib exhibited PK properties in animals compatible with once-daily oral dosing. Preclinical studies demonstrated a broad spectrum of antitumor activity that extended

to a range of histologically distinct xenografts, including lung, colon, breast, prostate, and ovarian cancer. In an orthotopic murine RCC model, treatment with 6.3 mg/kg/day of Cediranib resulted in a significant inhibition of primary tumor growth and microvessel density, with a notable decrease in lung metastases. Similar treatment prevented growth plate ossification in the long bones of growing rats and inhibited luteal development in the ovary, physiological processes that are highly dependent on neovascularization. Administration of 3 mg/kg/day Cediranib inhibited tumor xenografts growth by 69–100%. When administered concomitantly with other drugs (Gefitinib, ZD6126, or Irinotecan), Cediranib resulted in a greater tumor growth inhibition, with tumor regression induced in all cases and a 41% lower mean tumor volume when compared to pretreatment volume at 18 days of dosing. The drug was well tolerated in all studies.

Data of phase I clinical trial of Cediranib in patients with advanced cancer and liver metastases presented at the 40th ASCO meeting, June 2004. Cohorts of three to four patients received a single oral dose of the compound (0.5–20 mg/kg) followed by a 7-day washout period. An equal daily dosing was then administered for a total of 28 days. PK data of 16 patients indicated that Cediranib is rapidly absorbed with a median time to maximal plasma concentration of 3 h and a half-life of 20 h. The PK profile appeared to be linear following single and multiple doses. Treatment was well tolerated at the dose levels and the MTD has not been established at this time. Grade II dizziness was the only toxicity noted, in one patient from the 10 mg cohort. In April 2005, a phase I combination, multicenter, open-label, dose-escalating trials of Cediranib in patients with colorectal cancer and advanced NSCLC, was initiated in Canada. Patients received standard doses of Paclitaxel or Carboplatin, with daily doses of Cediranib escalating from 30 mg/day, every 21 days for up to eight courses. Data from this trial were presented at the 17th AACR-NCI-EORTC conference, November 2005. The treatment was well tolerated, with DLTs of fatigue and febrile neutropenia and mucositis (at 45 mg/day). Of nine evaluable patients, four had partial response. Four patients showed disease stabilization and one progressed. Different phase I data in patients with advanced prostate adenocarcinoma were presented at the 41st ASCO meeting, May 2005. Twenty patients received 1–30 mg oral Cediranib in a continuous 3-week cycle. Four grade III adverse events were observed in the 20 mg cohort, including back pain, fatigue, metastases to bone, and transient ischemic attack.

In November 2005, a phase II/III study in NSCLC patients started in Australia and Canada. In February 2006, Cediranib was undergoing a UK phase II/III trials in colorectal cancer. At that time, US phase II trials were underway in patients with advanced solid tumors, mesothelioma, melanoma, liver, ovarian, peritoneal, fallopian tube, kidney, and breast cancers. In May 2006, a US phase II trial began for neurofibromatosis type I and plexiform neurofibroma.

3.4.4
Neovastat (Ae941) (structure unknown)

Neovastat is a naturally occurring orally bioavailable antiangiogenic compound, extracted from shark cartilage, under investigation by AEterna Zentaris (Quebec, Canada). Neovastat possesses multiple antiangiogenic mechanisms of action that provide broad therapeutic potential for a number of diseases [294, 295]. The development of Neovastat first began due to the mistaken belief that sharks, whose skeletons consist mostly of cartilage, are not affected by cancer. Despite the fact that this assumption is not correct, several substances isolated from shark cartilage have been found to possess antitumor activity. Fractionation of liquid shark cartilage led to the characterization of some active components that have been tested for direct antitumor activity in vitro. As yet, however, no reports have identified the active components in Neovastat. Neovastat blocks two main mechanisms of angiogenesis activation, VEGF and matrix metalloproteinase (MMP)-2 and MMP-9. At the molecular level, Neovastat was shown to compete against the binding of VEGF to its receptor in endothelial cells and significantly inhibited the VEGF-dependent tyrosine phosphorylation of KDR, whereas it had no significant effect on Flt1 activity [296–300]. Moreover, the inhibition of receptor phosphorylation was correlated with a marked decrease in the ability of VEGF to induce pERK activation [296]. Neovastat in a concentration of up to 0.2 mg/mL inhibited VEGF-induced endothelial cell sprouting in a dose-dependent fashion. It also inhibited endothelial cell migration and vessel formation [296]. Neovastat (85 μg/mL) induced 50 and 100% cell death following 24 and 48 h treatment, respectively, in bovine aortic endothelial cells (BAECs) [119, 301]. Subchronic toxicity studies in animals did not indicate any significant toxicity associated with the administration of Neovastat. Toxicology studies in rats and monkeys demonstrated no DLT or target organ damage after 1 year of chronic exposure.

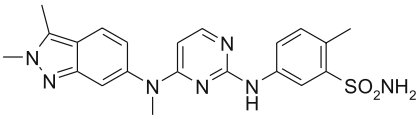
A US open-label, multicenter phase I/II study suggested that Neovastat was efficacious in the treatment of refractory metastatic lung cancer [215, 302, 303]. The study did not demonstrate any serious adverse events. Analysis of data from a group of 48 patients with unresectable late-stage NSCLC from phase I/II dose-tolerance trial showed that those receiving more than 2.6 mg/kg/day Neovastat were 50% less likely to die than those who received less than 2.6 mg/kg/day [302, 304]. Neovastat has now been monitored in over 800 patients, some of whom have taken the drug for over 4 years. Overall, Neovastat has an excellent safety profile with few side effects. Although one serious adverse event (hypoglycemia) was noted in type II diabetic patients, other grade III to IV toxicities have not been observed. Phase I/II trial of Neovastat (30–240 mL/day) conducted in 331 solid-tumor patients demonstrated the most frequent adverse events of nausea (7%), vomiting (3%), dyspepsia (2%) and anorexia (2%) [305, 306]. Phase I/II trial on patients with RCC, MM,

and prostate cancer performed in Canada and the USA, showed no DLT, good patient compliance, and improved conditions or disease stabilization were noted in some of the patients. Nevertheless, development of Neovastat for indications other than lung cancers were suspended or discontinued as a result of budget issues. A recent phase III study found that 200 patients with NSCLC given Neovastat in combination with induction chemotherapy (IC) and concomitant chemoradiotherapy (CRT) noted granulocytopenia as a common toxicity in the IC phase, and one patient suffered a myocardial infarction in the CRT phase. It appears, therefore, that Neovastat is suitable for long-term use either alone or in combination with other anticancer agents [215, 302–304, 307].

Identification of the active component of Neovastat may elucidate its specific mode of action and potentially limit the side effects identified at the present time. This is particularly pertinent if, as expected, life-long administration is required, because the effects of chronic exposure and interactions between Neovastat and other therapies are not yet known. The positive safety profile and the oral administration route of Neovastat, however, are advantages in comparison with current therapies and some angiogenesis inhibitors. Thus, should antiangiogenic therapy become a mainstream therapy, Neovastat could play a substantial role in the treatment of cancer.

3.4.5
Pazopanib (GW786034)

Pazopanib (GW786034) is a KDR TK inhibitor under development by GlaxoSmithKline (Brentford, UK, www.gsk.com) for the potential treatment of solid tumors [308]. Clinical data on Pazopanib were presented at the 40th ASCO meeting, June 2004. In a phase I, open-label, non-randomized, dose-escalating trial, 37 patients with various solid tumors were orally administered Pazopanib as part of a three-times a week schedule (50 or 100 mg each dose) or a daily administration schedule (50–2000 mg each dose). Four cases of stable disease were noted for patients in the trial for more than 27 weeks. Partial response was observed in a patient who received treatment for 46 weeks. Minimal responses of 15–18% tumor shrinkage were noted in two patients on the study for at least 12 weeks. An unconfirmed partial response was observed in a patient on the study for over 14 weeks. Apparent correlation between increase in blood pressure and Pazopanib dose was observed. DLT of grade III fatigue was obtained in the 200 mg dose. For the once-daily administration schedule the half-life was approximately 35 h. A parallel phase I randomized, double-blind trial was conducted in 63 patients. All six patients with RCC who received the therapeutic dose achieved a clinical benefit. Similar end-points were observed in a number of other tumor types including gastrointestinal, neuroendocrine, and lung. Common side effects included fatigue and hypertension. All together, phase I



Pazopanib

monotherapy trials had shown that the drug is well tolerated. Comparable results were obtained in a combination trial of Pazopanib with Lapatinib in 33 patients with solid tumors. The drug was well tolerated although it produced a side effect of diarrhea. Fatigue caused by the two drugs individually did not accumulate. Long-term treatment (greater than 1 year), at 300 mg twice daily did not result in any serious side effects. Disease stabilization was achieved in 14 patients and partial remission in three patients.

Phase II clinical trials of Pazopanib were initiated in November 2004 in patients with RCC [309]. Subsequently, GlaxoSmithKline initiated a phase II trial in patients with NSCLC in the USA, Europe, and Israel. By April 2006, phase III trials had started in patients with advanced/metastatic renal cancer.

3.4.6
XL647 and XL999 (structure unavailable)

XL647 (EXEL-647) and XL999 are two potent "spectrum selective" inhibitors under development by Exelixis (South San Francisco, CA, www.exelixis.com). These compounds aim at targeting both the tumor and its vasculature by inhibiting different RTKs implicated in driving tumor proliferation and vascularization [213, 290, 310, 311]. XL647 simultaneously inhibits the EGFR, HER2, KDR, and EphB4 TK with high potency and demonstrates excellent activity in target-specific cellular functional assays. Administration of XL647 resulted in a dose-dependent and sustained inhibition of KDR, EGFR, and ErbB2 phosphorylation. XL647 has good oral bioavailability and showed potent anticancer activity and sustained inhibition of target RTKs in vivo, following a single oral dose. XL647 induced tumor regression in established MDA-MB-231 and PC3 xenografts models. A single oral dose treatment in MDA-MB-231 xenograft model resulted in a complete and rapid loss of microvessels in the tumor, a decrease in cell proliferation and an increase in necrosis and hypoxia over time. In athymic, xenograft-bearing mice, treatment with 100 mg/kg of XL647 produced over 85% suppression of tumor growth. The compound has moderate clearance and a half-life of more than 8 h [312]. Exelixis initiated a phase I trial for XL647 in June 2004.

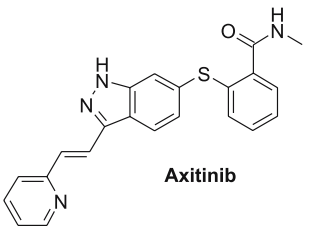
Phase I data of XL647 in 31 patients with NSCLC were presented at the 17th AACR-NCI-EORTC conference, November 2005. Patients received oral doses of XL647 ranging from 0.06 to 3.12 mg/kg on day 1 and days 4–8 in a 14-day cycle. The terminal half-life value after 5 days consecutive dosing was 70 h. One partial response and seven stable disease states has been achieved

and the drug was well tolerated [313]. In June 2006, data from an expanded dose escalation and PK phase I trial were presented at the 42nd ASCO meeting. Doses ranging from 0.06 to 7 mg/kg were tested in 37 patients. MTD was reached at 4.69 mg/kg given at 5 days in a 14-day cycle. Drug-related diarrhea was observed at the 7.0 mg/kg dose. Mean time T_{max} was 6–9 h and elimination half-life was 50–70 h [314]. In July 2006, Exelixis started a phase II trial of XL647 for chemotherapy-naive advanced NSCLC [315].

XL999 simultaneously inhibits the FGFR, KDR, PDGFR, and Flt3 TK with high potency and demonstrates excellent activity in target-specific cellular functional assays. In preclinical models of major tumor types, including human breast, lung, colon, and prostate cancer, XL999 demonstrated potent inhibition of tumor growth and has been shown to cause tumor regression. XL999 is suitable for both oral and intravenous dosing and shows sustained inhibition of target RTKs in vivo following a single oral dose. An in vitro functional angiogenesis assay demonstrated XL999-induced inhibition of tubule formation and migration on endothelial cells in culture in response to VEGF or bFGF. In nude mice, a single oral dose resulted in potent inhibition of KDR, PDGFR-beta, FGFR1, Flt3, and c-Kit. Daily administration of XL999 to nude mice bearing MDA-MB-231 xenograft resulted in a rapid destruction of the tumor vasculature, with tumor and endothelial cell death evident 2–4 h post-administration of the first dose. Longer exposure caused large decreases in vessel density and proliferating cells and large increases in tumor necrosis. Endothelial cells in the tumor vasculature were selectively targeted as endothelial cells elsewhere were not affected. Exelixis initiated a phase I trial for XL999 in September 2004.

In December 2005, phase II trials in RCC, colon, ovarian, and NSCLC were initiated. The monotherapy trials aimed at evaluating the drug in patients who have failed prior therapies or could not be treated with conventional therapies.

3.4.7
Axitinib (AG13736)



Axitinib

Pfizer (New York, NY, www.pfizer.com), in collaboration with its wholly-owned subsidiary Agouron Pharmaceuticals, is developing Axitinib (AG-

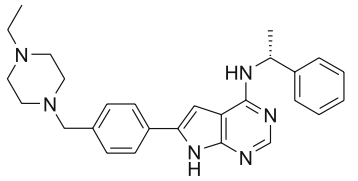
13736), a potent inhibitor of the VEGF/PDGF receptor TK, as an antiangiogenic agent for the potential treatment of cancer [316]. Axitinib is active against Flt1, KDR, Flt4, PDGFR-beta, and c-Kit with IC50 values of 1.2 nM, 0.25 nM, 0.25 nM, 2.5 nM, and 2.0 nM, respectively. The compound showed potent activity and specificity for the recombinant KDR kinase at subnanomolar concentrations. It was shown to inhibit proliferation and survival of VEGF-stimulated HUVEC cells. In a human colon carcinoma mouse model, oral administration of Axitinib twice-daily inhibited tumor growth associated with a significant decrease in microvessel density and increased necrosis. Axitinib significantly inhibited metastasis to the lung and lymph nodes in an orthotopically implanted human melanoma tumor in SCID mice with half-life of 2 h. Co-administration of Axitinib with Docetaxel resulted in a higher antitumor efficacy compared to that achieved by either agent alone. Quantitative MRI analysis revealed that Axitinib treatment produced changes in vascular permeability and antiangiogenic effects [317].

Phase I trails were initiated in April 2002 and data were presented at the 40th ASCO meeting, June 2004. Axitinib was orally administered at escalating doses to patients with various solid tumors including breast, thyroid, renal cell, lung, and other for cycles of 28 days. The MTD was found to be 5 mg/kg among fasted patients. DLTs at doses higher than the MTD were hypertension, seizures, elevated liver functions, mesenteric vain thrombosis, and pancreatitis and stomatitis. One patient with a cavitating lung lesion died from hemoptysis while on the treatment. At doses less than or equal to the MTD, the only DLTs observed were one case of stomatitis and six cases of dose-limiting hypertension. Durable responses were achieved with two patients and seven patients had stable disease for more than 4 months. PK studies showed that peak plasma concentrations occurred between 2 and 4 h and the terminal half-life was between 3 and 5 h. The dose of 5 mg/kg to fasted patients was recommended for phase II trials. By November 2004, Axitinib entered phase II studies in breast cancer and RCC. Data on a multicenter phase II study in 52 RCC patients were presented at the 41st ASCO meeting, May 2005. Patients received 5 mg Axitinib twice daily and demonstrated a partial response. After a 1-year follow-up, 36 patients remained on study with response or stable disease. Drug-related hypertension was experienced by 17 patients. Decreased tumor perfusion was observed in patients that responded to Axitinib treatment. The compound was well tolerated [318].

In July 2005, a phase II, randomized, open-label, active control, parallel-assignment trial of Axitinib in combination with Gemcitabine was initiated. The trial was designed to evaluate the efficacy of the combination in comparison with Gemcitabine alone. Data on a multicenter phase II trial in patients with advanced thyroid cancer were presented at the 42nd ASCO meeting, June 2006. In this study 32 patients with either refractory thyroid cancer or patients unsuitable for iodine treatment, were administered with 5 mg Axitinib

twice daily until disease progression or unacceptable toxicity occurred. Partial response was achieved in 22% of patients, tumor regression ranged from 36 to 67%, and sustained disease stability was achieved in 46% of patients, ranging from 4 to 13 months. Discontinuation was seen in 14 patients, citing adverse effects or disease progression. Common adverse effects were fatigue, proteinuria, diarrhea, and nausea.

3.4.8 AEE788



AEE788

AEE788 is a potent multitarget inhibitor of both EGF and VEGF RTK family members under development by Novartis (Basle, Switzerland, www.novartis.com) [187, 319]. At the enzymatic level, AEE788 inhibited EGFR, ErbB2, KDR, and Flt1 TK activity with IC50 values of 2 nM, 6 nM, 77 nM, and 59 nM, respectively [187]. AEE788 demonstrated an anti-proliferative activity against a range of EGFR and ErbB2-overexpressing cell lines and inhibited the proliferation of EGF- and VEGF-stimulated HUVEC cells [187]. Oral administration of AEE788 to tumor-bearing mice resulted in high and persistent compound levels within the tumor tissues. AEE788 also inhibited VEGF-induced angiogenesis in a murine implant model. Antiangiogenic activity was also apparent by measurement of tumor vascular permeability and interstitial leakage space using dynamic contrast enhanced magnetic resonance imaging methodology [187, 319]. In an in vitro study using the cell line JMAR SCCHN, AEE788 inhibited cell growth with an IC50 value of 7 μM at 72 h and induced 50% cell death after treatment with 14 μM at 48 h. Treatment of KAT-4 anaplastic thyroid cancer cells with AEE788 for a duration of 1 h inhibited autophosphorylation of EGFR and KDR, phosphorylation of ERK and AKT, and cell proliferation in a dose-dependent manner with an IC50 value of 7 μM [319]. Administration of AEE788 to nude mice implanted with JMAR tumors resulted in significant reduction in tumor growth [319]. A combinatorial treatment of AEE788 and Everolimus increased the antiproliferative effects of the drugs in comparison to that of single agents alone. Cell death, confirmed to occur via apoptosis, was dramatic at optimal concentrations of the combination.

Phase I clinical trials were initiated in April 2003 in patients who had not previously received treatment directed against EGFR, ErbB2, and VEGFR. Results were presented at the 16th EORTC-NCI-AACR meeting, September 2004. A group of 50 adult patients with advanced solid tumors received continuous, oral, daily administration of AEE788 at doses of 25–550 mg/day. A total of 41 patients were assessed. The mean exposure increased with dose duration, as did the exposure of the metabolite of AEE788, AQM-674. Exposure of the parent compound and active metabolite increased with dose until day 15 when steady state was achieved. The metabolite, AQM-674, was rapidly formed and eliminated in comparison to AEE788. At a dose of 300–400 mg, a predicted 80% inhibition of KDR phosphorylation occurred. The drug was widely distributed within the tissues and extensively metabolized. The once-daily regimen of up to 400 mg/day was found to be safe and well tolerated, achieving a therapeutic exposure profile. The half-life of the compound was noted to be above 24 h. DLTs were observed at the 550 mg dose. The most frequent adverse effects were diarrhea, fatigue, anemia, and nausea, which were experienced by 66, 50, and 42% of patients, respectively. A total of 14 out of 41 (34%) patients achieved stable disease and remained on the study for more than two cycles.

Data of a parallel phase I trial were presented at the 41st ASCO meeting, May 2005. A group of 37 patients with recurrent GBM were given 50–800 mg/day of AEE788 orally. Most frequently occurring adverse effects at all dose levels were primarily grade I/II and included diarrhea, fatigue, nausea, rash, vomiting, and decreased appetite. DLTs included grade II seizures (400 mg), grade IV stomatitis (550 mg), grade III fatigue with grade II proteinuria (550 mg) and grade III diarrhea. Stable disease was the overall best response, seen in six out of 36 patients (17%). Four patients treated with AEE788 (50–200 mg/day) had stable disease for 6–10 months. Most common adverse effects observed during all cycles at all dose levels were grade I/II and included diarrhea, fatigue, nausea, rash, anorexia, vomiting, stomatitis, and abdominal pain. Exposure of AEE788 appeared to increase over-proportionally with increased dose, while the metabolite AQM-674 appeared to increase proportionally with increased dose. Steady-state plasma concentrations of AEE788 were achieved by day 15 with once-daily dosing.

3.4.9 BIBF1120 (structure unavailable)

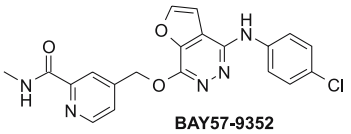
BIBF1120 is an orally available inhibitor for VEGFR, FGFR, and PDGFR kinases, under development by Boehringer Ingelheim (Ingelheim, Germany, www.boehringer-ingelheim.com) as an antiangiogenic agent for the potential treatment of cancer. BIBF1120 inhibits VEGFR2, FGFR1 and FGFR2 and PDGF alpha and beta with IC50 values of 13, 69, 137, 59, and 60 nM, respec-

tively. It also inhibits the growth of HUVEC cells with IC50 value of 9 nM. In nude mice with FaDu head and neck carcinoma xenografts, subcutaneous administration of the compound over more than 20 days led to a reduction in the tumor volume at the 100 mg/kg/day dose. MRI data showed that tumor blood supply and vessel permeability were reduced in nude mice bearing FaDu or HT29 colon carcinoma xenografts treated with 100 mg/kg oral BIBF1120 for three consecutive days. In Caki tumor model, treated with a dose of 100 mg/kg/day over 5 days, there was 80% inhibition of CD31-positive cells, an indicator of tumor vessel density. BIBF1120 and Docetaxel showed additive effects in a xenograft NCI-H460 model in nude mice. Tumor blood vessel density was reduced and apoptosis increased in tumors treated with the combination compared to either drug alone.

Data of phase I trials with the compound were presented at the 16th EORTC-NCI-AACR meeting, September 2004. The open-label, multiple-dose, PK study enrolled 25 patients with advanced cancer who were administered a single, oral dose of BIBF1120 on the first day, followed by a 1-day washout period and then 28 days of continuous administration of fixed oral doses ranging from 50 to 450 mg/day. The predominant drug-related adverse events were nausea, vomiting, diarrhea, abdominal pain, and reversible elevated liver enzymes. The gastrointestinal effects were mild-to-moderate and did not lead to discontinuation of treatment. The liver enzyme elevations were dose-limiting at 200, 300, and 450 mg/kg in some patients. A total of 13 patients were treated for more than two cycles. Stable disease was observed for two to 7 months among 11 patients. Three additional patients with stable disease continued to have treatment at 13, 15, and 21 months. One patient with RCC treated on 200 mg/kg showed a complete regression of pulmonary metastases. Subsequent results of this trial were presented at the 41st ASCO meeting, May 2005. DLTs were observed in six of 25 patients at 200–300 mg.

Data from different phase I study were presented at the 17th AACR-NCI-EORTC conference, September 2005. A group of 30 patients with advanced colorectal cancer were administered oral doses of BIBF1120 of between 50 and 500 mg/day. There were seven grade III or IV adverse events, including elevated liver enzymes, reduced CD4 count, and gastrointestrinal symptoms. MRI data showed that tumor blood flow or permeability was reduced by over 40% in 72% of patients. There was one partial response, in a patient treated with 250 mg/day. In June 2006, further data from this study were presented at the 42nd ASCO meeting. To that end, a total of 51 patients had been enrolled. The MTD was 400 mg/day. The main DLT was elevated liver enzyme levels. No grade IV adverse events were observed.

3.4.10
BAY57-9352

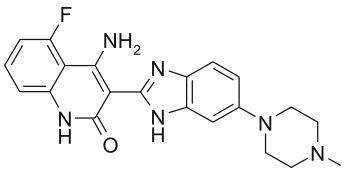


BAY57-9352

BAY57-9352 is a KDR inhibitor under development by Bayer Yakuhin (Osaka, Japan, www.bayer.co.jp) for the potential treatment of cancer [180]. It potently and selectively inhibited KDR, Flt4, c-Kit, and mouse PDGFR TK in vitro with IC50 values of 6, 4, 1, and 15 nM, respectively. BAY57-9352 blocked VEGF-dependent receptor autophosphorylation in mouse fibroblasts expressing human KDR, with an IC50 value of 19 nM. Its affect on KDR phosphorylation was detected in endothelial and smooth muscle cells as well. BAY57-9352 inhibited the proliferation of HUVEC cells and human aortic smooth muscle cells with IC50 values of 26 nM and 249 nM, respectively, with no effect on proliferation of MDA-MB-231 breast carcinoma, LS17T colorectal carcinoma, HCT-116 colorectal carcinoma, or PC-3 prostate carcinoma cells. Nevertheless, administration of 20 mg/kg BAY57-9352 reduced MDA-MB-231, Colo-205, DU-145, and H460 xenografts tumor growth in NCr nu/nu mice by 91, 79, 61, and 78%, respectively. Moreover, microvascular density and endothelial cell content around the MDA-MB-231 and Colo-205 tumor xenografts were significantly reduced within 24 h of the first administration.

In June 2006, phase I data were presented at the 42nd ASCO meeting. A group of 130 patients with advanced solid tumors received escalating doses of BAY-57-9352 in schedules of 14 days on followed by 7 days off in 28-day cycles. Doses ranged from 20 to 1500 mg twice daily. The drug was well tolerated and the MTD was not reached at the highest dose given. Preliminary data suggested that the drug induced disease stabilization.

3.4.11 CHIR258



CHIR258

CHIR258 (GFKI258 or TKI258) is a potent VEGF, FGF, and PDGF receptor kinase inhibitor for the potential treatment of cancer, under development by Novartis (formally Chiron) [320, 321]. CHIR258 has shown potent activity against several growth factor-related kinases, with IC50 values of 27, 2, 0.1, 10, and 8 nM against PDGFR-beta, c-Kit, Flt3, VEGFR1/2/3, and FGFR1/3, respectively. It showed minimal activity against 25 other kinases. The compound had a significant antitumor activity in more than ten models, including the KM12L4A human colon cancer [320]. It was also reported to induce regression in large tumors, and had potent antiangiogenic activity in vitro and in vivo. The absolute oral bioavailability of CHIR258 was greater than 70% in mice, rats, and monkeys; and 34% in dogs. Maximum plasma and tissue concentrations occurred approximately 4 h after an oral dose in mice and rats. The elimination half-life ranged from 2.7 to 3.6 h in plasma following an intravenous administration. CHIR258 inhibited the proliferation of a subset of cancer cell lines, with IC50 values of less than 25 nM. In in vivo studies, human colon tumor (KM12L4a) xenografts treated with CHIR258, demonstrated significant tumor regression and inhibition. Tumor regression and/or disease stabilization was observed in 90–100% of animals. In a mouse model of murine breast cancer (4T1), CHIR258 inhibited primary tumor growth in a dose-dependent manner (2–82%) and liver metastases were inhibited by more than 75% at all doses greater than 10 mg/kg/day. In further studies, CHIR258 was shown to potentiate the antitumor activity of the standard cytotoxic therapeutics Irinotecan, Trastuzumab and Gefitinib. Analysis of KM12L4a tumors after CHIR258 treatment indicated that phosphorylation of Flt1, KDR, PDGFR-beta, and FGFR were inhibited in a time- and dose-dependent manner.

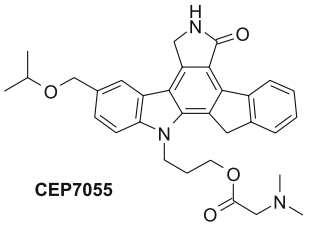
UK phase I studies in solid tumor and AML patients were initiated by January and October 2004, respectively. Patients received single oral doses of CHIR258, ranging between 50 and 400 mg for 7 days, followed by a 7-day rest period. The drug was well tolerated. Adverse events were generally mild to moderate, but there was one incidence of grade IV fatigue. All patients exhibited reductions or stabilization of peripheral blasts, and reduction or

stabilization in bone marrow blasts was seen in all but one of the nine evaluable patients.

A reduction in phosphorylated ERK was observed in patient peripheral blood lymphocytes 4–24 h following the first dose. Pharmacodynamic (PD) studies showed dose-dependent plasma concentration and supported once-daily dosing. By September 2004, the third cohort had completed treatment with 75 mg/day of CHIR258 and a 100 mg/day dose level had been initiated. No clinically significant toxicities had been detected at this point.

In June 2005, Chiron began US and UK phase I trials in MM. Data from this trial were presented at the 42nd ASCO meeting, June 2006. A total of 35 patients were treated in four intermittent dosing cohorts (25, 50, 75, and 100 mg/day) and three continuous dosing cohorts (100, 125, and 175 mg/day) all once daily. Treatment was 7 days on, 7 days off with a subsequent protocol amendment to daily dosing. The plasma PK values were linear between 25 and 175 mg doses with respect to C_{max} and AUC. DLTs occurred at 175 mg and the MTD was 125 mg. Treatment was associated with stable disease.

3.4.12 CEP7055



CEP7055

CEP7055 is the lead compound in a series of KDR TKI for the potential treatment of prostate and pancreatic cancers, under development by Cephalon (Frazer, PA, www.cephalon.com) and Sanofi-Aventis (Paris, France, www.sanofi-aventis.us) [184, 211, 322, 323]. It is a fully synthetic orally active ester of CEP5214, a very potent KDR inhibitor with poor water solubility [184, 211, 322]. CEP7055 demonstrated antitumor efficacy, as well as antiangiogenic and antimetastatic activity in animal models. It is 20% orally bioavailable in rats, and has a half-life of 4–5 h in monkeys. Chronic oral administration of CEP7055 at doses of 10–20 mg/kg/day resulted in significant inhibition of a variety of established murine and human subcutaneous tumor xenografts in nude mice. No DLT was noted following 10 or 28 days administration in monkeys. No adverse neurological, cardiac, or respiratory effects were observed. Treatment of human pancreatic ductal carcinoma-bearing mice with CEP7055 was well tolerated, and resulted in a significant reduction in primary pancreatic tumor mass, incidence of ascites, and the magni-

tude and extent of hepatic and peritoneal lymph node metastases relative to vehicle-treated mice. Oral administration of CEP7055 at 3 and 20 mg/kg/day to Balb/c mice inoculated with renal cancer cells (RENCA) tumors was also well tolerated and resulted in a decrease in metastatic score. Administration of CEP7055 in combination with Temozolomide led to an improvement in median survival of human GBM-bearing mice versus mice receiving Temozolomide monotherapy. In a dose-response study in the same model, chronic oral administration of CEP7055 alone at 24–95 mg/kg/day demonstrated a dose-related reduction in brain edema and hemorrhagic lesions. Significant reductions in neurological dysfunction were observed in GBM-bearing mice receiving CEP7055 alone and to a greater extent, in combination with Temozolomide.

Phase I data were presented at the 39th ASCO meeting, June 2003. A group of 19 patients with various solid tumors were given 10–120 mg/kg/day CEP7055 continuously for duration of 28 days followed by a 14-day washout period. Adverse events were generally mild; hypertension occurred in one patient on the 120 mg dose towards the end of the washout period.

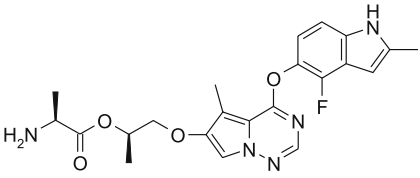
3.4.13
ZK304709 (structure unavailable)

ZK304709 (ZK-CDK) is an orally available dual specific CDK and VEGFR kinase inhibitor, under development by Schering (Berlin, Germany, www.schering.de) for the potential treatment of cancer [324]. ZK304709 inhibited CDK2 and KDR kinase activity with IC50 values of 4 and 30 nM, respectively. In xenograft mouse models ZK304709 reduced tumor blood supply and strongly induced apoptosis. Its dual kinase activity enables blocking of the cell cycle followed by preferential tumor cell apoptosis through CDK1 and 2 and blocking neoangiogenesis through VEGFR1/2/3 and PDGFR-beta. IC50 values for CDK2, CDK1, VEGFR1/2/3, and PDGFR-beta were 5 nM, 60 nM, 20 nM, and 55 nM, respectively [324].

Phase I trials were initiated on June 2004 and presented at the 42nd ASCO meeting in June 2006. Of the 40 patients enrolled on the dose-escalation trial, preliminary data were available for 38. Patients had eight daily dose levels of ZK304709, ranging from 15 to 360 mg, and completed a median of two treatment cycles. The drug was well tolerated when administered for 7 days in a 21-day cycle. Common adverse effects included nausea and vomiting. DLT were 180 mg/day, due to vomiting, and 360 mg/day, due to diarrhea. Dose escalation was terminated at 360 mg/day, due to a lack of dose-proportional exposure, without defining the maximum tolerated dose. In addition, data were presented from a similar phase I dose-escalation study where ZK304709 was administered for 14 days of a 28-day cycle. Patients received dose levels ranging from 15 to 285 mg/day with a median of two cycles completed. Disease stabilization was achieved in seven of 35 patients for four or more cy-

cles. Common adverse effects were vomiting, nausea and fatigue, with DLT being identified as grade III dizziness, hypertension, and fatigue. The drug was rapidly absorbed under non-fasting condition, with a T_{max} value of approximately 2 h.

3.4.14 BMS582664



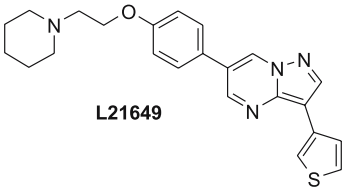
BMS582664

BMS582664 is a dual inhibitor of VEGFR family and FGFR family kinases under development by Bristol-Myers Squibb (New York, NY, www.bms.com) as an orally-active compound for the potential treatment of cancer. Preclinical data on BMS582664 were presented at the 96th AACR meeting, April 2005. BMS582664 inhibited the growth of a human lung carcinoma xenograft, L2987, by 85% when administered 80 mg/kg. The drug had excellent pharmaceutical properties, including solubility, and good oral bioavailability.

Clinical data on BMS582664 were presented at the 42nd ASCO meeting, June 2006. In an open-label dose-escalation phase I study, 26 patients with advanced or metastatic cancer received doses ranging between 180 and 1000 mg. Dose levels up to 800 mg were well tolerated with adverse events being hypertension, fatigue, and dizziness. The agent produced partial responses in two patients, while three patients were presented with stable disease of over 6 months.

3.4.15 L21649

Merck & Co (West Point, PA, www.merck.com) is developing L21649, a small molecular weight KDR and KDR/Flt3 kinase inhibitor for the potential treat-



L21649

ment of cancer and other angiogenic disorders. Preclinical data on two classes of KDR TK inhibitors showing that the lead compound, L21649 demonstrated good inhibition of VEGF stimulated HUVEC cells mitogenesis with an IC50 value of 18 nM and in vivo inhibition of KDR with an IC50 value of 130 nM [325, 326]. The compound had an IC50 value of 4 nM in an in vitro KDR kinase assay and a half-life of 5.1 h. Treatment of human HT1080 fibrosarcoma nude mouse xenograft model with L21649 (IC50 values of 19.5 nM in vitro, and 21 nM in vivo) was associated with partial or nearly complete inhibition of KDR phosphorylation and inhibition of tumor growth [325, 326]. Histology characterization revealed that treatment led to inhibition of tumor angiogenesis and cell proliferation, with an enhancement in tumor cell death [325, 326]. The inhibitors significantly blocked unstaged and staged growth of mammary and glioma tumor growth in vivo. Antitumor efficacy was associated with decreased levels of phosphorylated KDR in lungs and tumors, reduced microvessel density and vessel maturity and decreased endothelial cell proliferation. Treatments were tolerated with no significant changes in body weight.

Phase I data were presented at the 95th AACR meeting, March 2004. Normal healthy male volunteers were subjected to bone marrow aspirations prior to and 4 h following a single 25 mg oral dose of the compound or placebo. L21649 achieved plasma concentrations of 103.4 nM at 4 h post-dose. In July 2004, similar clinical data were presented at the 29th National Medicinal Chemistry symposium. The PK/PD correlated well, and at that time, it was believed that the plasma levels should be closer to the EC90 levels for maximal efficacy.

3.5 Additional Approaches

An escalating number of novel therapeutic compounds is still under discovery or preclinical studies, including RWJ-417975, a KDR TKI of Celltech Group and Johnson & Johnson (New Brunswick, NJ, www.jnj.com), RO4383596, a triple KDR/FGFR/PDGFR TKI of Hoffman La-Roche (Nutly, NJ www.rocheusa.com), IDDBCP167468, a KDR TKI by Abbott Laboratories (Abbott Park, IL, www.abbotte.com), DX-1235, a peptide inhibitor of KDR, by Dyax (Cambridge, MA, www.dyax.com), CX3543, a DNA G-quadruplex-interactive by Cylene Pharmaceuticals (San Diego, CA, www.cylenpharma.com), currently in phase I trial in patients with solid tumors or lymphomas, and others. In addition, a wide spectrum of compounds and antibodies targeting VEGF ligands exist under different developmental and clinical stages. Avastin (Bevacizumab), an anti-VEGF monoclonal antibody has developed and launched by Genentech (South San Francisco, CA, www.gene.com) in February 2004, as an antiangiogenesis therapy for the treatment of colorectal cancer and is currently at late stages of clinical trials for breast, prostate,

ovary, and fallopian tube cancers as well as NSCLC and RCC [327–330]. VEGF trap (AVE-0005), a soluble decoy receptor comprising portions of VEGFR-1 and 2, by Regeneron Pharmaceuticals (Tarrytown, NY, www.regeneron.com) together with Sanofi-Aventis, currently in phase I trail in patients with solid tumors and non-Hodgkin's lymphoma [331–333]. Veglin, an antisense oligonucleotide that inhibits VEGF signaling, by VasGene Therapeutics (Los Angeles, CA, www.vasgene.com) currently in phase I trial in patients with relapsed or refractory malignancies [334–336]. Trinam, a VEGF gene therapy utilizing an adenoviral vector under development by Ark Therapeutics Group (London, UK, www.arktherapeutics.com)

4 Future Perspective

Targeting cells that support tumor growth, for example the neovasculature of tumors, rather than cancer cells themselves, is a relatively new approach to cancer therapy. The control of angiogenesis in general and targeting VEGFR-2 in particular offers hope in the treatment of many disorders and may have wide spectrum applicability. Our knowledge of tumor angiogenesis and its impact on conventional cancer therapies has improved tremendously during the course of the last few years [337, 338]. The elucidation of structural abnormalities associated with tumor neovasculature, and of the underlying molecular mechanisms, has led to the identification of potential targets for therapeutic intervention by antiangiogenesis approaches. Antiangiogenesis therapies may offer a number of theoretical advantages over the conventional cytotoxic regimens. In principle, conventional therapy is hindered by the development of drug-resistant cancer cells. In contrast, endothelial cells possess a normal complement of chromosomes and are genetically stable, and are therefore less likely to accumulate mutations that allow them to develop drug resistance in a rapid manner [339–342]. In addition, endothelial cells are more sensitive than tumor cells to most cytotoxic agents. Thus, a low-dose chronic chemotherapy (or the so-called "metronomic approach"), designed for preferential antiangiogenic activity rather than tumoricidal activity, could be more efficacious and less toxic than conventional high-dose therapy [343, 344]. It should be taken into consideration, however, that tumor cells might reduce their sensitivity or become "resistant" to individual antiangiogenic therapy by increasing production of, or switching to other angiogenic factors [345]. Combinational use of multiple antiangiogenic agents should prove to be more effective in this scenario [346]. Finally, complete eradication of cancer cells is often unfeasible, partially due to an aberrant tumor vasculature that prevents uniform delivery of therapeutic doses of anticancer agents to the tumor tissues. By disrupting local blood supply, the antiangiogenic agents might detrimentally affect all tumor cells that are de-

pendent upon the vessels, thus minimizing the chance of residual tumor cells escaping [347].

Antiangiogenic agents may be predominantly effective in the context of combination therapy as they may enhance the delivery and therapeutic efficacy of other treatment modalities that directly target cancer cells. Despite the concern that a reduction of tumor blood supply would interfere with the delivery of chemotherapeutic agents and oxygen to the tumor tissues, it was suggested that that antiangiogenic therapies, when used properly, could "normalize" the tumor vasculature, thereby improving the efficiency of delivery of concurrently administered cytotoxic agents [348–350]. To this end, antiangiogenic therapies have been shown to potentate the antitumor effects of several conventional cytotoxic therapies (including both chemotherapies and radiation) in various animal models [199, 200, 351, 352].

The majority of drugs directed against VEGFR-2 currently in clinical investigation are small molecular weight TKIs. These molecules are orally available and therefore maybe more convenient for patients to handle. However, in contrast to antibodies, which are characterized by their high specificity to their targets and their minimal systemic toxicities to patients, small TKI molecules are less specific and often affect more than one kinase simultaneously, thus often leading to increased toxicity and lack of tolerance in clinical settings. Beneficially, antibodies and small TKI molecules are, however, not mutually exclusive. For example, combinations of growth factor receptor-specific antibody with receptor kinase-selective small molecule inhibitor have recently been shown to be more efficacious than each individual agent [353, 354].

Antiangiogenesis therapy is clearly an exciting area of research with potential for improving the care of patients with numerous cancers. The variety of antiangiogenic compounds ranks predominantly amongst novel and promising strategies for fighting cancer as well as other pathologies. With the recent approval of the anti-VEGF antibody, Avastin, the clinical antiangiogenesis approaches now look an increasingly realistic prospect. In particular, a number of carefully designed clinical trials are underway and it is hoped that answers to some of the open questions raised in this review will soon be forthcoming. Interestingly, there are currently more antiangiogenic agents in clinical trials than any other mechanistic category of anticancer drug. The next few years are clearly going to be an exciting test of the concept.

References

1. Smith SK (1997) Semin Reprod Endocrinol 15:221
2. Breier G (2000) Placenta 21(Suppl A):S11
3. Tonnesen MG, Feng X, Clark RA (2000) J Investig Dermatol Symp Proc 5:40
4. Gargett CE, Rogers PA (2001) Reproduction 121:181
5. Smith SK (2001) Trend Endocrinol Metab 12:147

6. Creamer D, Sullivan D, Bicknell R, Barker J (2002) Angiogenesis 5:231
7. Funatsu H, Yamashita H, Noma H, Shimizu E, Yamashita T, Hori S (2001) Jpn J Ophthalmol 45:577
8. Koch AE (2000) Ann Rheum Dis 59(Suppl 1):i65
9. Tonini T, Rossi F, Claudio PP (2003) Oncogene 22:6549
10. Arbiser JL (2004) Semin Cancer Biol 14:81
11. Folkman J (1971) N Engl J Med 285:1182
12. Folkman J (2002) Semin Oncol 29:15
13. Folkman J (2001) Semin Oncol 28:536
14. Yance DR Jr, Sagar SM (2006) Integr Cancer Ther 5:9
15. Dalgleish AG, O'Byrne K (2006) Cancer Treat Res 130:1
16. de Castro Junior G, Puglisi F, de Azambuja E, El Saghir NS, Awada A (2006) Crit Rev Oncol Hematol 59:40
17. Folkman J, Hanahan D (1991) Princess Takamatsu Symp 22:339
18. Giordano FJ, Johnson RS (2001) Curr Opin Genet Dev 11:35
19. Huss WJ, Hanrahan CF, Barrios RJ, Simons JW, Greenberg NM (2001) Cancer Res 61:2736
20. Bergers G, Benjamin LE (2003) Nat Rev Cancer 3:401
21. Fong TA, Shawver LK, Sun L, Tang C, App H, Powell TJ, Kim YH, Schreck R, Wang X, Risau W, Ullrich A, Hirth KP, McMahon G (1999) Cancer Res 59:99
22. Blagosklonny MV (2004) Cancer Cell 5:13
23. Wu Y, Hooper AT, Zhong Z, Witte L, Bohlen P, Rafii S, Hicklin DJ (2006) Int J Cancer 119:1519
24. Fan F, Wey JS, McCarty MF, Belcheva A, Liu W, Bauer TW, Somcio RJ, Wu Y, Hooper A, Hicklin DJ, Ellis LM (2005) Oncogene 24:2647
25. Ferrara N (2000) Recent Prog Horm Res 55:15
26. Karkkainen MJ, Petrova TV (2000) Oncogene 19:5598
27. Tille JC, Wang X, Lipson KE, McMahon G, Ferrara N, Zhu Z, Hicklin DJ, Sleeman JP, Eriksson U, Alitalo K, Pepper MS (2003) Exp Cell Res 285:286
28. Veikkola T, Karkkainen M, Claesson-Welsh L, Alitalo K (2000) Cancer Res 60:203
29. Hicklin DJ, Ellis LM (2005) J Clin Oncol 23:1011
30. Hanrahan V, Currie MJ, Gunningham SP, Morrin HR, Scott PA, Robinson BA, Fox SB (2003) J Pathol 200:183
31. Klagsbrun M, D'Amore PA (1996) Cytokine Growth Factor Rev 7:259
32. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z (1999) Faseb J 13:9
33. Connolly DT, Olander JV, Heuvelman D, Nelson R, Monsell R, Siegel N, Haymore BL, Leimgruber R, Feder J (1989) J Biol Chem 264:20017
34. Ferrara N, Henzel WJ (1989) Biochem Biophys Res Commun 161:851
35. Keck PJ, Hauser SD, Krivi G, Sanzo K, Warren T, Feder J, Connolly DT (1989) Science 246:1309
36. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N (1989) Science 246:1306
37. Senger DR, Asch BB, Smith BD, Perruzzi CA, Dvorak HF (1983) Nature 302:714
38. Houck KA, Ferrara N, Winer J, Cachianes G, Li B, Leung DW (1991) Mol Endocrinol 5:1806
39. Tischer E, Mitchell R, Hartman T, Silva M, Gospodarowicz D, Fiddes JC, Abraham JA (1991) J Biol Chem 266:11947
40. Keyt BA, Berleau LT, Nguyen HV, Chen H, Heinsohn H, Vandlen R, Ferrara N (1996) J Biol Chem 271:7788
41. Keyt BA, Nguyen HV, Berleau LT, Duarte CM, Park J, Chen H, Ferrara N (1996) J Biol Chem 271:5638

42. Soker S, Gollamudi-Payne S, Fidder H, Charmahelli H, Klagsbrun M (1997) J Biol Chem 272:31582
43. Maglione D, Guerriero V, Viglietto G, Delli-Bovi P, Persico MG (1991) Proc Natl Acad Sci USA 88:9267
44. Olofsson B, Pajusola K, Kaipainen A, von Euler G, Joukov V, Saksela O, Orpana A, Pettersson RF, Alitalo K, Eriksson U (1996) Proc Natl Acad Sci USA 93:2576
45. Nash AD, Baca M, Wright C, Scotney PD (2006) Pulm Pharmacol Ther 19:61
46. Kukk E, Lymboussaki A, Taira S, Kaipainen A, Jeltsch M, Joukov V, Alitalo K (1996) Development 122:3829
47. Onogawa S, Kitadai Y, Tanaka S, Kuwai T, Kimura S, Chayama K (2004) Cancer Sci 95:32
48. Li X, Eriksson U (2001) Int J Biochem Cell Biol 33:421
49. Achen MG, Jeltsch M, Kukk E, Makinen T, Vitali A, Wilks AF, Alitalo K, Stacker SA (1998) Proc Natl Acad Sci USA 95:548
50. Krishnan J, Kirkin V, Steffen A, Hegen M, Weih D, Tomarev S, Wilting J, Sleeman JP (2003) Cancer Res 63:713
51. Wong D, Luckhurst J, Toma H, Kuipers N, Loo S, Suarez A, Wilson JE, McManus BM (2001) J Heart Lung Transplant 20:156
52. Yamada Y, Nezu J, Shimane M, Hirata Y (1997) Genomics 42:483
53. Van Trappen PO, Steele D, Lowe DG, Baithun S, Beasley N, Thiele W, Weich H, Krishnan J, Shepherd JH, Pepper MS, Jackson DG, Sleeman JP, Jacobs IJ (2003) J Pathol 201:544
54. Lyttle DJ, Fraser KM, Fleming SB, Mercer AA, Robinson AJ (1994) J Virol 68:84
55. Kiba A, Sagara H, Hara T, Shibuya M (2003) Biochem Biophys Res Commun 301:371
56. Meyer M, Clauss M, Lepple-Wienhues A, Waltenberger J, Augustin HG, Ziche M, Lanz C, Buttner M, Rziha HJ, Dehio C (1999) Embo J 18:363
57. Ogawa S, Oku A, Sawano A, Yamaguchi S, Yazaki Y, Shibuya M (1998) J Biol Chem 273:31273
58. Shibuya M (2003) Cancer Sci 94:751
59. Ferrara N, Gerber HP, LeCouter J (2003) Nat Med 9:669
60. Robinson CJ, Stringer SE (2001) J Cell Sci 114:853
61. Barleon B, Siemeister G, Martiny-Baron G, Weindel K, Herzog C, Marme D (1997) Cancer Res 57:5421
62. de Vries C, Escobedo JA, Ueno H, Houck K, Ferrara N, Williams LT (1992) Science 255:989
63. Millauer B, Wizigmann-Voos S, Schnurch H, Martinez R, Moller NP, Risau W, Ullrich A (1993) Cell 72:835
64. Terman BI, Dougher-Vermazen M, Carrion ME, Dimitrov D, Armellino DC, Gospodarowicz D, Bohlen P (1992) Biochem Biophys Res Commun 187:1579
65. Lenton K (2002) J Biol Regul Homeost Agents 16:227
66. Meyer RD, Rahimi N (2003) Ann NY Acad Sci 995:200
67. Birnbaum D (1995) Jpn J Cancer Res 86:inside cover
68. Terman B, Khandke L, Dougher-Vermazan M, Maglione D, Lassam NJ, Gospodarowicz D, Persico MG, Bohlen P, Eisinger M (1994) Growth Factors 11:187
69. Wang D, Donner DB, Warren RS (2000) J Biol Chem 275:15905
70. Rahimi N (2006) Front Biosci 11:818
71. Peters KG, De Vries C, Williams LT (1993) Proc Natl Acad Sci USA 90:8915
72. Quinn TP, Peters KG, De Vries C, Ferrara N, Williams LT (1993) Proc Natl Acad Sci USA 90:7533

73. Sato Y, Kanno S, Oda N, Abe M, Ito M, Shitara K, Shibuya M (2000) Ann NY Acad Sci 902:201
74. Galland F, Karamysheva A, Pebusque MJ, Borg JP, Rottapel R, Dubreuil P, Rosnet O, Birnbaum D (1993) Oncogene 8:1233
75. Kaipainen A, Korhonen J, Pajusola K, Aprelikova O, Persico MG, Terman BI, Alitalo K (1993) J Exp Med 178:2077
76. Pajusola K, Aprelikova O, Korhonen J, Kaipainen A, Pertovaara L, Alitalo R, Alitalo K (1992) Cancer Res 52:5738
77. Andre T, Kotelevets L, Vaillant JC, Coudray AM, Weber L, Prevot S, Parc R, Gespach C, Chastre E (2000) Int J Cancer 86:174
78. Hewett PW, Murray JC (1996) Biochem Biophys Res Commun 221:697
79. Smith G, McLeod D, Foreman D, Boulton M (1999) Br J Ophthalmol 83:486
80. Hughes DC (2001) J Mol Evol 53:77
81. Joukov V, Pajusola K, Kaipainen A, Chilov D, Lahtinen I, Kukk E, Saksela O, Kalkkinen N, Alitalo K (1996) Embo J 15:290
82. Lee J, Gray A, Yuan J, Luoh SM, Avraham H, Wood WI (1996) Proc Natl Acad Sci USA 93:1988
83. Wise LM, Veikkola T, Mercer AA, Savory LJ, Fleming SB, Caesar C, Vitali A, Makinen T, Alitalo K, Stacker SA (1999) Proc Natl Acad Sci USA 96:3071
84. Romeo PH, Lemarchandel V, Tordjman R (2002) Adv Exp Med Biol 515:49
85. Tordjman R, Lepelletier Y, Lemarchandel V, Cambot M, Gaulard P, Hermine O, Romeo PH (2002) Nat Immunol 3:477
86. Mamluk R, Gechtman Z, Kutcher ME, Gasiunas N, Gallagher J, Klagsbrun M (2002) J Biol Chem 277:24818
87. Migdal M, Huppertz B, Tessler S, Comforti A, Shibuya M, Reich R, Baumann H, Neufeld G (1998) J Biol Chem 273:22272
88. Gluzman-Poltorak Z, Cohen T, Herzog Y, Neufeld G (2000) J Biol Chem 275:18040
89. Gluzman-Poltorak Z, Cohen T, Herzog Y, Neufeld G (2000) J Biol Chem 275:29922
90. Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M (1998) Cell 92:735
91. Neufeld G, Kessler O, Herzog Y (2002) Adv Exp Med Biol 515:81
92. Kreuter M, Bielenberg D, Hida Y, Hida K, Klagsbrun M (2002) Ann Hematol 81(Suppl 2):S74
93. Nakamura F, Goshima Y (2002) Adv Exp Med Biol 515:55
94. Neufeld G, Cohen T, Shraga N, Lange T, Kessler O, Herzog Y (2002) Trend Cardiovasc Med 12:13
95. Ikeda M, Hosoda Y, Hirose S, Okada Y, Ikeda E (2000) J Pathol 191:426
96. Gagnon ML, Bielenberg DR, Gechtman Z, Miao HQ, Takashima S, Soker S, Klagsbrun M (2000) Proc Natl Acad Sci USA 97:2573
97. Lee P, Goishi K, Davidson AJ, Mannix R, Zon L, Klagsbrun M (2002) Proc Natl Acad Sci USA 99:10470
98. Oh H, Takagi H, Otani A, Koyama S, Kemmochi S, Uemura A, Honda Y (2002) Proc Natl Acad Sci USA 99:383
99. Whitaker GB, Limberg BJ, Rosenbaum JS (2001) J Biol Chem 276:25520
100. Gill M, Dias S, Hattori K, Rivera ML, Hicklin D, Witte L, Girardi L, Yurt R, Himel H, Rafii S (2001) Circ Res 88:167
101. Peichev M, Naiyer AJ, Pereira D, Zhu Z, Lane WJ, Williams M, Oz MC, Hicklin DJ, Witte L, Moore MA, Rafii S (2000) Blood 95:952
102. Solovey A, Lin Y, Browne P, Choong S, Wayner E, Hebbel RP (1997) N Engl J Med 337:1584

103. Oberg C, Waltenberger J, Claesson-Welsh L, Welsh M (1994) Growth Factors 10:115
104. Yang K, Cepko CL (1996) J Neurosci 16:6089
105. Katoh O, Tauchi H, Kawaishi K, Kimura A, Satow Y (1995) Cancer Res 55:5687
106. Brown LF, Detmar M, Tognazzi K, Abu-Jawdeh G, Iruela-Arispe ML (1997) Lab Invest 76:245
107. Hatva E, Kaipainen A, Mentula P, Jaaskelainen J, Paetau A, Haltia M, Alitalo K (1995) Am J Pathol 146:368
108. Boocock CA, Charnock-Jones DS, Sharkey AM, McLaren J, Barker PJ, Wright KA, Twentyman PR, Smith SK (1995) J Natl Cancer Inst 87:506
109. Carmeliet P, Ferreira V, Breier G, Pollefeyt S, Kieckens L, Gertsenstein M, Fahrig M, Vandenhoeck A, Harpal K, Eberhardt C, Declercq C, Pawling J, Moons L, Collen D, Risau W, Nagy A (1996) Nature 380:435
110. Shalaby F, Ho J, Stanford WL, Fischer KD, Schuh AC, Schwartz L, Bernstein A, Rossant J (1997) Cell 89:981
111. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, Schuh AC (1995) Nature 376:62
112. Matthews W, Jordan CT, Gavin M, Jenkins NA, Copeland NG, Lemischka IR (1991) Proc Natl Acad Sci USA 88:9026
113. Cunningham SA, Stephan CC, Arrate MP, Ayer KG, Brock TA (1997) Biochem Biophys Res Commun 231:596
114. Fuh G, Li B, Crowley C, Cunningham B, Wells JA (1998) J Biol Chem 273:11197
115. Lu D, Kussie P, Pytowski B, Persaud K, Bohlen P, Witte L, Zhu Z (2000) J Biol Chem 275:14321
116. Muller YA, Li B, Christinger HW, Wells JA, Cunningham BC, de Vos AM (1997) Proc Natl Acad Sci USA 94:7192
117. Shinkai A, Ito M, Anazawa H, Yamaguchi S, Shitara K, Shibuya M (1998) J Biol Chem 273:31283
118. Wiesmann C, Fuh G, Christinger HW, Eigenbrot C, Wells JA, de Vos AM (1997) Cell 91:695
119. Kroll J, Waltenberger J (1997) J Biol Chem 272:32521
120. Seetharam L, Gotoh N, Maru Y, Neufeld G, Yamaguchi S, Shibuya M (1995) Oncogene 10:135
121. Waltenberger J, Claesson-Welsh L, Siegbahn A, Shibuya M, Heldin CH (1994) J Biol Chem 269:26988
122. Ito N, Wernstedt C, Engstrom U, Claesson-Welsh L (1998) J Biol Chem 273:23410
123. Abedi H, Zachary I (1997) J Biol Chem 272:15442
124. Gerber HP, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, Ferrara N (1998) J Biol Chem 273:30336
125. Guo D, Jia Q, Song HY, Warren RS, Donner DB (1995) J Biol Chem 270:6729
126. Takahashi T, Shibuya M (1997) Oncogene 14:2079
127. Rousseau S, Houle F, Kotanides H, Witte L, Waltenberger J, Landry J, Huot J (2000) J Biol Chem 275:10661
128. Warner AJ, Lopez-Dee J, Knight EL, Feramisco JR, Prigent SA (2000) Biochem J 347:501
129. Wu LW, Mayo LD, Dunbar JD, Kessler KM, Ozes ON, Warren RS, Donner DB (2000) J Biol Chem 275:6059
130. Takahashi N, Seko Y, Noiri E, Tobe K, Kadowaki T, Sabe H, Yazaki Y (1999) Circ Res 84:1194
131. Takahashi T, Shibuya M (2001) Biochem Biophys Res Commun 280:415

132. Takahashi T, Ueno H, Shibuya M (1999) Oncogene 18:2221
133. Takahashi T, Yamaguchi S, Chida K, Shibuya M (2001) Embo J 20:2768
134. Eliceiri BP, Paul R, Schwartzberg PL, Hood JD, Leng J, Cheresh DA (1999) Mol Cell 4:915
135. Eliceiri BP, Puente XS, Hood JD, Stupack DG, Schlaepfer DD, Huang XZ, Sheppard D, Cheresh DA (2002) J Cell Biol 157:149
136. Igarashi K, Isohara T, Kato T, Takigawa K, Shigeta K, Yamano T, Uno II (1998) Int J Mol Med 2:211
137. Igarashi K, Shigeta K, Isohara T, Yamano T, Uno I (1998) Biochem Biophys Res Commun 251:77
138. Dimmeler S, Dernbach E, Zeiher AM (2000) FEBS Lett 477:258
139. Gliki G, Abu-Ghazaleh R, Jezequel S, Wheeler-Jones C, Zachary I (2001) Biochem J 353:503
140. Hood J, Granger HJ (1998) J Biol Chem 273:23504
141. Zachary I, Gliki G (2001) Cardiovasc Res 49:568
142. Gille H, Kowalski J, Li B, LeCouter J, Moffat B, Zioncheck TF, Pelletier N, Ferrara N (2001) J Biol Chem 276:3222
143. Li B, Fuh G, Meng G, Xin X, Gerritsen ME, Cunningham B, de Vos AM (2000) J Biol Chem 275:29823
144. Larrivee B, Karsan A (2000) Int J Mol Med 5:447
145. Brown LF, Berse B, Jackman RW, Tognazzi K, Guidi AJ, Dvorak HF, Senger DR, Connolly JL, Schnitt SJ (1995) Hum Pathol 26:86
146. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Dvorak HF, Senger DR (1993) Am J Pathol 143:1255
147. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Senger DR, Dvorak HF (1993) Cancer Res 53:4727
148. Brown LF, Berse B, Tognazzi K, Manseau EJ, Van de Water L, Senger DR, Dvorak HF, Rosen S (1992) Kidney Int 42:1457
149. Brown LF, Harrist TJ, Yeo KT, Stahle-Backdahl M, Jackman RW, Berse B, Tognazzi K, Dvorak HF, Detmar M (1995) J Invest Dermatol 104:744
150. Brown LF, Tognazzi K, Dvorak HF, Harrist TJ (1996) Am J Pathol 148:1065
151. Guidi AJ, Abu-Jawdeh G, Berse B, Jackman RW, Tognazzi K, Dvorak HF, Brown LF (1995) J Natl Cancer Inst 87:1237
152. Guidi AJ, Abu-Jawdeh G, Tognazzi K, Dvorak HF, Brown LF (1996) Cancer 78:454
153. Guidi AJ, Schnitt SJ, Fischer L, Tognazzi K, Harris JR, Dvorak HF, Brown LF (1997) Cancer 80:1945
154. Olson TA, Mohanraj D, Carson LF, Ramakrishnan S (1994) Cancer Res 54:276
155. Plate KH, Breier G, Millauer B, Ullrich A, Risau W (1993) Cancer Res 53:5822
156. Wang ES, Teruya-Feldstein J, Wu Y, Zhu Z, Hicklin DJ, Moore MA (2004) Blood 104:2893
157. Dias S, Hattori K, Zhu Z, Heissig B, Choy M, Lane W, Wu Y, Chadburn A, Hyjek E, Gill M, Hicklin DJ, Witte L, Moore MA, Rafii S (2000) J Clin Invest 106:511
158. Wizigmann-Voos S, Breier G, Risau W, Plate KH (1995) Cancer Res 55:1358
159. Barnhill RL, Fandrey K, Levy MA, Mihm MC Jr, Hyman B (1992) Lab Invest 67:331
160. Barnhill RL, Piepkorn MW, Cochran AJ, Flynn E, Karaoli T, Folkman J (1998) Arch Dermatol 134:991
161. Gasparini G, Barbareschi M, Boracchi P, Verderio P, Caffo O, Meli S, Palma PD, Marubini E, Bevilacqua P (1995) Cancer J Sci Am 1:131
162. Gasparini G, Harris AL (1995) J Clin Oncol 13:765

163. Gasparini G, Toi M, Verderio P, Ranieri G, Dante S, Bonoldi E, Boracchi P, Fanelli M, Tominaga T (1998) Int J Oncol 12:1117
164. Gasparini G, Weidner N, Maluta S, Pozza F, Boracchi P, Mezzetti M, Testolin A, Bevilacqua P (1993) Int J Cancer 55:739
165. Lindmark G, Gerdin B, Pahlman L, Bergstrom R, Glimelius B (1994) Dis Colon Rectum 37:1219
166. Lindmark G, Gerdin B, Sundberg C, Pahlman L, Bergstrom R, Glimelius B (1996) J Clin Oncol 14:461
167. Macchiarini P, Fontanini G, Hardin MJ, Squartini F, Angeletti CA (1992) Lancet 340:145
168. Olivarez D, Ulbright T, DeRiese W, Foster R, Reister T, Einhorn L, Sledge G (1994) Cancer Res 54:2800
169. Tanigawa N, Amaya H, Matsumura M, Shimomatsuya T, Horiuchi T, Muraoka R, Iki M (1996) Cancer Res 56:2671
170. Tanigawa N, Matsumura M, Amaya H, Kitaoka A, Shimomatsuya T, Lu C, Muraoka R, Tanaka T (1997) Cancer 79:220
171. Weidner N, Carroll PR, Flax J, Blumenfeld W, Folkman J (1993) Am J Pathol 143:401
172. Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, Moore DH, Meli S, Gasparini G (1992) J Natl Cancer Inst 84:1875
173. Fong GH, Rossant J, Gertsenstein M, Breitman ML (1995) Nature 376:66
174. Hicklin DJ, Witte L, Zhu Z, Liao F, Wu Y, Li Y, Bohlen P (2001) Drug Discov Today 6:517
175. Lu D, Jimenez X, Zhang H, Bohlen P, Witte L, Zhu Z (2002) Int J Cancer 97:393
176. Lu D, Shen J, Vil MD, Zhang H, Jimenez X, Bohlen P, Witte L, Zhu Z (2003) J Biol Chem 278:43496
177. Posey JA, Ng TC, Yang B, Khazaeli MB, Carpenter MD, Fox F, Needle M, Waksal H, LoBuglio AF (2003) Clin Cancer Res 9:1323
178. Watanabe H, Mamelak AJ, Wang B, Howell BG, Freed I, Esche C, Nakayama M, Nagasaki G, Hicklin DJ, Kerbel RS, Sauder DN (2004) Exp Dermatol 13:671
179. Zhu Z, Hattori K, Zhang H, Jimenez X, Ludwig DL, Dias S, Kussie P, Koo H, Kim HJ, Lu D, Liu M, Tejada R, Friedrich M, Bohlen P, Witte L, Rafii S (2003) Leukemia 17:604
180. Boyer SJ (2002) Curr Top Med Chem 2:973
181. Ciardiello F, Bianco R, Caputo R, Caputo R, Damiano V, Troiani T, Melisi D, De Vita F, De Placido S, Bianco AR, Tortora G (2004) Clin Cancer Res 10:784
182. Drevs J, Muller-Driver R, Wittig C, Fuxius S, Esser N, Hugenschmidt H, Konerding MA, Allegrini PR, Wood J, Hennig J, Unger C, Marme D (2002) Cancer Res 62:4015
183. Fraley ME, Hoffman WF, Arrington KL, Hungate RW, Hartman GD, McFall RC, Coll KE, Rickert K, Thomas KA, McGaughey GB (2004) Curr Med Chem 11:709
184. Gingrich DE, Reddy DR, Iqbal MA, Singh J, Aimone LD, Angeles TS, Albom M, Yang S, Ator MA, Meyer SL, Robinson C, Ruggeri BA, Dionne CA, Vaught JL, Mallamo JP, Hudkins RL (2003) J Med Chem 46:5375
185. Laird AD, Cherrington JM (2003) Expert Opin Investig Drugs 12:51
186. Shawver LK, Slamon D, Ullrich A (2002) Cancer Cell 1:117
187. Traxler P, Allegrini PR, Brandt R, Brueggen J, Cozens R, Fabbro D, Grosios K, Lane HA, McSheehy P, Mestan J, Meyer T, Tang C, Wartmann M, Wood J, Caravatti G (2004) Cancer Res 64:4931
188. Fiedler W, Graeven U, Ergun S, Verago S, Kilic N, Stockschlader M, Hossfeld DK (1997) Blood 89:1870

189. Graeven U, Fiedler W, Karpinski S, Ergun S, Kilic N, Rodeck U, Schmiegel W, Hossfeld DK (1999) J Cancer Res Clin Oncol 125:621
190. Ratajczak MZ, Ratajczak J, Machalinski B, Majka M, Marlicz W, Carter A, Pietrzkowski Z, Gewirtz AM (1998) Br J Haematol 103:969
191. Dias S, Hattori K, Heissig B, Zhu Z, Wu Y, Witte L, Hicklin DJ, Tateno M, Bohlen P, Moore MA, Rafii S (2001) Proc Natl Acad Sci USA 98:10857
192. Zhang H, Li Y, Li H, Bassi R, Jimenez X, Witte L, Bohlen P, Hicklin D, Zhu Z (2004) Leuk Lymphoma 45:1887
193. Ria R, Vacca A, Russo F, Cirulli T, Massaia M, Tosi P, Cavo M, Guidolin D, Ribatti D, Dammacco F (2004) Thromb Haemost 92:1438
194. Rockwell P, Neufeld G, Glassman A, Caron D, Goldstein N (1995) Mol Cell Differ 3:91
195. Prewett M, Huber J, Li Y, Santiago A, O'Connor W, King K, Overholser J, Hooper A, Pytowski B, Witte L, Bohlen P, Hicklin DJ (1999) Cancer Res 59:5209
196. Paz K, Zhu Z (2005) Front Biosci 10:1415
197. Zhu Z, Witte L (1999) Invest New Drugs 17:195
198. Witte L, Hicklin DJ, Zhu Z, Pytowski B, Kotanides H, Rockwell P, Bohlen P (1998) Cancer Metastasis Rev 17:155
199. Bruns CJ, Shrader M, Harbison MT, Portera C, Solorzano CC, Jauch KW, Hicklin DJ, Radinsky R, Ellis LM (2002) Int J Cancer 102:101
200. Kozin SV, Boucher Y, Hicklin DJ, Bohlen P, Jain RK, Suit HD (2001) Cancer Res 61:39
201. Sweeney P, Karashima T, Kim SJ, Kedar D, Mian B, Huang S, Baker C, Fan Z, Hicklin DJ, Pettaway CA, Dinney CP (2002) Clin Cancer Res 8:2714
202. Zimmermann RC, Hartman T, Bohlen P, Sauer MV, Kitajewski J (2001) Microvasc Res 62:15
203. Pauli SA, Tang H, Wang J, Bohlen P, Posser R, Hartman T, Sauer MV, Kitajewski J, Zimmermann RC (2005) Endocrinology 146:1301
204. Manley PW, Bold G, Bruggen J, Fendrich G, Furet P, Mestan J, Schnell C, Stolz B, Meyer T, Meyhack B, Stark W, Strauss A, Wood J (2004) Biochim Biophys Acta 1697:17
205. Hunt S (2001) Curr Opin Mol Ther 3:418
206. Zhu Z, Rockwell P, Lu D, Kotanides H, Pytowski B, Hicklin DJ, Bohlen P, Witte L (1998) Cancer Res 58:3209
207. Zhu Z, Lu D, Kotanides H, Santiago A, Jimenez X, Simcox T, Hicklin DJ, Bohlen P, Witte L (1999) Cancer Lett 136:203
208. McLeod DS, Taomoto M, Cao J, Zhu Z, Witte L, Lutty GA (2002) Invest Ophthalmol Vis Sci 43:474
209. Traxler P, Bold G, Buchdunger E, Caravatti G, Furet P, Manley P, O'Reilly T, Wood J, Zimmermann J (2001) Med Res Rev 21:499
210. Underiner TL, Ruggeri B, Gingrich DE (2004) Curr Med Chem 11:731
211. Bayes M, Rabasseda X, Prous JR (2003) Methods Find Exp Clin Pharmacol 25:483
212. Motzer RJ, Hoosen S, Bello CL, Christensen JG (2006) Expert Opin Investig Drugs 15:553
213. Ryan AJ, Wedge SR (2005) Br J Cancer 92(Suppl 1):S6
214. Hannah AL (2005) Curr Mol Med 5:625
215. Sridhar SS, Shepherd FA (2003) Lung Cancer 42(Suppl 1):S81
216. Antonian L, Zhang H, Yang C, Wagner G, Shawver LK, Shet M, Ogilvie B, Madan A, Parkinson A (2000) Drug Metab Dispos 28:1505
217. Bischof M, Abdollahi A, Gong P, Stoffregen C, Lipson KE, Debus JU, Weber KJ, Huber PE (2004) Int J Radiat Oncol Biol Phys 60:1220

218. Haspel HC, Scicli GM, McMahon G, Scicli AG (2002) Microvasc Res 63:304
219. Huss WJ, Barrios RJ, Greenberg NM (2003) Mol Cancer Ther 2:611
220. Mendel DB, Laird AD, Smolich BD, Blake RA, Liang C, Hannah AL, Shaheen RM, Ellis LM, Weitman S, Shawver LK, Cherrington JM (2000) Anticancer Drug Des 15:29
221. Vajkoczy P, Menger MD, Vollmar B, Schilling L, Schmiedek P, Hirth KP, Ullrich A, Fong TA (1999) Neoplasia 1:31
222. Zhao Y, Yang CY, Haznedar J, Antonian L (2001) J Pharm Biomed Anal 25:821
223. Giles FJ, Stopeck AT, Silverman LR, Lancet JE, Cooper MA, Hannah AL, Cherrington JM, O'Farrell AM, Yuen HA, Louie SG, Hong W, Cortes JE, Verstovsek S, Albitar M, O'Brien SM, Kantarjian HM, Karp JE (2003) Blood 102:795
224. Kuenen BC, Rosen L, Smit EF, Parson MR, Levi M, Ruijter R, Huisman H, Kedde MA, Noordhuis P, van der Vijgh WJ, Peters GJ, Cropp GF, Scigalla P, Hoekman K, Pinedo HM, Giaccone G (2002) J Clin Oncol 20:1657
225. Stopeck A, Sheldon M, Vahedian M, Cropp G, Gosalia R, Hannah A (2002) Clin Cancer Res 8:2798
226. Dowlati A, Robertson K, Radivoyevitch T, Waas J, Ziats NP, Hartman P, Abdul-Karim FW, Wasman JK, Jesberger J, Lewin J, McCrae K, Ivy P, Remick SC (2005) Clin Cancer Res 11:7938
227. O'Donnell A, Padhani A, Hayes C, Kakkar AJ, Leach M, Trigo JM, Scurr M, Raynaud F, Phillips S, Aherne W, Hardcastle A, Workman P, Hannah A, Judson I (2005) Br J Cancer 93:876
228. Hoff PM, Wolff RA, Bogaard K, Waldrum S, Abbruzzese JL (2006) Jpn J Clin Oncol 36:100
229. Salzberg M, Pless M, Rochlitz C, Ambrus K, Scigalla P, Herrmann R (2006) Invest New Drugs 24:299
230. Fiedler W, Mesters R, Tinnefeld H, Loges S, Staib P, Duhrsen U, Flasshove M, Ottmann OG, Jung W, Cavalli F, Kuse R, Thomalla J, Serve H, O'Farrell AM, Jacobs M, Brega NM, Scigalla P, Hossfeld DK, Berdel WE (2003) Blood 102:2763
231. Giles FJ, Cooper MA, Silverman L, Karp JE, Lancet JE, Zangari M, Shami PJ, Khan KD, Hannah AL, Cherrington JM, Thomas DA, Garcia-Manero G, Albitar M, Kantarjian HM, Stopeck AT (2003) Cancer 97:1920
232. Heymach JV, Desai J, Manola J, Davis DW, McConkey DJ, Harmon D, Ryan DP, Goss G, Quigley T, Van den Abbeele AD, Silverman SG, Connors S, Folkman J, Fletcher CD, Demetri GD (2004) Clin Cancer Res 10:5732
233. Mesters RM, Padro T, Bieker R, Steins M, Kreuter M, Goner M, Kelsey S, Scigalla P, Fiedler W, Buchner T, Berdel WE (2001) Blood 98:241
234. Peterson AC, Swiger S, Stadler WM, Medved M, Karczmar G, Gajewski TF (2004) Clin Cancer Res 10:4048
235. Stadler WM, Cao D, Vogelzang NJ, Ryan CW, Hoving K, Wright R, Karrison T, Vokes EE (2004) Clin Cancer Res 10:3365
236. Zangari M, Anaissie E, Stopeck A, Morimoto A, Tan N, Lancet J, Cooper M, Hannah A, Garcia-Manero G, Faderl S, Kantarjian H, Cherrington J, Albitar M, Giles FJ (2004) Clin Cancer Res 10:88
237. Fury MG, Zahalsky A, Wong R, Venkatraman E, Lis E, Hann L, Aliff T, Gerald W, Fleisher M, Pfister DG (2006) Invest New Drugs (published online 19 Sept 2006)
238. Anon (2002) Expert Rev Anticancer Ther 2:5
239. Laird AD, Christensen JG, Li G, Carver J, Smith K, Xin X, Moss KG, Louie SG, Mendel DB, Cherrington JM (2002) Faseb J 16:681
240. Laird AD, Vajkoczy P, Shawver LK, Thurnher A, Liang C, Mohammadi M, Schlessinger J, Ullrich A, Hubbard SR, Blake RA, Fong TA, Strawn LM, Sun L, Tang C,

Hawtin R, Tang F, Shenoy N, Hirth KP, McMahon G, Cherrington JM (2000) Cancer Res 60:4152
241. Hoekman K (2001) Cancer J 7(Suppl 3):S134
242. Shaheen RM, Davis DW, Liu W, Zebrowski BK, Wilson MR, Bucana CD, McConkey DJ, McMahon G, Ellis LM (1999) Cancer Res 59:5412
243. Kim DW, Jo YS, Jung HS, Chung HK, Song JH, Park KC, Park SH, Hwang JH, Rha SY, Kweon GR, Lee SJ, Jo KW, Shong M (2006) J Clin Endocrinol Metab 91(10):4070
244. Tokuyama J, Kubota T, Saikawa Y, Yoshida M, Furukawa T, Otani Y, Kumai K, Kitajima M (2005) Anticancer Res 25:17
245. Kuenen BC, Giaccone G, Ruijter R, Kok A, Schalkwijk C, Hoekman K, Pinedo HM (2005) Clin Cancer Res 11:6240
246. Lu B, Geng L, Musiek A, Tan J, Cao C, Donnelly E, McMahon G, Choy H, Hallahan DE (2004) Int J Radiat Oncol Biol Phys 58:844
247. Nakamura T, Ozawa S, Kitagawa Y, Ueda M, Kubota T, Kitajima M (2006) Oncol Rep 15:79
248. Jiang XT, Tao HQ, Zou SC (2006) Zhonghua Wei Chang Wai Ke Za Zhi 9:335
249. Zhou Q, Olivo M, Lye KY, Moore S, Sharma A, Chowbay B (2005) Cancer Chemother Pharmacol 56:569
250. Yorozuya K, Kubota T, Watanabe M, Hasegawa H, Ozawa S, Kitajima M, Chikahisa LM, Yamada Y (2005) Oncol Rep 14:677
251. Marzola P, Degrassi A, Calderan L, Farace P, Crescimanno C, Nicolato E, Giusti A, Pesenti E, Terron A, Sbarbati A, Abrams T, Murray L, Osculati F (2004) Clin Cancer Res 10:739
252. Garofalo A, Naumova E, Manenti L, Ghilardi C, Ghisleni G, Caniatti M, Colombo T, Cherrington JM, Scanziani E, Nicoletti MI, Giavazzi R (2003) Clin Cancer Res 9:3476
253. Abdollahi A, Lipson KE, Han X, Krempien R, Trinh T, Weber KJ, Hahnfeldt P, Hlatky L, Debus J, Howlett AR, Huber PE (2003) Cancer Res 63:3755
254. Xiong HQ, Herbst R, Faria SC, Scholz C, Davis D, Jackson EF, Madden T, McConkey D, Hicks M, Hess K, Charnsangavej CA, Abbruzzese JL (2004) Invest New Drugs 22:459
255. Sistla A, Sunga A, Phung K, Koparkar A, Shenoy N (2004) Drug Dev Ind Pharm 30:19
256. Takahashi H, Obata R, Tamaki Y (2006) J Ocul Pharmacol Ther 22:213
257. Faivre S, Delbaldo C, Vera K, Robert C, Lozahic S, Lassau N, Bello C, Deprimo S, Brega N, Massimini G, Armand JP, Scigalla P, Raymond E (2006) J Clin Oncol 24:25
258. Cabebe E, Wakelee H (2006) Drugs Today (Barc) 42:387
259. Abrams TJ, Lee LB, Murray LJ, Pryer NK, Cherrington JM (2003) Mol Cancer Ther 2:471
260. Baratte S, Sarati S, Frigerio E, James CA, Ye C, Zhang Q (2004) J Chromatogr A 1024:87
261. Bayes M, Rabasseda X, Prous JR (2004) Methods Find Exp Clin Pharmacol 26:473
262. Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, Schreck RE, Abrams TJ, Ngai TJ, Lee LB, Murray LJ, Carver J, Chan E, Moss KG, Haznedar JO, Sukbuntherng J, Blake RA, Sun L, Tang C, Miller T, Shirazian S, McMahon G, Cherrington JM (2003) Clin Cancer Res 9:327
263. O'Farrell AM, Abrams TJ, Yuen HA, Ngai TJ, Louie SG, Yee KW, Wong LM, Hong W, Lee LB, Town A, Smolich BD, Manning WC, Murray LJ, Heinrich MC, Cherrington JM (2003) Blood 101:3597
264. Sun L, Liang C, Shirazian S, Zhou Y, Miller T, Cui J, Fukuda JY, Chu JY, Nematalla A, Wang X, Chen H, Sistla A, Luu TC, Tang F, Wei J, Tang C (2003) J Med Chem 46:1116
265. von Mehren M (2003) Curr Treat Options Oncol 4:441

266. Abrams TJ, Murray LJ, Pesenti E, Holway VW, Colombo T, Lee LB, Cherrington JM, Pryer NK (2003) Mol Cancer Ther 2:1011
267. Morimoto AM, Tan N, West K, McArthur G, Toner GC, Manning WC, Smolich BD, Cherrington JM (2004) Oncogene 23:1618
268. Schueneman AJ, Himmelfarb E, Geng L, Tan J, Donnelly E, Mendel D, McMahon G, Hallahan DE (2003) Cancer Res 63:4009
269. Bello CL, Sherman L, Zhou J, Verkh L, Smeraglia J, Mount J, Klamerus KJ (2006) Anticancer Drugs 17:353
270. Fiedler W, Serve H, Dohner H, Schwittay M, Ottmann OG, O'Farrell AM, Bello CL, Allred R, Manning WC, Cherrington JM, Louie SG, Hong W, Brega NM, Massimini G, Scigalla P, Berdel WE, Hossfeld DK (2005) Blood 105:986
271. O'Farrell AM, Foran JM, Fiedler W, Serve H, Paquette RL, Cooper MA, Yuen HA, Louie SG, Kim H, Nicholas S, Heinrich MC, Berdel WE, Bello C, Jacobs M, Scigalla P, Manning WC, Kelsey S, Cherrington JM (2003) Clin Cancer Res 9:5465
272. Prenen H, Cools J, Mentens N, Folens C, Sciot R, Schoffski P, Van Oosterom A, Marynen P, Debiec-Rychter M (2006) Clin Cancer Res 12:2622
273. Bold G, Altmann KH, Frei J, Lang M, Manley PW, Traxler P, Wietfeld B, Bruggen J, Buchdunger E, Cozens R, Ferrari S, Furet P, Hofmann F, Martiny-Baron G, Mestan J, Rosel J, Sills M, Stover D, Acemoglu F, Boss E, Emmenegger R, Lasser L, Masso E, Roth R, Schlachter C, Vetterli W (2000) J Med Chem 43:2310
274. Drevs J, Hofmann I, Hugenschmidt H, Wittig C, Madjar H, Muller M, Wood J, Martiny-Baron G, Unger C, Marme D (2000) Cancer Res 60:4819
275. Furet P, Bold G, Hofmann F, Manley P, Meyer T, Altmann KH (2003) Bioorg Med Chem Lett 13:2967
276. Morgan B, Thomas AL, Drevs J, Hennig J, Buchert M, Jivan A, Horsfield MA, Mross K, Ball HA, Lee L, Mietlowski W, Fuxuis S, Unger C, O'Byrne K, Henry A, Cherryman GR, Laurent D, Dugan M, Marme D, Steward WP (2003) J Clin Oncol 21:3955
277. Thomas AL, Morgan B, Drevs J, Unger C, Wiedenmann B, Vanhoefer U, Laurent D, Dugan M, Steward WP (2003) Semin Oncol 30:32
278. Jost L, Gschwind HP, Jalava T, Wang Y, Guenther C, Souppart C, Rottmann A, Denner K, Waldmeier F, Gross G, Masson E, Laurent D (2006) Drug Metab Dispos 34:1817
279. Tyagi P (2005) Clin Colorectal Cancer 5:24
280. Wood JM, Bold G, Buchdunger E, Cozens R, Ferrari S, Frei J, Hofmann F, Mestan J, Mett H, O'Reilly T, Persohn E, Rosel J, Schnell C, Stover D, Theuer A, Towbin H, Wenger F, Woods-Cook K, Menrad A, Siemeister G, Schirner M, Thierauch KH, Schneider MR, Drevs J, Martiny-Baron G, Totzke F (2000) Cancer Res 60:2178
281. Gourley M, Williamson JS (2000) Curr Pharm Des 6:417
282. Thomas AL, Morgan B, Horsfield MA, Higginson A, Kay A, Lee L, Masson E, Puccio-Pick M, Laurent D, Steward WP (2005) J Clin Oncol 23:4162
283. Hennequin LF, Stokes ES, Thomas AP, Johnstone C, Ple PA, Ogilvie DJ, Dukes M, Wedge SR, Kendrew J, Curwen JO (2002) J Med Chem 45:1300
284. Wedge SR, Ogilvie DJ, Dukes M, Kendrew J, Curwen JO, Hennequin LF, Thomas AP, Stokes ES, Curry B, Richmond GH, Wadsworth PF (2000) Cancer Res 60:970
285. Checkley D, Tessier JJ, Wedge SR, Dukes M, Kendrew J, Curry B, Middleton B, Waterton JC (2003) Magn Reson Imaging 21:475
286. Pradel C, Siauve N, Bruneteau G, Clement O, de Bazelaire C, Frouin F, Wedge SR, Tessier JL, Robert PH, Frija G, Cuenod CA (2003) Magn Reson Imaging 21:845

287. Carlomagno F, Vitagliano D, Guida T, Ciardiello F, Tortora G, Vecchio G, Ryan AJ, Fontanini G, Fusco A, Santoro M (2002) Cancer Res 62:7284
288. Wedge SR, Ogilvie DJ, Dukes M, Kendrew J, Chester R, Jackson JA, Boffey SJ, Valentine PJ, Curwen JO, Musgrove HL, Graham GA, Hughes GD, Thomas AP, Stokes ES, Curry B, Richmond GH, Wadsworth PF, Bigley AL, Hennequin LF (2002) Cancer Res 62:4645
289. McCarty MF, Wey J, Stoeltzing O, Liu W, Fan F, Bucana C, Mansfield PF, Ryan AJ, Ellis LM (2004) Mol Cancer Ther 3:1041
290. Sandstrom M, Johansson M, Andersson U, Bergh A, Bergenheim AT, Henriksson R (2004) Br J Cancer 91:1174
291. Ciardiello F, Caputo R, Damiano V, Caputo R, Troiani T, Vitagliano D, Carlomagno F, Veneziani BM, Fontanini G, Bianco AR, Tortora G (2003) Clin Cancer Res 9:1546
292. Checkley D, Tessier JJ, Kendrew J, Waterton JC, Wedge SR (2003) Br J Cancer 89:1889
293. Heffelfinger SC, Yan M, Gear RB, Schneider J, LaDow K, Warshawsky D (2004) Lab Invest 84:989
294. Anon (1999) Drugs R D 1:135
295. Gingras D, Labelle D, Nyalendo C, Boivin D, Demeule M, Barthomeuf C, Beliveau R (2004) Invest New Drugs 22:17
296. Beliveau R, Gingras D, Kruger EA, Lamy S, Sirois P, Simard B, Sirois MG, Tranqui L, Baffert F, Beaulieu E, Dimitriadou V, Pepin MC, Courjal F, Ricard I, Poyet P, Falardeau P, Figg WD, Dupont E (2002) Clin Cancer Res 8:1242
297. Dupont E, Falardeau P, Mousa SA, Dimitriadou V, Pepin MC, Wang T, Alaoui-Jamali MA (2002) Clin Exp Metastasis 19:145
298. Dupont E, Wang B, Mamelak AJ, Howell BG, Shivji G, Zhuang L, Dimitriadou V, Falardeau P, Sauder DN (2003) J Cutan Med Surg
299. Gingras D, Batist G, Beliveau R (2001) Expert Rev Anticancer Ther 1:341
300. Gingras D, Boivin D, Deckers C, Gendron S, Barthomeuf C, Beliveau R (2003) Anticancer Drugs 14:91
301. Boivin D, Gendron S, Beaulieu E, Gingras D, Beliveau R (2002) Mol Cancer Ther 1:795
302. Latreille J, Batist G, Laberge F, Champagne P, Croteau D, Falardeau P, Levinton C, Hariton C, Evans WK, Dupont E (2003) Clin Lung Cancer 4:231
303. Shepherd FA (2001) Lung Cancer 34(Suppl 3):S81
304. Sauder DN, Dekoven J, Champagne P, Croteau D, Dupont E (2002) J Am Acad Dermatol 47:535
305. Anon (2001) Expert Rev Anticancer Ther 1:3
306. Batist G, Patenaude F, Champagne P, Croteau D, Levinton C, Hariton C, Escudier B, Dupont E (2002) Ann Oncol 13:1259
307. Weber MH, Lee J, Orr FW (2002) Int J Oncol 20:299
308. Dev IK, Dornsife RE, Hopper TM, Onori JA, Miller CG, Harrington LE, Dold KM, Mullin RJ, Johnson JH, Crosby RM, Truesdale AT, Epperly AH, Hinkle KW, Cheung M, Stafford JA, Luttrell DK, Kumar R (2004) Br J Cancer 91:1391
309. Hutson TE, Bukowski RM (2006) Clin Genitourin Cancer 4:296
310. Shi W, Siemann DW (2005) In Vivo 19:1045
311. Heymach JV (2005) Br J Cancer 92(Suppl 1):S14
312. Zirrolli JA, Bradshaw EL, Long ME, Gustafson DL (2005) J Pharm Biomed Anal 39:705
313. Tuccillo C, Romano M, Troiani T, Martinelli E, Morgillo F, De Vita F, Bianco R, Fontanini G, Bianco RA, Tortora G, Ciardiello F (2005) Clin Cancer Res 11:1268
314. Morgensztern D, Govindan R (2006) Expert Rev Anticancer Ther 6:545

315. Lee D (2005) Clin Lung Cancer 7:89
316. Rini BI (2005) Clin Genitourin Cancer 4:175
317. Li KL, Wilmes LJ, Henry RG, Pallavicini MG, Park JW, Hu-Lowe DD, McShane TM, Shalinsky DR, Fu YJ, Brasch RC, Hylton NM (2005) J Magn Reson Imaging 22:511
318. Rugo HS, Herbst RS, Liu G, Park JW, Kies MS, Steinfeldt HM, Pithavala YK, Reich SD, Freddo JL, Wilding G (2005) J Clin Oncol 23:5474
319. Yigitbasi OG, Younes MN, Doan D, Jasser SA, Schiff BA, Bucana CD, Bekele BN, Fidler IJ, Myers JN (2004) Cancer Res 64:7977
320. Lee SH, Lopes de Menezes D, Vora J, Harris A, Ye H, Nordahl L, Garrett E, Samara E, Aukerman SL, Gelb AB, Heise C (2005) Clin Cancer Res 11:3633
321. Trudel S, Li ZH, Wei E, Wiesmann M, Chang H, Chen C, Reece D, Heise C, Stewart AK (2005) Blood 105:2941
322. Ruggeri B, Singh J, Gingrich D, Angeles T, Albom M, Yang S, Chang H, Robinson C, Hunter K, Dobrzanski P, Jones-Bolin S, Pritchard S, Aimone L, Klein-Szanto A, Herbert JM, Bono F, Schaeffer P, Casellas P, Bourie B, Pili R, Isaacs J, Ator M, Hudkins R, Vaught J, Mallamo J, Dionne C (2003) Cancer Res 63:5978
323. Jones-Bolin S, Zhao H, Hunter K, Klein-Szanto A, Ruggeri B (2006) Mol Cancer Ther 5:1744
324. Siemeister G, Luecking U, Wagner C, Detjen K, Mc Coy C, Bosslet K (2006) Biomed Pharmacother 60:269
325. Fraley ME, Hoffman WF, Rubino RS, Hungate RW, Tebben AJ, Rutledge RZ, McFall RC, Huckle WR, Kendall RL, Coll KE, Thomas KA (2002) Bioorg Med Chem Lett 12:2767
326. Manley PJ, Balitza AE, Bilodeau MT, Coll KE, Hartman GD, McFall RC, Rickert KW, Rodman LD, Thomas KA (2003) Bioorg Med Chem Lett 13:1673
327. Anon (2002) Drugs R D 3:28
328. Anon (2003) Clin Colorectal Cancer 3:85
329. Anon (2004) Med Lett Drugs Ther 46:47
330. Ratner M (2004) Nat Biotechnol 22:1198
331. Holash J, Davis S, Papadopoulos N, Croll SD, Ho L, Russell M, Boland P, Leidich R, Hylton D, Burova E, Ioffe E, Huang T, Radziejewski C, Bailey K, Fandl JP, Daly T, Wiegand SJ, Yancopoulos GD, Rudge JS (2002) Proc Natl Acad Sci USA 99:11393
332. Konner J, Dupont J (2004) Clin Colorectal Cancer 4(Suppl 2):S81
333. Saishin Y, Saishin Y, Takahashi K, Lima e Silva R, Hylton D, Rudge JS, Wiegand SJ, Campochiaro PA (2003) J Cell Physiol 195:241
334. Forster Y, Meye A, Krause S, Schwenzer B (2004) Cancer Lett 212:95
335. He R, Liu B, Yang C, Yang RC, Tobelem G, Han ZC (2003) Cancer Gene Ther 10:879
336. Ruan GR, Liu YR, Chen SS, Fu JY, Chang Y, Qin YZ, Li JL, Yu H, Wang H (2004) Leuk Res 28:763
337. Jain RK (2002) Semin Oncol 29:3
338. Munn LL (2003) Drug Discov Today 8:396
339. Boehm T, Folkman J, Browder T, O'Reilly MS (1997) Nature 390:404
340. Geng L, Donnelly E, McMahon G, Lin PC, Sierra-Rivera E, Oshinka H, Hallahan DE (2001) Cancer Res 61:2413
341. Kerbel RS (1997) Nature 390:335
342. Kerbel RS (2001) Cancer Metastasis Rev 20:1
343. Klement G, Baruchel S, Rak J, Man S, Clark K, Hicklin DJ, Bohlen P, Kerbel RS (2000) J Clin Invest 105:R15
344. Klement G, Huang P, Mayer B, Green SK, Man S, Bohlen P, Hicklin D, Kerbel RS (2002) Clin Cancer Res 8:221

345. Yu JL, Rak JW, Coomber BL, Hicklin DJ, Kerbel RS (2002) Science 295:1526
346. Jung YD, Mansfield PF, Akagi M, Takeda A, Liu W, Bucana CD, Hicklin DJ, Ellis LM (2002) Eur J Cancer 38:1133
347. Huang X, Molema G, King S, Watkins L, Edgington TS, Thorpe PE (1997) Science 275:547
348. Jain RK (2001) Nat Med 7:987
349. Jain RK, Carmeliet PF (2001) Sci Am 285:38
350. Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ, Jain RK (2004) Cancer Res 64:3731
351. Lambin P, Landuyt W (2003) Verh K Acad Geneeskd Belg 65:29
352. Lee CG, Heijn M, di Tomaso E, Griffon-Etienne G, Ancukiewicz M, Koike C, Park KR, Ferrara N, Jain RK, Suit HD, Boucher Y (2000) Cancer Res 60:5565
353. Huang S, Armstrong EA, Benavente S, Chinnaiyan P, Harari PM (2004) Cancer Res 64:5355
354. Matar P, Rojo F, Cassia R, Moreno-Bueno G, Di Cosimo S, Tabernero J, Guzman M, Rodriguez S, Arribas J, Palacios J, Baselga J (2004) Clin Cancer Res 10:6487

Top Med Chem (2007) 1: 383–405
DOI 10.1007/7355_2006_010

Published online: 13 January 2007

Novel Small-Molecule Inhibitors of Src Kinase for Cancer Therapy

Tomi K. Sawyer

Pfizer Research Technology Center, 620 Memorial Drive, Cambridge, MA 02139, USA
tomi.k.sawyer@pfizer.com

1 Introduction 384

2 Src Kinase Genetics and Signal Transduction 386

3 Src Kinase Inhibitor: Structural Biology and Drug Design 388

4 Src Kinase Inhibitor: Chemical Diversity and Biological Properties 391
4.1 Pyrimidinylaminothiazole Template-Based Inhibitor BMS-354825 (Dasatinib, Sprycel™) 393
4.2 Quinazoline Template-Based Inhibitors AZM475271 (M475271) and AZD0530 394
4.3 Quinoline Template-Based Inhibitor SKI-606 (Bosutinib) 394
4.4 Pyridopyrimidinone Template-Based Inhibitors PD180970, PD173955 and PD166326 395
4.5 Pyrazolopyrimidine Template-Based Inhibitors PP1 and PP2 395
4.6 Pyrrolopyrimidine Template-Based Inhibitors CGP-76030 and CGP-76775 . 396
4.7 Indolinone Template-Based Inhibitor SU-6656 396
4.8 Purine Template-Based Inhibitors AP23464, AP23848, AP23846, AP23994, AP23451, and AP23588 397
4.9 Other Template-Based Inhibitors 398

5 Src Kinase Inhibitor: Drug Development for Cancer Therapy 399

6 Concluding Remarks 399

References 400

Abstract Drug discovery for cancer therapy is making extraordinary progress within the realm of advancing novel oncogenic protein kinase inhibitor lead compounds of varying chemical structure and biological mechanism. About 30 years ago, the first oncogenic protein kinase, $pp60^{src}$ (Src), was discovered and provided a prototype of the now-existing superfamily of Tyr, Ser/Thr and dual-specificity protein kinases. This review highlights Src kinase with respect to its known genetic and signal transduction pathways. Furthermore, several key Src kinase inhibitors are highlighted with respect to structural biology, drug design, chemical diversity, and biological properties. Noteworthy are a few novel small molecules that have enabled preclinical proof-of-concept studies as well as providing promising clinical candidates for cancer therapy.

Keywords Src · Src family kinases · Kinase selectivity · Structure-based design · Mechanism-based design

Abbreviations

ATP	Adenosine triphosphate
B-ALL	B-cell acute lymphoblastic leukemia
CML	Chronic myelogenous leukemia
EGFR	Epidermal growth factor receptor
FAK	Focal adhesion kinase
FDA	Food and Drug Administration
IL6-R	Interleukin-6 receptor
KO	Knockout
SCF	Stem cell factor
SFK	Src family kinase
STAT-3	Signal transducer and activator of transcription-3
VEGFR	Vascular endothelial growth factor receptor

1 Introduction

Cancer drug discovery is evolving with respect to the identification of key therapeutic targets for strategies focused on the ultimate objective to advance effective and safe-acting medicines. In this regard, oncogenic protein kinases are well-recognized as promising therapeutic targets, and their functional roles in cell growth, survival, differentiation, motility, cell–cell interactions, and/or cell–matrix interactions have provided a basis for mechanism-based strategies to create novel small-molecule inhibitors. Significant progress over the past decade has established small-molecule inhibitors of oncogenic protein kinase inhibitors of varying chemical and biological scope [1–20]. Noteworthy have been the development of novel proof-of-concept lead compounds, clinical candidates and, in a few cases, breakthrough medicines [21–25].

Of the nearly 300 cancer genes that have been reported to date, protein kinases represent the largest group (nearly 10%) having structural homology. Over the last 25 years, dating back to the identification of the non-receptor tyrosine kinase, $pp60^{src}$ (Src) [26], the protein kinase complement of the human genome sequence has been elucidated [27]. Many cancers have been correlated to somatic mutations of protein kinases, of which both receptor and non-receptor tyrosine kinases have emerged as particularly significant therapeutic targets for cancer drug discovery. Oncogenic transformation of protein kinases in humans may arise from fusion products of genomic re-arrangements (e.g., chromosomal translocations), mutations (e.g., gain-of-function), deletions, and overexpression resulting from

Table 1 Some known protein tyrosine, serine/threonine and dual specificity kinases

Receptor tyrosine kinases	***Receptor serine/threonine kinase***
Epidermal growth factor receptor (EGFR)	Transforming growth factor receptor-β (TGFR-β)
Fibroblast growth factor receptor (FGFR)	
Vascular endothelial growth factor receptor (VEGFR)	***Non-receptor serine/threonine kinases and dual specificity kinases***
Platelet-derived growth factor receptor (PDGFR)	cAMP-dependent protein kinase (PKA)
Stem cell reeptor (KIT)	Phosphoinositol-3-kinase (PI-3K)
Hematopoietic class III receptor (Flt)	Cyclin-dependent kinase (CDK)
Insulin receptor (IRK)	Mitogen-activated protein kinase (MAPK)
Insulin-like growth factor receptor (IGFR)	MAPKK (ERK)
Colony-stimulating factor receptor (CSFR)	MAPKKK (MEK)
Nerve growth factor receptor (NGFR)	Raf kinase
Hepatocyte growth factor receptor (Met)	Aurora kinases
Glial-derived neurotrophic factor receptor (RET)	Protein kinase-C (PKC)
	Protein kinase-B (PKB/Akt)
Non-receptor tyrosine kinases	mTor (FRAP)
Src and Src-family kinase (SFK)	Polo-like kinases (Plk)
Abl	Integrin-linked kinase (ILK)
FAK, Pyk2	Glycogen synthase kinase-3 (GSK-3)
Janus kinase (JAK) family	

gene amplification (Table 1) [10]. Such transformations typically result in enhanced or constitutive kinase activity, which then effects subsequent altered downstream signal transduction. Gene knockout (KO) and related functional genomic and cellular biology studies have further characterized a number of protein kinases in terms of signal transduction pathways and in vivo phenotypes as related to cancer or other diseases (e.g., Src gene KO and osteopetrosis).

As summarized in Table 2, some examples of oncogenic protein tyrosine kinases include EGFR, HER-2, HER-3, VEGFR-1 (Flt-1), VEGFR-2 (Flk-1/KDR), Flt-3, Flt-4, PDGFR-α, PDGFR-β, KIT, RET, MET, IGF-1R, Abl, Src, and Src family kinases (SFKs), FAK, Pyk2, and JAK family. Furthermore, a few examples of protein serine/threonine and dual-specificity kinases that have been identified as key therapeutic targets with respect to oncogenic signaling include TGFβR, CDK family, Raf, MEK, PKC, PI-3K, Akt, mTOR, and aurora kinases.

Table 2 Some functional genomic relationships of protein kinases to cancer phenotypes (adapted from [10])

Therapeutic target protein kinase	Gene modification	Cancer (or other disease)
EGFR/ErbB1	Overexpression, point mutations	Breast, NSCL, ovarian, glioblastoma
ErbB2/HER2/Neu	Overexpression, point mutations	Breast, ovarian, gastric, NSCL, colon
ErbB3/HER3	Overexpression	Breast
IGF-1R	Overexpression	Cervical, sarcomas
PDGFR-α	Overexpression	Glioma, glioblastoma, ovarian, GIST
PDGFR-β	Fusions (Tel-PDGFR-β)	Leukemias
	Overexpression	Glioma
Flt3, Flt4	Point mutation	Leukemias, angiosarcoma
KIT	Point mutations, overexpression	GIST, AML, myelodysplastic syndromes
MET	Point mutations, overexpression	Renal, hepatocellular
RET	Point mutations, fusions	Thyroid, parathyroid, adrenal
VEGFR1/Flt1	Expression	Tumor angiogenesis
VEGFR2/Flk1	Expression	Tumor angiogenesis
Src	C-terminal truncation, point mutations, overexpression	Colon, breast, pancreatic; metastasis
	Deletion (KO)	Osteopetrosis
Yes	Overexpression	Colon, melanoma
FAK	Overexpression	Metastases, adhesion, invasion
Pyk2	Overexpression	Metastases, adhesion, invasion
Abl	Fusions (Bcr-Abl), pont mutations	CML, ALL
JAK1, JAK3	Overexpression	Leukemias
JAK2	Translocation	Leukemias

2 Src Kinase Genetics and Signal Transduction

Src is the prototype of the superfamily of protein tyrosine kinases and was one of the first protein kinases to be characterized by various genetic, cellular, and structure–function studies to help understand its role in signal transduction pathways as well as in disease processes, including cancer, osteoporosis, and both tumor- and inflammation-mediated bone loss [28–38]. In fact, studies on Src provided some of the first evidence correlating protein kinase activity and substrate protein phosphorylation in the regulation of signal transduction pathways relative to normal cellular activity as well as malignant transformations. Src family kinases include Fyn, Yes, Yrk, Blk, Fgr, Hck, Lyn,

and Frk subfamily members Frk/Rak and Iyk/Bsk. A broad spectrum of functional properties exists for these SFKs, including cell growth, differentiation, survival, cytoskeletal alterations, adhesion, and migration. Gene expression profiling (using loss-of-function screening via RNA-mediated interference or small-molecule Src kinase inhibition) has been utilized to study the relationship between Src activity and cancer invasive phenotype in a panel of human colon cancer cell lines as well as to identify and validate numerous members of a "transcriptional cascade" pathway for metastatic activity.

The signal transduction pathways of Src-dependent activities in cancer cells (e.g., cell growth, differentiation, survival, cytoskeletal alterations, cell–cell and cell–matrix adhesion, and cell migration) have been determined (Fig. 1) as a result of a plethora of pioneering investigations [39–57]. Elevated Src expression and/or activity has been correlated with tumor growth in specific cancers having HER-2 or c-Met receptors by studies using Src-specific antisense DNA. Activation of HER-2 and downstream signaling pathways have been determined to lead to increased Src protein synthesis and decreased Src protein degradation, resulting in Src up-regulation and activation in HER-2-driven breast cancer invasion and metastasis. Elevated Src expression and/or activity has been found in breast cancer cell lines and malignant breast tumors. Src has been implicated in metastatic colon cancer, head and neck cancers, and pancreatic cancer. Activating Src mutations in advanced human colon cancer have also been identified. Src has been implicated in malignant transformations for certain cancers, such as breast cancer and multiple myeloma, via the epidermal growth factor receptor (EGFR) or interleukin-6 receptor (IL6-R) signaling pathways, respectively, which commonly activate the transcription factor known as the signal transducer and activator of transcription-3 (STAT-3). Aberrant activation of STAT signaling

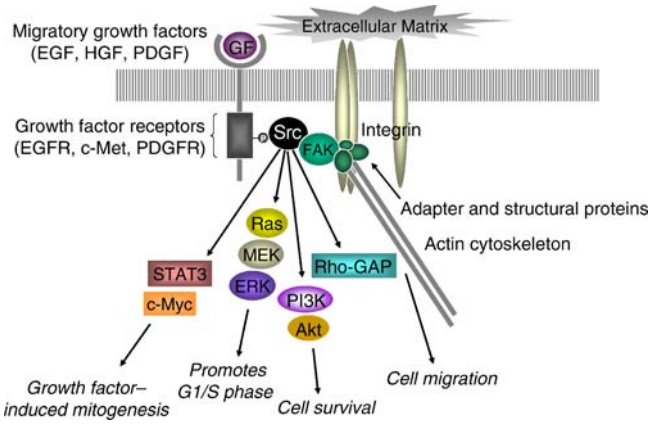


Fig. 1 Some Src-dependent signal transduction pathways related to cancer cell growth, survival and migrations (adapted from Sawyer et al. [32])

pathways has been linked to oncogenesis with respect to the prevention of apoptosis. Src cooperates with EGFR to modulate colon cancer adhesion and invasion properties, and increased Src activity has been correlated with the progression of colon cancer (i.e., metastasis). Relative to integrin receptors, Src and focal adhesion kinase (FAK) are critical to modulate the dynamic relationship between cell–cell and cell–matrix interactions. Src-induced deregulation of E-cadherin in colon cancer cells has been determined to require integrin signaling.Further, Src tyrosine kinase activity is required for adhesion turnover associated with cancer cell migration. Collectively, such findings are consistent with the correlation of Src kinase activity (via overexpression of CSK and/or dominant-negative mutants of Src) with cellular and in vivo metastasis. With respect to vascular endothelial growth factor receptor (VEGFR), Src is intimately involved in VEGF-mediated angiogenesis and vascular permeability. In particular, the ability of VEGF to disrupt endothelial barrier function, which has been correlated to tumor cell extravasation and metastasis, is mediated through Src tyrosine kinase.

3 Src Kinase Inhibitor: Structural Biology and Drug Design

Src and SFKs possess both catalytic and non-catalytic regulatory motifs (i.e., the SH3 and SH2 domains) which are functionally important in signal tran-

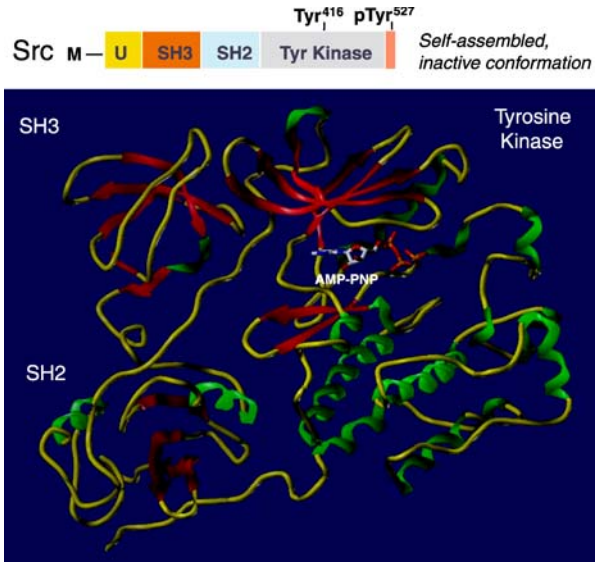


Fig. 2 X-ray structure of Src SH3-SH2-tyrosine kinase complexed with AMP-PNP in a down-regulated, inactive conformation of the protein (adapted from Xu et al. [60])

duction processes. The molecular basis of Src activation has been further revealed by structural biology studies, including X-ray structures of near full-length Src (i.e., SH3-SH2-tyrosine kinase) [58–60]. These studies have shown that Src exists in an assembled, inactive conformation by virtue of its SH3 and SH2 domains (Fig. 2). Specifically, the inactive conformation involves intramolecular binding of the SH2 domain with the C-terminal tail (phosphorylated at Tyr-527) as well as intramolecular binding of the SH3 domain with a linker sequence between the SH2 domain and the N-terminal lobe of the tyrosine kinase. The process of Src activation is believed to involve displacement of the imperfect intramolecular SH3 and SH2 interactions within the inactive conformation by intermolecular binding with SH3 and/or SH2 cog-

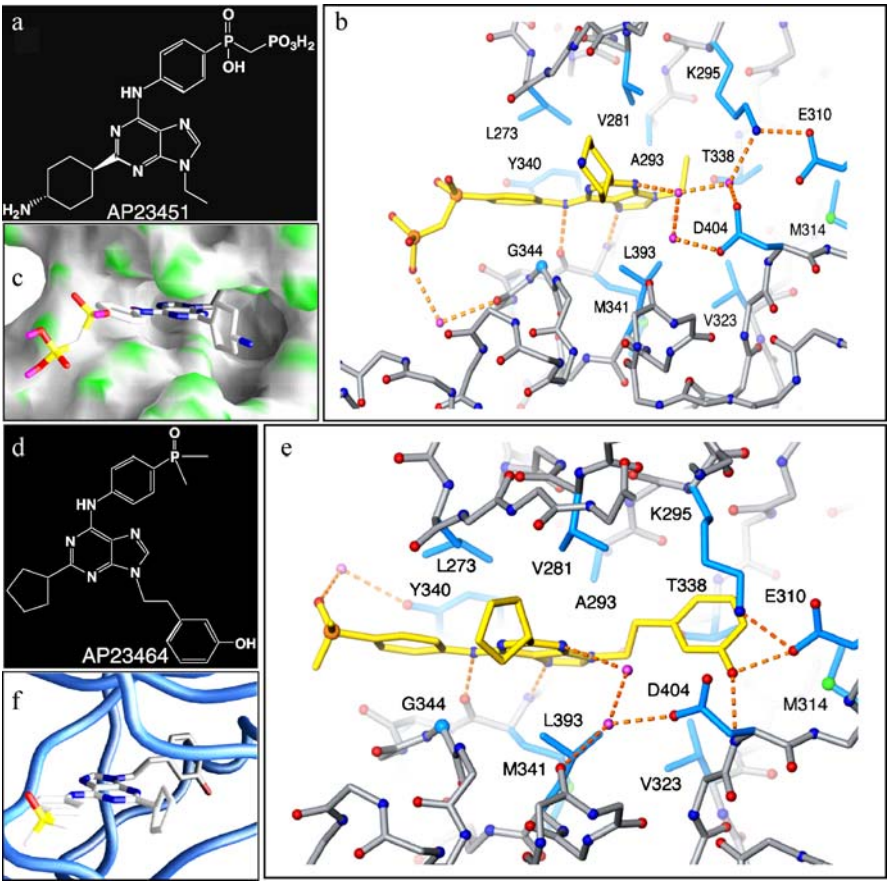


Fig. 3 X-ray structure of Src tyrosine kinase complexed with ATP-mimetic inhibitors AP23464 and AP23451 in active conformations of the protein (adapted from Dalgarno et al. [61])

nate proteins, and subsequent phosphorylation at Tyr-416 (kinase domain) and dephosphorylation at Tyr-527.

Recently, several X-ray structures of the Src kinase have been determined with respect to a number of small-molecule complexes, including AP23464 and AP23451 [61] (Fig. 3), purvalanol and CPG77675 [62], and a des-methyl analog of STI-571 (imatinib) [63].

Also related to Src kinase structural biology have been studies on two SFKs, namely Lck and Fyn. Importantly, the X-ray structure of Lck kinase was the first SFK determined [64] as complexes with AMP-PNP, staurosporine and PP2. Furthermore, a Fyn kinase–staurosporine complex has been recently described [65]. Extrapolating from the above Src kinase inhibitor crystal structures with respect to the hydrophobic specificity pocket and the active conformation of the protein to bind ATP-competitive inhibitors of varying templates and functional group elaboration, a working hypothesis of known Src kinase inhibitors (vide infra) can be suggested (Fig. 4).

Finally, a strategy to exploit protein engineering to mutate the ATP-binding pockets of protein kinases with the objective of enhancing selectivity for synthetic ATP analogs or inhibitors has been developed [66–68] using Src tyrosine kinase as a prototype model. In brief, mutation of a conserved amino acid in the ATP binding pocket was made to create a unique new site

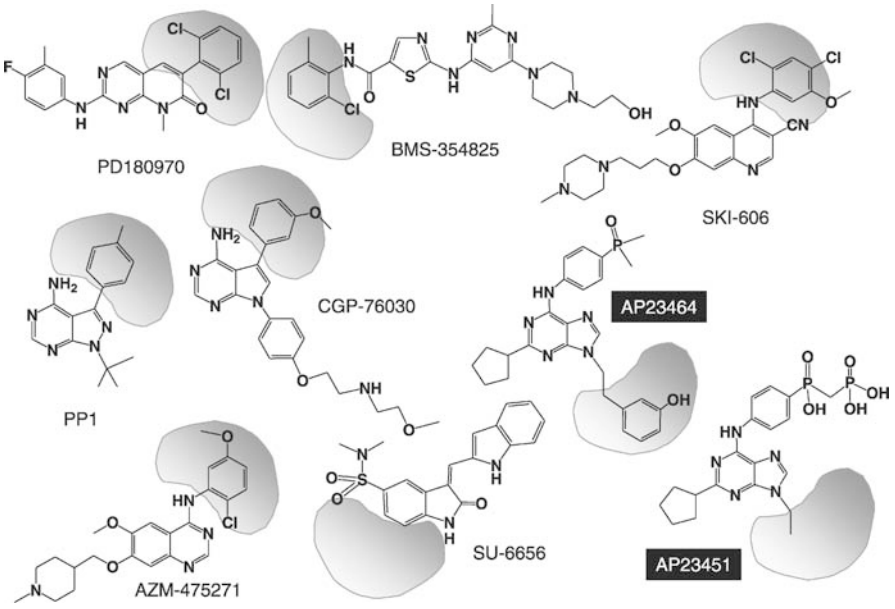


Fig. 4 Schematic models of Src kinase binding (active conformation) with known small-molecule inhibitors in terms of the hydrophobic specificity pocket relative to AP23464 and AP23451 [61]

that would accommodate a synthetic ATP substrate analog, namely, [γ-^{32}P]-N^6-(benzyl)-ATP. This then provided a matched set of enzyme–substrate to explore signal transduction pathways with respect to the identification of cellular substrates under varying experimental conditions.

4 Src Kinase Inhibitor: Chemical Diversity and Biological Properties

The design of Src kinase inhibitors has focused on a number of strategies [7, 24, 31–33, 37, 38, 69, 70], including ATP template-related mimetics, peptide substrate analogs, natural products, and other unique small molecules (e.g., lead compounds from biological screening of corporate chemical collections and/or combinatorial libraries as well as lead compounds from structure-based de novo design and virtual screening). A schematic model of the Src kinase active site (Fig. 5) illustrates ATP and peptide substrate binding relative to predicted conserved H-bonding interactions and the proximate hydrophobic specificity pocket.

First- and second-generation Src (and SFK) tyrosine kinase inhibitors BMS-354825 (**1**), AZM-475271 (**2**), AZD-0530 (**3**), SKI-606 (**4**), PD180970 (**5**), PD173955 (**6**), PD166326 (**7**), PP1 (**8**), PP2 (**9**), CGP-76030 (**10**), CGP-77675 (**11**), SU-6656 (**12**), AP23464 (**13**), AP23848 (**14**) AP23846 (**15**), AP23994 (**16**), AP23451 (**17**), and AP23588 (**18**) are particularly noteworthy small molecules based on a variety of different templates (Fig. 6), which yet exemplify ATP-

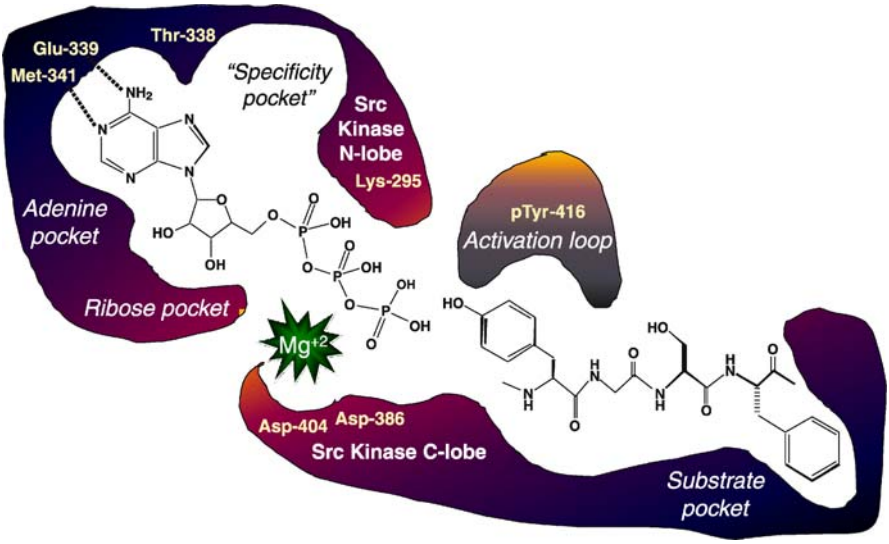


Fig. 5 Schematic model of Src tyrosine kinase complexed with ATP and peptide substrate

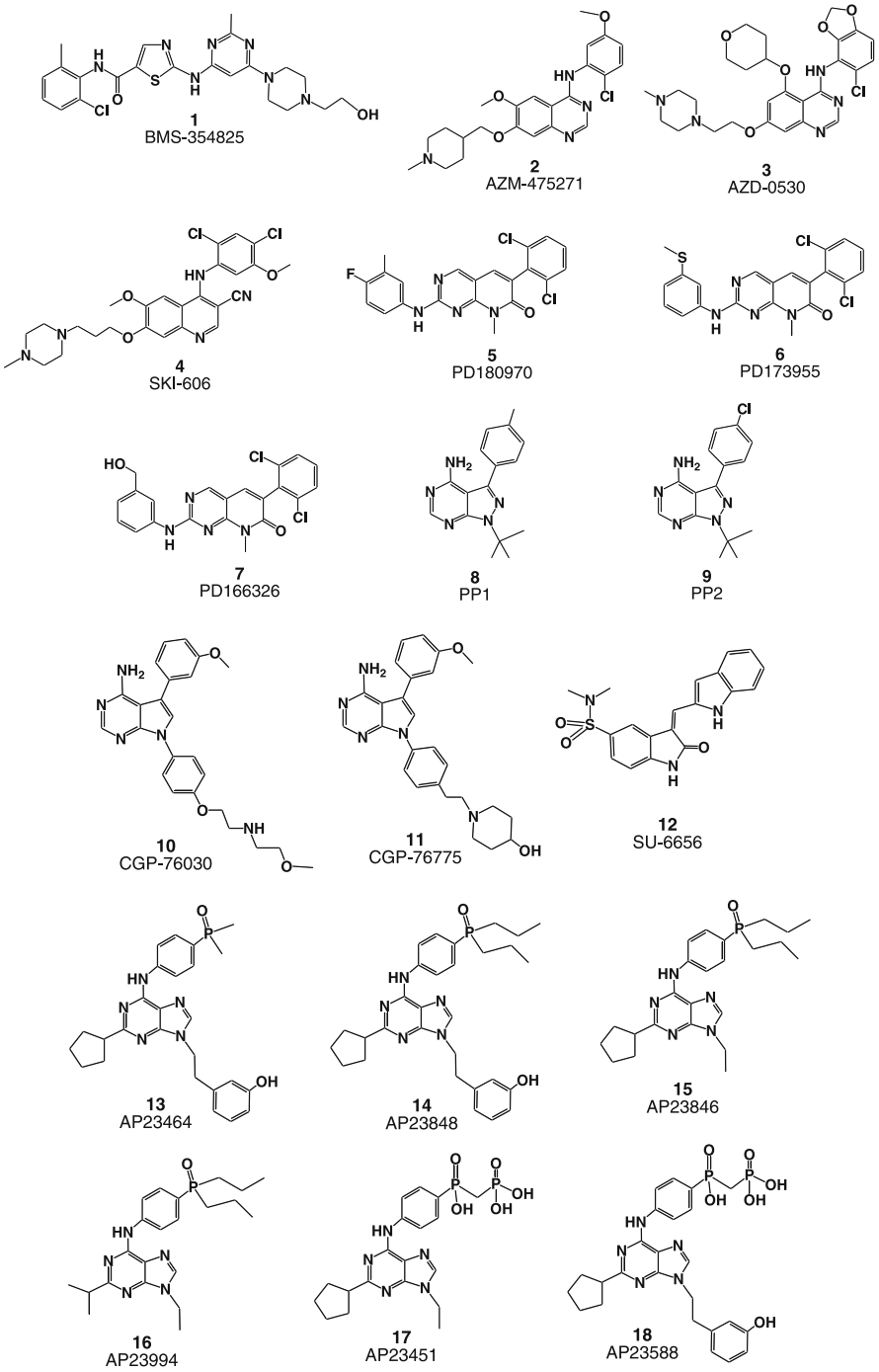


Fig. 6 Some known Src kinase inhibitor lead compounds (see text for discussion)

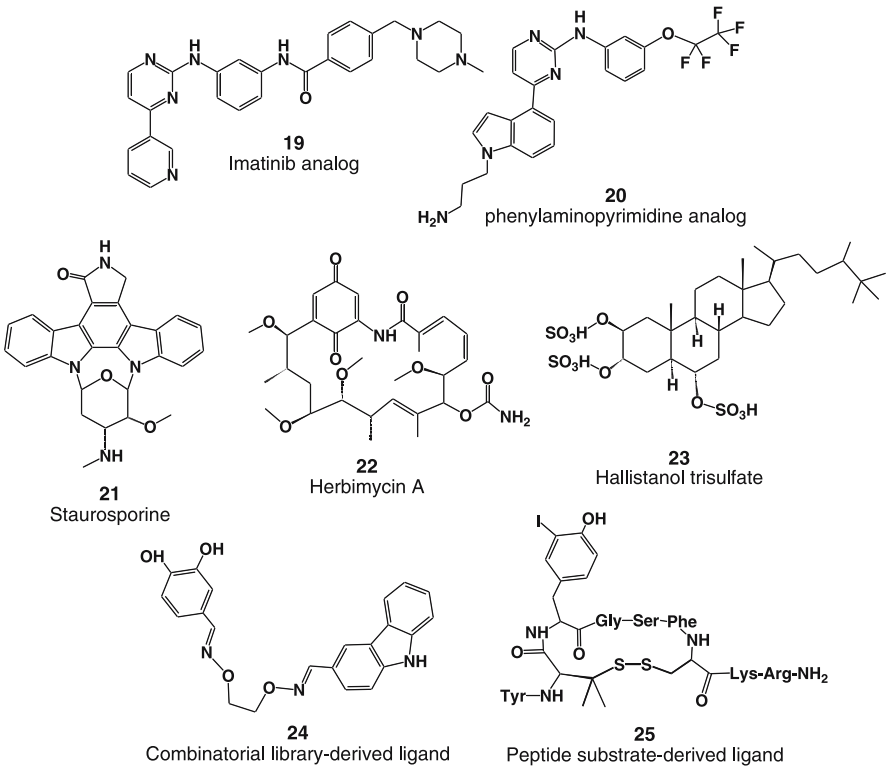


Fig. 6 (continued)

competitive binding ligands. BMS-354825, SKI-606, and AZD-0530 are in clinical trials for Src-dependent (as well as Abl-dependent) cancers. Details of the chemistry, biology and, in some cases, in vivo efficacy of the above small-molecule inhibitors of Src kinase are described below.

4.1 Pyrimidinylaminothiazole Template-Based Inhibitor BMS-354825 (Dasatinib, Sprycel™)

BMS-354825 (**1**) is a highly potent inhibitor of Src kinase (IC_{50} = 0.5 nM), Bcr-Abl kinase (IC_{50} < 1 nM), and KIT (IC_{50} = 5 nM), and it is also a relatively potent inhibitor of both PDGFR-β (IC_{50} = 28 nM) and EGFR (IC_{50} = 180 nM). Furthermore, it has been tested in vitro and in vivo against both Src- and Abl-dependent cancers [71–76]. Although an X-ray structure of BMS-35825 complexed with Src kinase has not yet been described, it has been successfully determined with Abl kinase. The Abl kinase X-ray structures show BMS-354825 binding to the ATP pocket relative to a number of H-bonding

interactions between the inhibitor and protein, and the disubstituted benzamide moiety fitting well into the hydrophobic specificity pocket. BMS-354825 is a very effective inhibitor of a number of Bcr-Abl mutants (except for T315I) that are otherwise resistant to the FDA approved Brc-Abl kinase inhibitor imatinib (Gleevec™). Relative to in vivo Bcr-Abl-driven disease models of chronic myelogenous leukemia (CML), BMS-354825 was first shown to be effective and such work provided impetus to clinical testing. Recently, BMS-354825 has received FDA approval for the treatment of CML. BMS-354825 is a highly potent inhibitor of Src and the SFK Lyn in human prostate cancer cells in terms of kinase activity, downstream signaling via FAK and Crk-associated substrate ($p130^{CAS}$), and related cellular functions (including cell adhesion, migration, and invasion). Therefore, BMS-354825 has potential for the treatment of metastatic prostate cancers. BMS-354825 is also a potent inhibitor on pancreatic tumor cells with respect to reducing Src expression and production of VEGF and IL-8. Furthermore, in an orthotopic in vivo model of pancreatic cancer it was determined that BMS-354825 significantly reduced tumor size and incidence of metastasis.

4.2 Quinazoline Template-Based Inhibitors AZM475271 (M475271) and AZD0530

AZM475271 (**2**) and AZD0530 (**3**) [77–81], quinazoline template-based molecules, are potent inhibitors of Src tyrosine kinase and have been determined to be effective inhibitors of tumor growth in Src-transformed 3T3 tumor xenograft mice at doses as low as 6 mg/kg po once daily. In an orthotopic model of implanted pancreatic cancer cells in nude mice, AZM475271 provided further in vivo proof-of-concept with respect to the use of a Src kinase inhibitor for cancer invasion and metastasis. The combination of AZM475271 with gemcitabine demonstrated significant antitiumor and antimetastic activity in this model. In studies involving lung adenocarcinoma cells, AZM475271 reduced growth, invasion, and VEGF-mediated neovascularization, resulting in growth inhibition of subcutaneous tumors and lung metastasis. AZD0530 [82, 83] is a highly potent, selective and orally-effective inhibitor of Src kinase with very good pharmacokinetic properties. It effects potent inhibition of tumor growth in Src-transformed 3T3-fibroblast xenograft models, and further increases survival in a highly aggressive, orthotopic model of pancreatic cancer. AZD0530 is in phase I clinical trials.

4.3 Quinoline Template-Based Inhibitor SKI-606 (Bosutinib)

SKI-606 (**4**) [84–88] is a highly potent inhibitor of Src kinase (IC_{50} = 1.1 nM) and Abl kinase (IC_{50} = 1 nM). The compound is also a potent inhibitor

of Src-dependent cell proliferation (IC_{50} = 100 nM) and is selective for Src over non-SFKs. SKI-606 has been found to be orally active in s.c. colon tumor xenograft models, effecting reduced Src autophosphorylation (Tyr^{418}) in HT29 and Colo205 tumors. Interestingly, SKI-606 was shown to inhibit HT29 tumor growth upon once-daily administration, whereas twice-daily administration was necessary to inhibit Colo205, HCT116, and DLD1 tumor growth. Collectively, such results implicated the potential development of SKI-606 for the treatment of colorectal cancer. Finally, with respect to its Abl kinase inhibitory properties, SKI-606 effects potent antiproliferative activity against CML cells in vitro, and in vivo studies further showed that SKI-606, at high dose, causes complete regression of CML xenografts in nude mice. SKI-606 is in phase II clinical trials.

4.4
Pyridopyrimidinone Template-Based Inhibitors PD180970, PD173955 and PD166326

PD180970 (**5**), PD173955 (**6**), and PD166326 (**7**) [89–98] have been determined to be potent inhibitors of Src and Abl kinase with varying selectivities to PDGFR, FGFR, EGFR, and Kit kinases. PD173955 effects potent antiproliferative activity in cancer cell lines (e.g., MDA-MB-468 breast cancer cells), and it exemplifies a novel class of antimotic inhibitors involving Src and Yes kinases, which have roles in cellular progression through the initial phase of mitosis. PD180970 is a highly potent inhibitor of Abl (IC_{50} = 2.2 nM), and induces apoptosis in CML cells in vitro. PD180970 was also shown to block Stat5 signaling and induce apoptosis in a Bcr-Abl high-expressing cell line that is resistant toimatinib. Furthermore, PD180970 is an effective inhibitor of several imatinib-resistant Bcr-Abl mutants in vitro, with the exception of T315I. Finally, PD166326, has been shown to be highly potent against Bcr-Abl tyrosine kinase and several Bcr-Abl mutants in vitro. In mice with the CML-like disease, PD166326 inhibited Bcr/Abl kinase activity after a single oral dose as well as effecting marked antileukemic activity in vivo. Finally, PD166326 was determined to prolong the survival of mice with imatinib-resistant CML (i.e., H396P and M351T mutants of Bcr-Abl).

4.5
Pyrazolopyrimidine Template-Based Inhibitors PP1 and PP2

PP1 (**8**) and its pyrazolopyrimidine analog PP2 (**9**) [99–108] were first described as potent inhibitors of SFKs with marked selectivity versus ZAP-70, JAK2, EGF-R, and PKA kinases. PP1 provided an early key inhibitor of Src kinase to enable determination of its roles in VEGF-mediated angiogenesis and vascular permeability, Src-driven human breast cancer cell lines with respect to both heregulin-dependent or independent growth, and Src-related,

collagen type-I-induced E-cadherin down-regulation and consequent effects on cell proliferation and metastatic properties. PP1 and its chemically similar analog, PP2, are both also effective inhibitors of Bcr-Abl kinase in vitro. PP2 studies on Bcr-Abl signaling pathways related to proliferation and survival in K-562 cells (Bcr-Abl-driven) implicated the roles of SFKs in growth and apoptosis, including blocking both Stat5 and Erk activation. Furthermore, both PP1 and PP2 have been determined to be effective kinase inhibitors of the stem cell factor (SCF) receptor c-Kit. Also, PP1 inhibited mutant constitutively active forms of c-Kit kinase (D814V and D814Y) that are known to exist in mast cell disorders.

4.6
Pyrrolopyrimidine Template-Based Inhibitors CGP-76030 and CGP-76775

CGP-76030 (**10**) and CGP-76775 (**11**) [109–112] were first described as potent and selective inhibitors of Src tyrosine kinase in vitro and in vivo relative to animal models of osteoporosis, and subsequently in cancer cell lines (e.g., pancreatic and leukemia). In osteoclasts, CGP-77675 was selective for Src versus Cdc2, EGFR, Abl, and FAK, and it was an effective inhibitor of bone resorption in vitro and in vivo. Specifically, CGP-77675 inhibited osteoclasts (i.e., parathyroid hormone-induced bone resorption in rat fetal long bone cultures). It also dose-dependently reduced the hypercalcemia induced in mice by interleukin-1 as well as effected partial prevention of bone loss and micro-architectural changes in young ovariectomized rats. In PC3 prostate cancer cells, CGP-76030 has been determined to reduce growth, adhesion, motility, and invasion. In Bcr-Abl-driven chronic myelogenous leukemia cell lines, CGP-76030 has been shown to be an effective inhibitor of Bcr-Abl tyrosine kinase and several imatinib-resistant Bcr-Abl mutants (except for T315I) in vitro. Interestingly, in Bcr-Abl-driven B-cell acute lymphoblastic leukemia (B-ALL), CGP-76030 blocked proliferation in vitro and prolonged survival of B-ALL mice, not through Bcr-Abl inhibition, but rather through SFKs (i.e., Lyn, Hck and Fgr).

4.7
Indolinone Template-Based Inhibitor SU-6656

SU-6656 (**12**) [113–115] is a potent inhibitor of Src kinase (as well as Lck, Fyn, and Yes kinases) and is an effective inhibitor of PDGF-stimulated DNA synthesis and Myc induction in a fibroblast cell line. SU-6656 has also been a useful tool for investigating the role of Src and Ras-ERK signal transduction in Src-transformed cells with respect to Rac1, as well as implicating Vav2 and Tiam1 as downstream effectors of Src to modulate Rac1-dependent pathways. In endothelial cells, SU-6656 is effective in increasing radiation-induced apoptosis and vascular endothelium destruction, and in vivo studies have

found that SU-6656 (administered before fractionated irradiation) increased radiation-induced destruction of blood vessels within tumor windows as well as tumor growth delay.

4.8
Purine Template-Based Inhibitors AP23464, AP23848, AP23846, AP23994, AP23451, and AP23588

AP23464 (**13**), AP23848 (**14**), AP23846 (**15**), AP23994 (**16**), AP23451 (**17**), and AP23588 (**18**) [7, 24, 32, 37, 38, 69, 116–129] are highly potent inhibitors of Src kinase (IC_{50} or K_i range $\sim$ 1–10 nM). AP23464 has been utilized to examine the functional relationship of Src and FAK in adhesion turnover associated with migration of colon cancer cells and to provide mechanistic proof-of-concept correlating Src tyrosine kinase as a key therapeutic target. Specifically, Src kinase-dependent phosphorylation of FAK (at Tyr-925) in colon cancer cells was determined to correlate with cell–matrix adhesion turnover associated with cell migration. AP23846 has been investigated in ovarian cancer cells relative to tumor growth inhibition and Src-dependent inhibition correlation to enhanced cytotoxicity of docetaxel in both chemosensitive and chemoresistant ovarian cancer cell lines. Furthermore, AP23994, an orally bioavailable analog of AP23846, was effective in vivo to significantly decreased tumor burden in ovarian cancer models (SKOV3ip1 and HeyA8-MDR), relative to the untreated controls. Consistent with in vitro studies, the greatest effect on tumor reduction in vivo was observed in combination therapy with docetaxel. Furthermore, Src inhibition alone by AP23994 and in combination with docetaxel significantly down-regulated tumoral production of vascular endothelial growth factor and interleukin 8, and effected antiangiogenic activities, including decreased microvessel density, and significantly affected vascular permeability. Other studies have shown AP23846 to effectively reduce cellular migration in pancreatic adenocarcinoma cells in vitro as well as angiogenesis for implanted tumor cells in vivo.

AP23451 is a novel bone-targeted (by virtue of diphosphonate functionalization) inhibitor of Src kinase (K_i = 8 nM) and it effects significant reduction of osteoclast activity in vitro and in vivo with respect to osteoclast formation and osteoclast-dependent bone resorption. Specifically, AP23451 inhibits osteoclast formation and induced osteoclast apoptosis in vitro in the 0.1–1 μM range. Neither a non-bone-targeted analog of AP23451 nor a bone-targeted aniline (substructural moiety of AP23451) are biologically active in vitro or in vivo, hence implicating the bone-targeting drug design of this lead compound. Furthermore, additional mechanistic studies have shown that the effects of AP23451 to induce osteoclast apoptosis are not prevented by addition of geranylgeraniol, which otherwise prevents alendronate-induced apoptosis. In vivo, AP23451 dose-dependently prevents parathyroid hormone-induced bone resorption hypercalcemia and ovariectomy-induced bone loss. Finally,

AP23451 administration to mice inoculated with MDA-231 breast cancer cells effectively prevents metastasis-induced osteolysis similar to bisphosphonate zoledronic (Zometa™). However, it also significantly reduces the volume of tumor cells inside the bone marrow cavities of the mice as opposed to a lack of inhibitory effect on tumor cell volume in mice treated with zoledronic acid. AP23588 is also a bone-targeted Src kinase inhibitor which has been determined to possess both anti-resorptive and anabolic properties in vitro with respect to reducing osteoclast activity and stimulating osteoblast activity, respectively.

Beyond Src kinase inhibition, it is noteworthy to discuss the above series of purine template-based inhibitors in the context of their potent inhibitory properties against Abl and Kit kinases. AP23464 is markedly potent in vitro against Abl kinase (IC_{50} = 1 nM), including imatinib-resistant Bcr-Abl kinase mutants (e.g., nucleotide binding P-loop mutants Q252H, Y253F, and E255K; the C-terminal loop mutant M351T; and the activation loop mutant H396P), except for the so-called "gatekeeper" mutant T315I. Mechanistic studies on AP23464 have shown it to effectively ablate Bcr-Abl tyrosine phosphorylation, block cell cycle progression, and promote apoptosis in Bcr-Abl-expressing cells. Interestingly, AP23846 was determined to inhibit mutant T315I Bcr-Abl with submicromolar potency and provided a prototype lead compound for this particularly challenging mutant Bcr-Abl. Finally, AP23464 and its analog AP23848 are potent inhibitors of Kit kinase, including selectivity to inhibit mutant D816V versus wild-type. Both compounds inhibit phosphorylation of Kit as well as downstream targets Akt and signal transducer and activator of transcription 3 (STAT3) to effect cell-cycle arrest and apoptosis. AP23848 was shown to inhibit mutant Kit phosphorylation and tumor growth in a mouse model.

4.9 Other Template-Based Inhibitors

Other Src kinase inhibitors have also been advanced [63, 130–135], including novel phenylaminopyrimidines (**19** and **20**), natural products (**21–23**), a combinatorial library-based molecule (**24**), and a substrate-based analog (**25**). The phenylaminopyrimidines (**19** and **20**) exemplify small-molecule inhibitors that bind to an inactive conformation of Src kinase, as based on a X-ray crystal structure of compound (**19**) complexed with Src kinase. Although both compounds are not highly potent (i.e., Src kinase $IC_{50} = \sim 1\ \mu M$), it is likely that optimization of molecular recognition may be achieved using structure-based drug design. The natural product inhibitors of Src kinase (compounds **21–23**) illustrate novel templates and, in the case of staurosporine (**21**), an X-ray crystal structure in complex with Lck kinase has been determined. Staurosporine as well as herbimycin A (**22**) and halistanol trisulfate (**23**) provide novel templates relative to the chemical diversity of inhibitors of Src

kinase, albeit their potencies are relatively low compared to many of the aforementioned ATP-mimetics, which generally have Src kinase IC_{50} values in the 1–10 nM range. Noteworthy, nevertheless, is Herbimycin A with respect to its effective in vitro and in vivo anti-resorptive activities in rodent osteoclast and bone resorption models. The combinatorial chemistry-derived Src kinase inhibitor **24** exemplifies a particularly unique small-molecule lead compound in terms of its potency (Src IC_{50} = 64 nM) and selectivity (75-fold selectivity for Src kinase over both Lyn and Fyn kinases, and > 1000-fold selectivity over Lck kinase). Finally, the peptide substrate-based inhibitor **25** illustrates the use of combinatorial chemistry with drug design focused on the integration of both conformational and topographical constraints to achieve relatively potent ($IC_{50} \sim 100$ nM range) and moderately SFK-selective Src kinase inhibitors.

5
Src Kinase Inhibitor: Drug Development for Cancer Therapy

Following the milestone discovery of Src kinase about 30 years ago, there has been extraordinary progress in advancing structural, biochemical, cellular, and in vivo studies of Src kinase towards delineating its role(s) in both normal physiology and pathophysiological states, including cancer and bone disease. Src kinase has been established to be functionally involved in cellular proliferation, survival, and migration. Such activities provide an opportunity to leverage strategies for drug development, especially for cancer therapy. Particularly noteworthy has been recent achievements in clinical trials involving several Src kinase inhibitors, including BMS-354825, AZD-0530, and SKI-606, of which the successful FDA approval for BMS-354825 (Dasatinib, Sprycel™) is especially noteworthy. Nevertheless, it is important to note that these small-molecule ligands are dual Src/Abl kinase inhibitors and their drug development has been highly focused on exploiting their highly effective Abl kinase inhibitory properties for the treatment of chronic myelogenous leukemia and imatinib-resistance. Such work provides precedence for further drug development of Src kinase inhibitors, including for cancer therapy and related bone disease (e.g., osteoporosis and osteolytic bone metastasis).

6
Concluding Remarks

The legacy of Src kinase reflects a multidisciplinary campaign involving a plethora of scientists throughout the world, from academia to industry, and with many milestone contributions to both basic research and drug discov-

ery. This chapter highlights some aspects of such progress within the scope of snapshots along the 30 years that have transpired since the discovery of Src kinase. Without question, this will not be the proverbial last chapter in the story of Src kinase as there is much yet to understand about its complex biochemical, cellular, and in vivo roles. From a chemistry perspective, the opportunity for structure-based drug design to further create novel small-molecule inhibitors of Src kinase has been enabled by recent X-ray crystal structures of a number of novel ligands. Hopefully, the good works of so many scientists from both past and recent efforts will continue to advance Src kinase inhibitors as part of a molecular armamentarium of chemical and biological medicines for the war against disease.

Acknowledgements I wish to especially acknowledge my former colleagues at ARIAD Pharmaceuticals as well as key collaborators who have contributed in great ways to Src kinase inhibitor drug discovery, as included in this chapter. My colleagues include David Dalgarno, William Shakespeare, Chester Metcalf III, Yihan Wang, Raji Sundaramoorthi, Dong Zou, Mathew Thomas, Wei-Sheng Huang, Jan Romero, Xiaotian Zhu, Tim Clackson, and Manfred Weigele of ARIAD Pharmaceuticals. My collaborators include Valerie Brunton and Margaret Frame (Beatson Cancer Research Institute, University of Glasgow), Gary Gallick, Justin Summy, Anil Sood, and Liz Han (MD Anderson Cancer Center, University of Texas), Brian Druker, Thomas O'Hare, Amy Corbin and Michael Deininger (Oregon Health and Sciences University), George Daley and Azam Mohammed (Children's Hospital, Harvard Medical School), and Brendan Boyce and Xianping Ping (University of Rochester Medical Center). I have been privileged and blessed to have such outstanding colleagues and collaborators to work with and as friends to share what has been an intriguing drug discovery sojourn.

References

1. Vieth M, Higgs RE, Robertson DH, Shapiro M, Gragg EA, Hemmerle H (2004) Biochim Biophys Acta 1697:243–257
2. Cherry M, Williams DH (2004) Curr Med Chem 11:663–673
3. Muegge I, Enyedy IJ (2004) Curr Med Chem 11:693–707
4. Dancey J, Sausville EA (2003) Nat Rev Drug Discov 2:296–313
5. Drevs J, Medinger M, Schmidt–Gersbach C, Weber R, Unger C (2003) Curr Drug Targets 4:113–121
6. Diller DJ, Li R (2003) J Med Chem 46:4638–4647
7. Sawyer TK, Shakespeare WC, Wang Y, Sundaramoorthi R, Huang WS, Metcalf CA III, Thomas M, Lawrence BM, Rozamus L, Noehre J, Zhu X, Narula S, Bohacek RS, Weigele M, Dalgarno DC (2005) Med Chem 1:293–319
8. Laird AD, Cherrington JM (2003) Exp Opin Inv Drug 12:51–64
9. Nam N-H, Paranga K (2003) Curr Drug Targets 4:159–179
10. Blume-Jensen P, Hunter T (2002) Nature 411:355–365
11. Cohen P (2002) Nat Rev Drug Disc 1:309–315
12. Fabbro D, Ruetz S, Buchdunger E, Cowan-Jacob SW, Fendrich G, Liebetanz J, Mestan J, O'Reilly T, Traxler P, Chaudhuri B, Fretz H, Zimmermann J, Meyer T, Caravatti G, Furet P, Manley PW (2002) Pharmacol Ther 93:79–98

13. Brunelleschi S, Penego L, Santoro MM, Gaudino G (2002) Curr Pharm Des 8:1959–1972
14. Scapin G (2002) Drug Disc Today 11:601–611
15. Huse M, Kuriyan J (2002) Cell 109:275–282
16. Naumann T, Matter H (2002) J Med Chem 45:2366–2378
17. Woolfrey JR, Weston GS (2002) Curr Pharm Des 8:1527–1545
18. Bridges AJ (2001) Chem Rev 101:2541–2572
19. Dumas J (2001) Exp Opin Ther Pat 11:405–429
20. Tsatsanis C, Spandidos DA (2000) Int J Mol Med 5:583–590
21. De Jong MJ, Verweij J (2006) Eur J Cancer 42:1351–1356
22. Mikalsen T, Gerits N, Moens U (2006) Biotechnol Annu Rev 12:153–223
23. Ventura JJ, Nebreda AR (2006) Clin Transl Oncol 8:153–160
24. Sawyer TK (2004) Curr Med Chem Anticancer Agents 4:449–455
25. Becker J (2004) Nat Biotechnol 22:15–18
26. Thomas SM, Brugge JS (1997) Annu Rev Cell Dev Biol 13:513–609
27. Manning G, Whyte DB, Marrtinez R, Hunter T, Sudarasanam S (2002) Science 298:1912–1934
28. Yeatman TJ (2004) Nat Rev Cancer 4:470–480
29. Roskoski R (2004) Biochem Biophys Res Commun 324:1155–1164
30. Summy JM, Gallick GE (2003) Cancer Metastasis Rev 22:337–358
31. Warmuth M, Damoiseaux R, Liu Y, Fabbro D, Gray N (2004) Curr Pharm Des 9:2043–2059
32. Sawyer T, Boyce B, Dalgarno D, Iuliucci J (2001) Exp Opin Inv Drug 10:1327–1344
33. Susa M, Missbach M, Green J (2000) Trends Pharmacol Sci 21:489–495
34. Parsons SJ, Parsons JT (2004) Oncogene 18:7906–7909
35. Biscardi JS, Tice DA, Parsons SJ (1999) Adv Cancer Res 76:61–119
36. Thomas SM, Brugge JB (1997) Ann Rev Cell Dev Biol 13:513–609
37. Sawyer TK, Wang Y, Shakespeare WC, Metcalf CA III, Sundaramoorthi R, Narula S, Dalgarno DC (2005) In: Waksman G (ed) Proteomics and protein–protein interactions: biology chemistry bioinformatics and drug design series: protein reviews, vol 3. Springer, Berlin Heidelberg New York, pp 219–253
38. Metcalf CA III, van Schravendijk MR, Dalgarno DC, Sawyer TK (2002) Curr Pharm Design 8:2049–2075
39. Maa M-C, Leu TH, McCarley DJ, Schatzman RC, Parsons SJ (1995) Proc Natl Acad Sci USA 92:6981–6985
40. Mao W, Irby R, Coppola D, Fu L, Wloch M, Turner J, Yu H, Garcia R, Jove R, Yeatman TJ (1997) Oncogene 15:3083–3090
41. Staley CA, Parikh NU, Gallick GE (1997) Cell Growth Differ 8:269–274
42. Ellis LM, Staley CA, Liu W, Fleming RY, Parikh NU, Bucana CD, Gallick GE (1998) J Biol Chem 273:1052–1057
43. Irby RB, Mao W, Coppola D, Kang J, Loubeau JM, Trudeau W, Karl R, Fujita DJ, Jove R, Yeatman TJ (1998) Nat Genet 21:187–190
44. Egan C, Pang A, Durda D, Cheng HC, Wang JH, Fujita DJ (1999) Oncogene 18:1227–1237
45. Verbeek BS, Vroom TM, Adriaansen-Slot SS, Ottenhoff-Kalff AE, Geertzema JG, Hennipman A, Rijksen G (1996) J Pathol 180:383–388
46. Talamonti MS, Roh MS, Curley SA, Gallick GE (1993) J Clin Invest 91:3–60
47. Van Oijen MG, Rijkseng G, Ten Broek FW, Slootweg PJ (1998) J Oral Pathol Med 27:147–152

48. Lutz MP, Esser IB, Flossmann-Kast BB, Vogelmann R, Luhrs H, Friess H, Buchler MW, Adler G (1998) Biochem Biophys Res Commun 243:503–508
49. Karni R, Jove R, Levitzki A (1999) Oncogene 18:4654–462
50. Tsai YT, Su YH, Fang SS, Huang TN, Qiu Y, Jou YS, Shih HM, Kung HJ, Chen RH (2000) Mol Cell Biol 20:2043–2054
51. Turkson J, Bowman T, Garcia R, Caldenhoven E, De Groot RP, Jove R (1998) Mol Cell Biol 18:2545–2552
52. Avizienyte E, Wyke AW, Jones RJ, Mclean GW, Westhoff MA, Brunton VG, Frame MC (2002) Nat Cell Biol 8:632–638
53. Nakagawa T, Tanaka S, Suzuki H, Takayanagi H, Miyazaki T, Nakamura K, Tsuruo T (2000) Int J Cancer 88:384–391
54. Sakamoto M, Takamuri M, Ino Y, Miuru A, Genda T, Hirohasi S (2001) Jpn J Cancer Res 92:941–946
55. Boyer B, Bourgeois Y, Poupon M-R (2002) Oncogene 21:2347–2356
56. Weis S, Cui J, Barnes L, Cheresh D (2004) J Cell Biol 167:223–229
57. Eliceiri BP, Paul R, Schwartzbert PI, Hood JD, Leng J, Cheresch DA (1999) J Mol Cell 4:915–924
58. Xu W, Harrison SC, Eck MJ (1997) Nature 285:595–602
59. Williams JC, Weijland A, Gonfloni S, Thomson A, Courtneidge SA, Superti-Furga G, Wierenga RK (1997) J Mol Biol 274:757–775
60. Xu W, Doshi A, Lei M, Eck M, Harrison SC (1999) Mol Cell 3:629–636
61. Dalgarno D, Stehle T, Narula S, Schelling P, van Schravendijk MR, Adams S, Andrade L, Keats J, Ram M, Jin L, Grossman T, MacNeil I, Metcalf C III, Shakespeare W, Wang Y, Keenan T, Sundaramoorthi R, Bohacek R, Weigele M, Sawyer T (2006) Chem Biol Drug Des 67:46–57
62. Breitenlechner CB, Kairies NA, Honold K, Scheiblich S, Koll H, Greiter E, Koch S, Schafer W, Huber R, Engh RA (2005) J Mol Biol 353:222–231
63. Cowan-Jacob SW, Fendrich G, Manley PW, Jahnke W, Fabbro D, Liebetanz J, Meyer T (2005) Structure 13:861–871
64. Zhu X, Kim JL, Newcomb JR, Rose PE, Stover DR, Toledo LM, Zhao H, Morgenstern KA (1999) Structure 7:651–661
65. Kinoshita T, Matsubara M, Ishiguro H, Okita K, Tada T (2006) Biochem Biophys Res Commun 346:840–844
66. Kraybill BC, Elkin LL, Blethrow JD, Morgan DO, Shokat KM (2002) J Am Chem Soc 124:12118–12128
67. Bishop AC, Kung C-Y, Shah K, Witucki L, Shokat KM, Liu Y (1999) J Am Chem Soc 121:627–631
68. Liu Y, Shah K, Yang F, Witucki L, Shokat KM (1998) Chem Biol 5:91–101
69. Shakespeare WC, Metcalf CA III, Wang Y, Sundaramoorthi R, Keenan T, Weigele M, Bohacek RS, Dalgarno DC, Sawyer TK (2003) Curr Opin Drug Disc Dev 6:729–741
70. Trevino JG, Summy JM, Gallick GE (2006) Mini Rev Med Chem 6:681–687
71. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL (2004) Science 305:399–401
72. Lombardo LJ, Lee FY, Chen P, Norris D, Barrish JC, Behnia K, Castaneda S, Cornelius LAM, Das J, Doweyko AM, Fairchild C, Hunt JT, Inigo I, Johnston K, Kamath A, Kan D, Klei H, Marathe P, Pang S, Peterson R, Pitt S, Schieven GL, Schmidt RJ, Tokarski J, Wen M-L, Wityak J, Borzilleri RM (2004) J Med Chem 47:6658–6661
73. Burgess MR, Skaggs BJ, Shah NP, Lee FY, Sawyers CL (2005) Proc Natl Acad Sci USA 102:3395–3400
74. Trevino JG, Summy JM, Lesslie DP, Parikh NU, Hong DS, Lee FY, Donato NJ, Abbruzzese JL, Baker CH, Gallick GE (2006) Am J Pathol 168:962–972

75. Johnson FM, Saigal B, Talpaz M, Donato NJ (2005) Clin Cancer Res 11:6924–6932
76. Nam S, Kim D, Cheng JQ, Zhang S, Lee JH, Buettner R, Mirosevich J, Lee FY, Jove R (2005) Cancer Res 65:9185–9189
77. Ple PA, Green TP, Hennequin LF, Curwen J, Fennell M, Allen J, Lambert-Van Der Brempt C, Costello G (2004) J Med Chem 47:871–887
78. Barlaam B, Fennell M, Germain H, Green T, Hennequin L, Morgentin R, Olivier A, Ple P, Vautier M, Costello G (2005) Bioorg Med Chem Lett 15:5446–5449
79. Ali N, Yoshizumi M, Fujita Y, Izawa Y, Kanematsu Y, Ishizawa K, Tsuchiya K, Yano S, Sone S, Tamaki T (2005) J Pharmacol Sci 98:130–141
80. Zheng R, Yano S, Matsumori Y, Nakataki E, Muguruma H, Yoshizumi M, Sone S (2005) Clin Exp Metastasis 22:195–204
81. Yezhelyev MV, Koehl G, Guba M, Brabletz T, Jauch KW, Ryan A, Barge A, Green T, Fennell M, Bruns CJ (2004) Clin Cancer Res 10:8028–8036
82. Hennequin LF, Allen J, Breed J, Curwen J, Fennell M, Green TP, Lambert-van der Brempt C, Morgentin R, Norman RA, Olivier A, Otterbein L, Ple PA, Warin N, Costello G (2006) J Med Chem 49:6465–6488
83. Hiscox S, Morgan L, Green TP, Barrow D, Gee J, Nicholson RI (2006) Breast Cancer Res Treat 97:263–274
84. Boschelli DH, Ye F, Wang YD, Dutia M, Johnson SL, Wu B, Miller K, Powell DW, Yaczko D, Young M, Tischler M, Arndt K, Discafani C, Etienne C, Gibbons J, Grod J, Lucas J, Weber JM, Boschelli F (2001) J Med Chem 44:3965–3977
85. Golas JM, Lucas J, Etienne C, Golas J, Discafani C, Sridharan L, Boghaert E, Arndt K, Ye F, Boschelli DH, Li F, Titsch C, Huselton C, Chaudhary I, Boschelli F (2005) Cancer Res 65:5358–5364
86. Boschelli DH, Wang YD, Johnson S, Wu B, Ye F, Barrios Sosa AC, Golas JM, Boschelli F (2004) J Med Chem 47:599–601
87. Boschelli DH, Wang YD, Ye F, Wu B, Zhang N, Dutia M, Powell DW, Wissner A, Arndt K, Weber JM, Boschelli F (2001) J Med Chem 44:822–833
88. Wang YD, Miller K, Boschelli DH, Ye F, Wu B, Floyd MB, Powell DW, Wissner A, Weber JM, Boschelli F (2000) Bioorg Med Chem Lett 10:2477–2480
89. Dorsey JF, Jove R, Kraker AJ, Wu J (2000) Cancer Res 60:3127–3131
90. Huang M, Dorsey JF, Epling-Burnette PK, Nimmanapalli R, Landowski TH, Mora LB, Niu G, Sinibaldi D, Bai F, Kraker A, Yu H, Moscinski L, Wei S, Djeu J, Dalton WS, Bhalla K, Loughran TP, Wu J, Jove R (2002) Oncogene 21:8804–8816
91. La Rosee P, Corbin AS, Stoffregen EP, Deininger MW, Druker BJ (2002) Cancer Res 62:7149–7173
92. Hamby JM, Connolly CJ, Schroeder MC, Winters RT, Showalter HD, Panek RL, Major TC, Olsewski B, Ryan MJ, Dahring T, Lu GH, Keiser J, Amar A, Shen C, Kraker AJ, Slintak V, Nelson JM, Fry DW, Bradford L, Hallak H, Doherty AM (1997) J Med Chem 40:2296–2303
93. Klutchko SR, Hamby JM, Boschelli DH, Wu Z, Kraker AJ, Amar AM, Hartl BG, Shen C, Klohs WD, Steinkampf RW, Driscoll DL, Nelson JM, Elliott WL, Roberts BJ, Stoner CL, Vincent PW, Dykes DJ, Panek RL, Lu GH, Major TC, Dahring TK, Hallak H, Bradford LA, Showalter HD, Doherty AM (1998) J Med Chem 41:3276–3292
94. Kraker AJ, Hartl BG, Amar AM, Barvian MR, Showalter HD, Moore CW (2000) Biochem Pharmacol 60:885–898
95. Wisniewski D, Lambek CL, Liu C, Strife A, Veach DR, Nagar B, Young MA, Schindler T, Bornmann WG, Bertino JR, Kuriyan J, Clarkson B (2002) Cancer Res 62:4244–4255

96. Nimmanapalli R, O'Bryan E, Huang M, Bali P, Burnette PK, Loughran T, Tepperberg J, Jove R, Bhalla K (2002) Cancer Res 62:5761–5769
97. Wolff NC, Veach DR, Tong WP, Bornmann WG, Clarkson B, Ilaria RL Jr (2005) Blood 105:3995–4003
98. Moasser MM, Srethapakdi M, Sachar KS, Kraker AJ, Rosen N (1999) Cancer Res 59:6145–6152
99. Hanke JH, Gardner JP, Dow RL, Changelian PS, Brissette WH, Weringer EJ, Pollok BA, Connelly PA (1996) J Biol Chem 271:695–701
100. Eliceiri BP, Paul R, Schwartzbert PI, Hood JD, Leng J, Cheresch DA (1999) J Mol Cell 4:915–924
101. Paul R, Zhang ZG, Eliceiri BP, Jiang Q, Boccia AD, Zhang RL, Chopp M, Cheresh DA (2001) Nat Med 7:222–227
102. Belsches-Jablonski AP, Biscardi JS, Peavy DR, Tice DA, Romney DA, Parsons SJ (2000) Oncogene 20:1464–1475
103. Menke A, Philipp C, Vogelmann R, Seidel B, Lutz MP, Adler G, Wedlich D (2001) Cancer Res 61:3508–3517
104. Tatton L, Morley GM, Chopras R, Khwaja A (2003) J Biol Chem 278:4847–4853
105. Warmuth M, Simon N, Mitina O, Mathes R, Fabbro D, Manley PW, Buchdunger E, Forster K, Moarefi I, Hallek M (2003) Blood 101:664–672
106. Wilson MB, Schreiner SJ, Choi H-J, Kamens J, Smithgall TS (2002) Oncogene 21:8075–8088
107. Tatton L, Morley GM, Chopra R, Khwaja A (2003) J Biol Chem 278:4847–4853
108. Warmuth M, Simon N, Mitina O, Mathes R, Fabbro D, Manley PW, Buchdunger E, Forster K, Moarefi I, Hallek M (2003) Blood 101:664–672
109. Missbach M, Jeschke M, Feyen J, Muller K, Glatt M, Green J, Susa M (1999) Bone 24:437–449
110. Recchia I, Rucci N, Funari A, Migliaccio S, Taranta A, Longo M, Kneissel M, Susa M, Fabbro D, Teti A (2004) Bone 34:65–79
111. Recchia I, Rucci N, Festuccia C, Bologna M, MacKay AR, Migliaccio S, Longo M, Susa M, Fabbro D, Teti A (2003) Eur J Cancer 39:1927–1935
112. Hu Y, Liu Y, Pelletier S, Buchdunger E, Warmuth M, Fabbro D, Hallek M, Van Etten RA, Li S (2004) Nat Genet 36:453–461
113. Blake RA, Broome MA, Liu X, Wu J, Gishizky M, Sun L, Courtneidge SA (2000) J Mol Cell Biol 20:9018–9027
114. Servitja JM, Marinissen MJ, Sodhi A, Bustelo XR, Gutkind JS (2003) J Biol Chem 278:34339–34346
115. Cuneo KC, Geng L, Tan J, Brousal J, Shinohara ET, Osusky K, Fu A, Shyr Y, Wu H, Hallahan DE (2006) Int J Radiat Oncol Biol Phys 64:1197–1203
116. Brunton VG, Avizienyte E, Fincham VJ, Serrels B, Metcalf CA III, Sawyer TK, Frame MC (2005) Cancer Res 65:1335–1342
117. Boyce BF, Xing L, Shakespeare W, Wang Y, Dalgarno D, Iuliucci J, Sawyer T (2003) Kidney Intern 85:52–55
118. Wang Y, Metcalf CA III, Shakespeare WC, Sundaramoorthi R, Keenan TP, Bohacek RS, van Schravendijk MR, Violette SM, Narula SS, Dalgarno DC, Haraldson C, Keats J, Liou S, Mani U, Pradeepan S, Ram M, Adams S, Weigele M, Sawyer TK (2003) Bioorg Med Chem Lett 13:3067–3070
119. Boyce BF, Xing L, Yao Z, Yamashita T, Shakespeare WC, Wang Y, Metcalf CA III, Sundaramoorthi R, Dalgarno DC, Iuliucci JD, Sawyer TK (2006) Clin Cancer Res 12:6291s–6295s

120. Han LY, Landen CN, Trevino JG, Halder J, Lin YG, Kamat AA, Kim TJ, Merritt WM, Coleman RL, Gershenson DM, Shakespeare WC, Wang Y, Sundaramoorth R, Metcalf CA III, Dalgarno DC, Sawyer TK, Gallick GE, Sood AK (2006) Cancer Res 66:8633–8639
121. Trevino JG, Gray MJ, Nawrocki ST, Summy JM, Lesslie DP, Evans DB, Sawyer TK, Shakespeare WC, Watowich SS, Chiao PJ, McConkey DJ, Gallick GE (2006) Angiogenesis 9:101–110
122. Boyce BF, Xing L, Yao Z, Shakespeare WC, Wang Y, Metcalf CA III, Sundaramoorthi R, Dalgarno DC, Iuliucci JD, Sawyer TK (2006) Ann NY Acad Sci 1068:447–457
123. Lesslie DP, Summy JM, Parikh NU, Fan F, Trevino JG, Sawyer TK, Metcalf CA III, Shakespeare WC, Hicklin DJ, Ellis LM, Gallick GE (2006) Br J Cancer 94:1710–1717
124. Carragher NO, Walker SM, Scott Carragher LA, Harris F, Sawyer TK, Brunton VG, Ozanne BW, Frame MC (2006) Oncogene 25:5726–5740
125. Summy JM, Trevino JG, Lesslie DP, Baker CH, Shakespeare WC, Wang Y, Sundaramoorthi R, Metcalf CA III, Keats JA, Sawyer TK, Gallick GE (2005) Mol Cancer Ther 4:1900–1911
126. Brunton VG, Avizienyte E, Fincham VJ, Serrels B, Metcalf CA III, Sawyer TK, Frame MC (2005) Cancer Res 65:1335–1342
127. O'Hare T, Pollock R, Stoffregen EP, Keats JA, Abdullah OM, Moseson EM, Rivera VM, Tang H, Metcalf CA III, Bohacek RS, Wang Y, Sundaramoorthi R, Shakespeare WC, Dalgarno D, Clackson T, Sawyer TK, Deininger MW, Druker BJ (2004) Blood 104: 2532–2539
128. Azam M, Nardi V, Shakespeare WC, Metcalf CA III, Bohacek RS, Wang Y, Sundaramoorthi R, Sliz P, Veach DR, Bornmann WG, Clarkson B, Dalgarno DC, Sawyer TK, Daley GQ (2006) Proc Natl Acad Sci USA 103:9244–9249
129. Corbin AS, Demehri S, Griswold IJ, Wang Y, Metcalf CA III, Sundaramoorthi R, Shakespeare WC, Snodgrass J, Wardwell S, Dalgarno D, Iuliucci J, Sawyer TK, Heinrich MC, Druker BJ, Deininger MW (2005) Blood 106:227–234
130. Zimmerman J, Buchdunger E, Mett H, Meyer T, Lydon NB (1997) Bioorg Med Chem Lett 7:187–192
131. Nakano H, Kobayashi E, Takahashi I, Tamaoki T, Kuzuu Y, Iba H (1987) J Antibiot (Tokyo) 40:706–708
132. Yoneda T, Lowe C, Lee C-H, Gutierrez G, Niewolna M, Williams PJ, Izbicka E, Uehara Y, Mundy GR (1993) J Clin Invest 91:2791–2795
133. Slate DL, Lee RH, Rodriguez J, Crews P (1994) Biochem Biophys Res Comm 203:260–264
134. Maly DJ, Choong IC, Ellman JA (2000) Proc Natl Acad Sci USA 97:2419–2424
135. Alfaro-Lopez J, Yuan W, Phan BC, Kamath J, Lou Q, Lam KS, Hruby VJ (1998) J Med Chem 41:2252–2260

Top Med Chem (2007) 1: 407–444
DOI 10.1007/7355_2006_008

Published online: 11 November 2006

Bcr-Abl Kinase Inhibitors

Diane H. Boschelli

Chemical and Screening Sciences, Wyeth Research, 401 N. Middletown Road, Pearl River, NY 10965-1215, USA
bosched@wyeth.com

1 Bcr-Abl: The Hallmark of Chronic Myelogenous Leukemia (CML) 408

2 Imatinib, a Bcr-Abl Kinase Inhibitor Effective in Treating CML 409

3 New Bcr-Abl Kinase Inhibitors 411
3.1 Second Generation 2-Phenylaminopyrimidines 411
3.1.1 AMN107 411
3.1.2 NS-187 415
3.2 Additional Bcr-Abl Inhibitors 416
3.2.1 ON012380 416
3.2.2 Adaphostin 417
3.2.3 AG1024 418

4 Dual Inhibitors of Bcr-Abl and Src Kinases 418
4.1 PD166326 419
4.2 CGP76030 422
4.3 AP23464 and AP23848 423
4.4 SKI-606 425
4.5 BMS-354825 427
4.6 AZD0530 431

5 Key Issues 432
5.1 Direct Comparison of AMN107 and BMS-354825 432
5.2 Combinations of Imatinib and New Bcr-Abl Inhibitors 433
5.3 Overcoming the T315I Mutation 434

6 Present Status and Future Outlook 435

References 437

Abstract The hallmark of chronic myelogenous leukemia (CML) is the expression of Bcr-Abl, a constitutively active form of the Abl tyrosine kinase. Imatinib, a 2-phenylaminopyrimidine Bcr-Abl inhibitor developed by Novartis and marketed under the tradename of Gleevec (Glivec), is highly effective in treating CML patients with early stage disease. However, patients with advanced disease often become resistant to imatinib. The predominant form of this resistance is the development of mutations in the Bcr-Abl protein. These point mutations can be amino acid residues that make direct contact with imatinib or residues that do not allow Bcr-Abl to adopt the inactive conformation. Since imatinib can only bind to the inactive conformation of the protein, both types of mutations prevent this inhibitor from binding. Several approaches have been taken to identify additional

Bcr-Abl inhibitors including: (1) more potent analogs of imatinib; (2) non-ATP competitive inhibitors of Bcr-Abl; and (3) dual inhibitors of both Bcr-Abl and members of the Src family of kinases (SFKs) that bind to the active form of Bcr-Abl. The progress made on the development of these new agents, including compounds with activity against the highly resistant T315I mutation of Bcr-Abl, will be discussed.

Keywords Bcr-Abl · Abl · CML · Imatinib · T315I

Abbreviations

CML	chronic myelogenous leukemia
ALL	acute lymphoblastic leukemia
SFK	Src family kinase
GST	gastrointestinal stromal tumors
ASH	American Society of Hematology
AACR	American Association for Cancer Research
ASCO	American Society of Clinical Oncology

1 Bcr-Abl: The Hallmark of Chronic Myelogenous Leukemia (CML)

Chronic myelogenous leukemia (CML) is a hematopoietic stem cell disease that results in white blood cell hyperproliferation [1–6]. CML is associated with the presence of a genetic abnormality known as the Philadelphia chromosome. Fusion of a piece of chromosome 9 that contains a portion of the *ABL* gene with a piece of chromosome 22 that contains part of the *BCR* gene generates *BCR-ABL*, an oncogene that leads to the expression of Bcr-Abl. Bcr-Abl is the constitutively active form of the Ableson (Abl) kinase and drives the uncontrolled growth of the white blood cells. Although this genetic abnormality was first observed in CML there is also a Philadelphia chromosome positive form of acute lymphoblastic leukemia (ALL) [7–10].

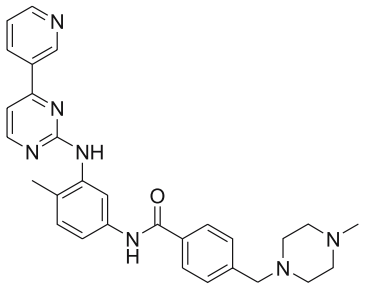
The incidence of CML is rather low, with only 1 to 2 cases per 100 000 people being diagnosed each year [6]. The disease proceeds through three phases: the initial or chronic phase followed by an accelerated phase that leads to blast crisis. While some CML patients in chronic phase can be cured by bone marrow transplant, survival rates are lower for those with later stage disease and many CML patients are not eligible for transplantation due to their age or lack of an available donor match. Chemotherapy with IFN-α can prolong life span but severe side effects are often observed, highlighting the need for an improved treatment for CML. Since this disease is characterized by a single oncogene, CML is the perfect candidate for targeted therapy: the inhibition of Bcr-Abl activity.

2
Imatinib, a Bcr-Abl Kinase Inhibitor Effective in Treating CML

Ten years ago, Novartis reported that the 2-phenylaminopyrimidine (**1**) was a potent Bcr-Abl kinase inhibitor [11] (Scheme 1). This compound was initially referred to as CGP 57148, or STI-571, and was later given the generic name of imatinib. Imatinib had an IC_{50} of 38 nM in an Abl kinase assay, and also inhibited platelet-derived growth factor receptor (PDGFR) tyrosine kinase [12] and another tyrosine kinase, c-Kit [13]. However, an IC_{50} of greater than 100 μM was observed for the inhibition of Src kinase activity [12]. Src is a member of a family of highly homologous non-receptor tyrosine kinases (SFKs) that includes Lck, Hck, Fyn and Yes [14]. Since there is a strong structural similarity between Abl and the SFKs, the lack of activity against Src was rather surprising [15].

Imatinib inhibited the growth of several cell lines transformed by wild-type Bcr-Abl and also inhibited Bcr-Abl autophosphorylation in these cells [11, 16–19]. The lines used included PB-3c mast cells, BALB/c-3T3 fibroblasts and MO7e myeloid cells. Imatinib also blocked the proliferation of several Bcr-Abl-dependent human CML lines, including KU812, K562 and MC3. Imatinib was shown to be efficacious in an animal model of CML [20]. Treatment of nude mice implanted with KU812 cells with a 160 mg/kg oral dose of imatinib every eight hours resulted in no tumor growth when the animals were observed for 240 days. Activity of imatinib in this and other animal studies led to the initiation of clinical trials in 1998 [21, 22].

Shortly thereafter, in 2001, imatinib was approved by the FDA for the treatment of CML and is marketed in the United States under the trade name of Gleevec. The drug is also used in several other countries under the trade name of Glivec. It is currently the first-line of treatment for CML patients and revolutionized the treatment of this disease. The remarkable story of the dis-



Scheme 1

covery of imatinib and its rapid progression to clinical use has been outlined in many review articles [23–27] and is also the subject of a book by Daniel Vasella, the chairman and CEO of Novartis during this exciting time [28]. As mentioned earlier imatinib is also an inhibitor of c-Kit [13]. This kinase is constitutively activated in gastrointestinal stromal tumors (GIST) [29] and treatment of GIST patients with imatinib resulted in high response rates [30]. Imatinib was approved by the FDA in 2002 for the treatment of GIST and it is currently the first line of therapy for this disease [31].

While imatinib is highly effective in treating early-stage CML, it was not long before it was observed that those patients with late-stage disease, either accelerated or blast phase, often developed resistance to the drug. The initial report of resistance appeared in 2001 and it was noted that while in some cases the decreased patient response was due to amplification of the *BCR-ABL* gene, a more common reason was mutation of the Thr 315 residue in the catalytic domain of Abl kinase to an Ile residue (T315I) [32]. Since then several alternative mechanisms of imatinib resistance were observed including binding of the drug to acid glycoproteins, overexpression of the multiple drug resistance gene, and upregulation of alternate pathways including SFKs [33]. However, the most common source of imatinib resistance remained mutations of Abl kinase. In addition to T315I, other mutations were soon identified including E255K/V, Y253F/H and M351T [34, 35]. The E255K/V and Y253F/H mutations are in the ATP phosphate-binding loop (P-loop) while the M351T mutation is proximal to the activation loop. It was shown that this activation loop can also contain mutations, including H396P.

Crystallography studies showed that imatinib binds to an inactive form of Abl [36, 37]. In this bound conformation the activation loop of the Abl kinase domain is distinct from that of both the inactive and active forms of the SFKs, explaining why imatinib does not inhibit these kinases. The crystal structure also revealed that the Thr 315 residue was involved in a key hydrogen bonding interaction with the C-2 amino group of imatinib.

The remainder of this work will focus on the approaches to designing and identifying new inhibitors of Bcr-Abl including: (1) more potent analogs of imatinib; (2) non-ATP competitive inhibitors of Bcr-Abl; and (3) dual inhibitors of both Bcr-Abl and members of the Src family of kinases (SFKs) that bind to the active form of Bcr-Abl. The status of the new Bcr-Abl inhibitors currently in the clinic will be summarized.

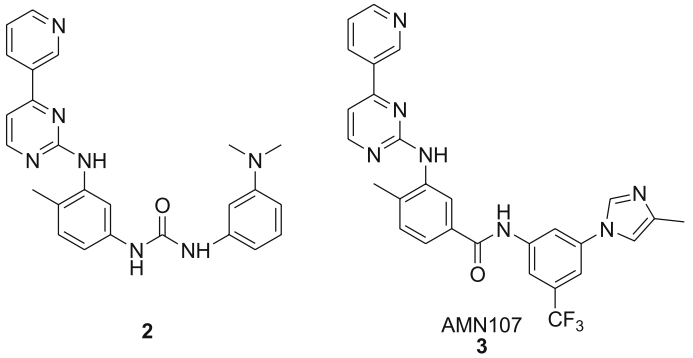
3
New Bcr-Abl Kinase Inhibitors

3.1
Second Generation 2-Phenylaminopyrimidines

3.1.1
AMN107

Subsequent to the discovery of imatinib, Novartis continued to pursue additional compounds as Abl inhibitors. A new Abl inhibitor with increased potency that bound more tightly to the enzyme might overcome some of the resistance associated with imatinib. It was postulated that replacement of the amide functionality of imatinib with a urea group should retain the key hydrogen-bonding interactions with Abl [38]. The 4-(3-pyridinyl)-2-(2-methylphenyl)aminopyrimdine portion of the molecule was held constant and parallel synthesis was used to prepare a library of ureas via reaction of a carbamate with a variety of amines. Some of these urea derivatives were potent inhibitors in a recombinant Abl kinase assay but they did not retain this activity in a cellular Bcr-Abl autophosphorylation assay. Analog **2** had an IC_{50} of 48 nM in the cell free assay but an IC_{50} of 570 nM in the cell assay (Scheme 2). Replacement of the dimethylamino group of **2** with the (4-methylpiperazin-1-yl)methyl group present in imatinib resulted in decreased activity, with a 10 μM concentration of this analog providing only 45% inhibition in the kinase assay.

The search for additional new Abl inhibitors continued and at the 2004 meeting of the American Society of Hematology (ASH), Novartis disclosed an Abl kinase inhibitor with improved activity over imatinib [39]. The first literature report on AMN107 (**3**) appeared in early 2005 [40] (Scheme 2). The structure of AMN107 diverges from that of imatinib in two areas. First, the



Scheme 2

position of the amide is reversed so that the carbonyl group is now linked to the phenyl group of the 2-(phenylamino)pyrimidine and second, there is a different substitution pattern on the pendant phenyl ring. AMN107 contains a 3-(4-methyl-1H-imidazole)-5-trifluoromethyl phenyl group as opposed to the 4-(4-methylpiperazinyl)methyl phenyl group present in imatinib. A crystal structure with AMN107 showed that it binds to the inactive form of Abl, with a similar binding mode to that observed for imatinib. As expected, reversal of the amide bond allowed AMN107 to retain the key hydrogen bonding interactions to Abl present in imatinib, including the hydrogen bond to Thr315. One major difference was that while the terminal nitrogen of the 4-(4-methylpiperazin-1-yl)methyl group of imatinib makes contact with His361 and Ile360 in the C-terminal lobe, these contacts are not made with the imidazole group of AMN107. The crystal structure also showed that a fluorine atom of the trifluoromethyl group interacted with the carbonyl group of Asp381 [41]. The analog of AMN107 where the trifluoromethyl group was replaced by a methyl group had decreased activity in a Bcr-Abl autophosphorylation assay. No additional SAR was reported for this compound.

In assays with BaF3 cells expressing wild-type Bcr-Abl, AMN107 had improved activity over imatinib. The IC_{50} values for AMN107 and imatinib in a Bcr-Abl autophosphorylation assay were 21 and 220 nM, respectively, while in a proliferation assay IC_{50}s of 25 and 650 nM were observed. A similar effect was seen in 32D cells expressing wild-type Bcr-Abl, with AMN107 having IC_{50}s of 20 and 9.2 nM for the inhibition of autophosphorylation and cell proliferation, respectively. Increased potency was also seen with AMN107 in assays with BaF3 cells expressing some of the imatinib resistant mutated forms of Bcr-Abl. In cells harboring the E255V mutation, one of the most clinically relevant mutations, the IC_{50}s in the autophosphorylation assay for AMN107 and imatinib were 250 and 6500 nM, respectively. In proliferation assays with these cells, the IC_{50}s for AMN107 and imatinib were 680 and 6400 nM, respectively. AMN107 had even greater activity in cell assays with the M351T mutant, having IC_{50}s of 31 and 33 nM for autophosphorylation and proliferation, respectively, compared with IC_{50}s of 595 and 1290 for imatinib. Most of the mutants examined were about 10–30 fold more sensitive to AMN107 than to imatinib. However, AMN107 was not active against cells harboring the T315I mutation when tested at 10 μM. AMN107 inhibited PDGFR and c-Kit with roughly the same potency as that of imatinib, making AMN107 a more selective Abl inhibitor than imatinib. As was seen with imatinib, a 3 μM concentration of AMN107 did not inhibit several kinases including Her-1, IGFR-1, Flt-3, VEGFR-2, FGFR-1, CDK-1, and c-Src.

AMN107 was profiled in additional imatinib resistant cells [42]. Imatinib resistant variants of KBM5 and KBM7 CML lines were selected that either had the T315I Abl mutation, KBM5 $STI571^{R1.0}$, or an amplification of the Bcr-Abl fusion gene, KBM7 $STI571^{R1.0}$. These lines were chosen to evaluate the activity of AMN107 against two of the most common forms of imatinib re-

sistance. In proliferation assays imatinib had IC_{50}s of 480 and 259 nM against the KBM5 and KBM7 lines, respectively. AMN107 was about 50-fold more potent than imatinib having IC_{50}s of 11 and 4.0 nM for the proliferation of the KBM5 and KBM7 lines. As anticipated from the earlier studies, neither imatinib or AMN107 had much effect on the proliferation of the KBM5 $STI571^{R1.0}$ line, having IC_{50}s of 6.4 and 2.4 μM, respectively. When tested in the KBM7 $STI571^{R1.0}$ line, imatinib had an IC_{50} of only 2.5 μM while that of AMN107 was 97 nM, making AMN107 27-fold more potent. Similar results were seen in Bcr-Abl autophosphorylation assays with these cells.

The effect of both AMN107 and imatinib on the cell cycle of K562 cells was examined [43, 44]. After a 24-hour incubation, a 10 nM dose of AMN107 caused a G1 arrest, with a 200 nM dose of imatinib required to produce the same result. After a 48-hour incubation, these same doses resulted in apoptosis, making AMN107 20-fold more potent than imatinib. A 5 μM dose of AMN107 led to increased apoptosis in cells isolated from imatinib resistant patients. Treatment of K562 cells with a 50 nM dose of AMN107 caused a decrease in the phosphorylation of the Tyr177 residue of Bcr-Abl. Phosphorylation at this site allows Bcr-Abl to bind the adapter protein Grb2 [45, 46]. It was also shown that treatment of K562 cells with AMN107 resulted in reduction of the phosphorylation of several downstream targets of Bcr-Abl including STAT5 and CrkL.

While imatinib is efficacious in treating Philadelphia chromosome positive CML, it is not as effective a treatment for patients with Philadelphia chromosome positive ALL [47]. As a prelude to clinical investigation, AMN107 was studied for its effect on Bcr-Abl positive ALL cell lines [48]. In proliferation and Bcr-Abl phosphorylation assays with two patient-derived ALL cell lines, namely Z-119 and Z-181, AMN107 was 30–40-fold more potent than imatinib.

AMN107 was tested in vivo in a severe combined immunodeficient (SCID) mouse model of CML using 32D cells harboring wild-type Bcr-Abl that also expressed the luciferase gene [40]. These cells permitted the use of bioluminescence imaging to non-invasively evaluate the response to drug treatment. Dosing of AMN107 at 75 mg/kg orally once a day for 16 days resulted in increased survival of the treated animals, over an observation period of 105 days. In addition, AMN107 was effectively transported into tissues such as the spleen. In a similar model employing 32D cells transformed by the E255V mutant, AMN107 again increased survival time, while imatinib did not. In a corresponding in vivo study with the M351T mutant, there was a further extension in the survival time compared to that seen with the E255V mutant, correlating with the increased activity seen with AMN107 in the autophosphorylation and proliferation cell assays with this mutant.

The in vivo activity of AMN107 was assessed in a second SCID mouse model of blast-phase CML [42]. In this model, mice were implanted with KBM5 cells and the tumors allowed to grow for 20 days. AMN107 was then administered ip for 20 days at doses of 10, 20 and 30 mg/kg. A dose response was

observed, with the 30 mg/kg treatment resulting in increased median survival time to 49 days from 27 days for the control animals.

AMN107 entered clinical trials in 2004. There was a preliminary report at the 2004 ASH meeting [49], and more details of the clinical findings were presented at the 2005 meetings of the American Society of Clinical Oncology (ASCO) [50, 51], American Association for Cancer Research (AACR) [52] and ASH [53]. The Phase I trial included patients with imatinib-resistant CML in blast crisis, accelerated and chronic phase, along with a smaller number of patients with Philadelphia chromosome positive ALL. As of June 15, 2005 119 patients had been treated with AMN107 [53]. It was noted that the C_{max} and AUC showed a dose-dependent increase from 50 to 400 mg, but did not increase at the higher doses. The peak concentration was seen after three hours and the half-life of AMN107 was 15 hours. The maximum tolerated dose of AMN107 was 600 mg twice a day with this dose causing increased levels of neutropenia and hyperbilirubinemia. A twice daily dose of 400 mg was selected for the Phase II trial. CML patients with no known Abl mutations prior to treatment with AMN107 had a hematological response rate of 72% and a cytogenetic response rate of 59%. For those patients having a known Abl mutation, the hematological and cytogenetic response rates were 60% and 41%, respectively. The response rate correlated with levels of the phosphorylation of CrkL, a Bcr-Abl substrate, seen in the patient peripheral blood or bone marrow samples [54]. A dose of 200 mg of AMN107 caused a significant reduction in the amount of CrkL phosphorylation as measured by Western blot.

In the initial report from the Phase I trial, the most prevalent Bcr-Abl mutations seen in patients treated with AMN107 were M351T and G250E [51]. Since AMN107 and imatinib do not make the same contacts with Abl, these two compounds would be expected to have different resistance profiles. A cell-based method that produced a mutation pattern for imatinib similar to that seen in the clinic [55] was applied to AMN107 [56]. Cells were grown in the presence of increasing compound concentrations and the resultant resistant colonies were picked and analyzed. A smaller number of mutations were observed with AMN107 compared to imatinib. Only seven amino acids were affected, and some of these mutations were not seen with imatinib either in the mutagenesis study or in the clinic. Increasing concentrations of AMN107 suppressed all but the T315I mutation. Another mutagenesis study also found a different mutation pattern for AMN107 compared to that of imatinib [57]. Exposure to AMN107 resulted in 20 colonies with single point mutations, five of which were unique to AMN107. Once again the most resistant mutation was the T315I.

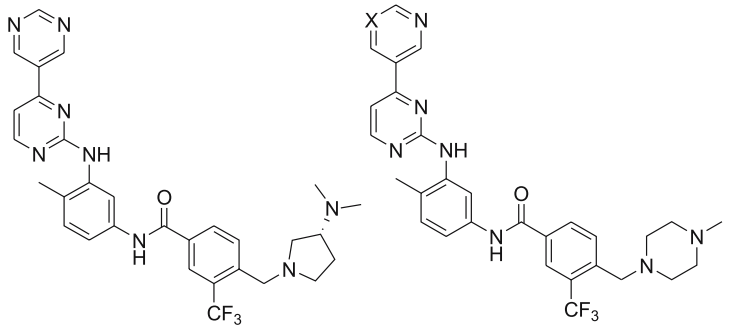
These impressive results led to AMN107 being dubbed both Super Gleevec and Son of Gleevec. Phase II trials have been initiated and these continuing studies will determine if AMN107 will enter the market for not only the treatment of imatinib-resistant CML but perhaps replace imatinib as the first line agent.

3.1.2 NS-187

In addition to Novartis, other companies have studied 2-phenylaminopyrimidines as kinase inhibitors. In 2005, Nippon Shinyaku identified a new Abl kinase inhibitor from this class of compounds [58]. NS-187 (**4**) differs from imatinib in that it has a pyrimidine ring at C-4 of the pyrimidine, as opposed to a pyridine ring, and different substituents on the pendant phenyl ring (Scheme 3). In an isolated Abl kinase assay NS-187 had an IC_{50} of 5.8 nM, while imatinib had an IC_{50} of 106 nM. When NS-187 was tested against a panel of kinases at 10 μM, activity was seen against Arg, Blk, Flt3, Lyn, PDGFRα, PDGFRβ and p70S6K. The most potent activity was seen for the inhibition of Arg, Fyn and Lyn, with the IC_{50} for Lyn being 19 nM. Interestingly, NS-187 had an IC_{50} of 1.7 μM for Src. The selectivity for Lyn over Src was attributed to the presence of a Cys residue in Src at the position that corresponds to a Gln residue in Abl and Lyn. It was postulated that the Gln in Abl and Lyn can form a hydrogen bond to NS-187, that can not be formed between the Cys of Src and NS-187.

In autophosphorylation assays in K562 cells and in BaF3 cells expressing wild-type Bcr-Abl, NS-187 had IC_{50}s of 11 and 63 nM, respectively. NS-187 also suppressed the growth of K562 cells and inhibited the phosphorylation of Abl downstream targets, including CrkL. In autophosphorylation assays with BaF3 cells transfected with the E255K and T315I Abl mutants, NS-187 had IC_{50}s of 340 nM and greater than 10 μM, respectively. The inactivity against the T315I mutation was confirmed by an in vitro kinase assay. When NS-187 was profiled against 13 Abl mutants, activity was seen against all but the T315I mutant.

Pharmacokinetic studies with NS-187 provided an oral bioavailability of 32% and the maximum daily tolerated dose in mice was 200 mg/kg. NS-187



Scheme 3

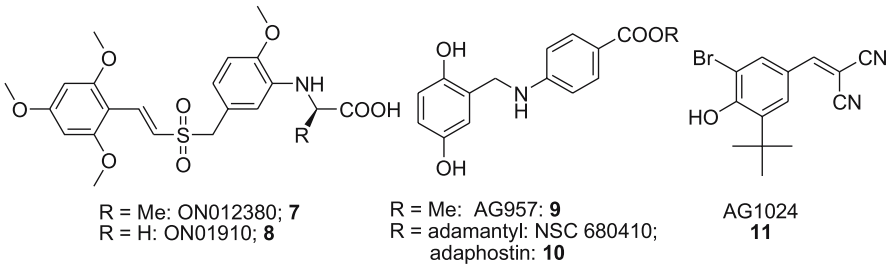
was tested in a xenograft model using KU812 cells. When dosed orally at 0.2 and 20 mg/kg twice a day for 10 days the 0.2 mg/kg dose resulted in significant inhibition of tumor growth while the 20 mg/kg dose completely inhibited tumor growth with no adverse effects. In a study in a xenograft model using BaF3 cells transformed with wild-type Bcr-Abl, NS-187 prolonged the life span of the animals in a dose-dependent manner. In a xenograft model using BaF3 cells transfected with the E255K Abl mutant, treatment with NS-187 also led to prolonged survival. The xenograft studies were expanded to BaF3 cells with additional Abl mutations including M244V, G250E, Q252H, Y253F, M351T, H396P and T315I [59]. With the exception of those animals with the T315I mutant, treatment with NS-187 resulted in an increased life span.

A SAR study of NS-187 appeared subsequent to these initial reports. Compounds were assayed for their ability to inhibit the proliferation of K562 cells with no information provided as to their activity in an Abl kinase assay [60]. Imatinib was used as a starting point and addition of a halogen at C-3 of the pendant phenyl ring led to increased activity in the cell assay. Further increased activity was observed with a C-3 trifluoromethyl group with **5** having an IC_{50} of 5 nM, compared to an IC_{50} of 180 nM for imatinib (Scheme 3). Replacement of the pyridine ring of **5** with a pyrimidine ring did not alter the activity, with **6** having an IC_{50} of 4 nM (Scheme 3). Replacement of the N-methylpiperazine ring of **6** with five additional cyclic amines gave compounds with IC_{50}s of 4–17 nM. NS-187 had an IC_{50} of 11 nM and it was selected for further study on the basis of its preferable pharmacokinetics and toxicity, although these data were not provided. The enantiomer of NS-187 was slightly more active in the cell proliferation assay having an IC_{50} of 4 nM. The authors concluded their manuscript with the statement that they expect NS-187 will advance to clinical trials.

3.2 Additional Bcr-Abl Inhibitors

3.2.1 ON012380

While the previously discussed 2-phenylaminopyrimidines bound in the ATP site of Bcr-Abl, another tactic has been to target a different area of the kinase. One advantage of this approach is that such a compound would be predicted to retain activity against the imatinib resistant mutations in the kinase domain, including the T315I mutation. By screening a library of non-ATP competitive kinase inhibitors, a group at the Fels Institute identified ON012380 (**7**) as a potent inhibitor of Bcr-Abl kinase activity having an IC_{50} of 9.0 nM [61] (Scheme 4). Kinetic assays confirmed that this compound was a non-ATP competitive inhibitor. The IC_{50} for imatinib in the Bcr-Abl kinase assay was 98 nM, and combination of ON012380 and 10 nM imatinib low-



Scheme 4

ered the IC_{50} to 0.83 nM, consistent with the different binding properties of ON012380 and imatinib.

When tested against a panel of kinases, ON012380 had activity against Fyn, IGFR, Lyn and PDGFR, with IC_{50}s of 86, 91, 85 and 80 nM, respectively. Interestingly, the IC_{50} for Src was greater than 10 μM. In a kinase assay using T315I mutated Abl, the IC_{50} for ON012580 decreased from 9.0 to 1.5 nM. This same effect was seen in a proliferation assay with 32Dcl3 cells containing the T315I and 15 additional Abl mutations, all of which were slightly more sensitive to ON012380 than the wild-type cells.

ON012380 was tested in an in vivo model where nude mice were injected iv with the 32Dc13 cells containing the T315I Abl mutation. After one day, the animals were dosed with both ON012380 and imatinib at 100 mg/kg ip in a saline vehicle for 14 days. Blood samples were examined on days 7 and 14. The blood of the ON012380 treated animals had a reduced number of T315I cells compared to the blood of both the control and the imatinib treated animals. No toxicity was observed in the animals treated with ON012380. ON012380 is being further evaluated for use in CML by Onconova Therapeutics [62].

A structurally related compound, ON01910 (**8**), was reported to be a 10 nM non-ATP competitive inhibitor of polo-like kinase 1 (Plk1) [63] (Scheme 4). Interestingly, the activity of ON012380 against Plk1 has not been reported. ON01910 is a 32 nM inhibitor of Bcr-Abl, and also inhibits Fyn and PDGFR, with IC_{50}s of 182 and 18 nM, but it has IC_{50}s of greater than 10 μM for both IGFR and Lyn. Since the structures of the two compounds only differ by the presence of a methyl group on ON012380, the differences in activity against IGFR and Lyn are rather striking. Onconova is currently investigating ON01910 (**8**) in Phase I clinical trials as an anti-cancer agent but at this time no results have been disclosed [62].

3.2.2 Adaphostin

In 1994, the tyrphostin AG957 (**9**) was identified as an inhibitor of Bcr-Abl that blocked the proliferation of K562 cells [64] (Scheme 4). Solubility and

pharmacokinetic issues with this compound led to a series of structural modifications that resulted in an adamantyl ester derivative NSC 680410, also known as adaphostin (**10**) [65]. Although adaphostin had reduced activity compared to AG957 in an in vitro kinase assay with IC_{50}s of 13.6 and 2.90 μM, respectively, it was slightly more potent in inhibiting the proliferation of K562 cells with IC_{50}s of 9.75 and 16.6 μM, respectively. It was also reported that the adamantyl group improved the pharmacokinetic properties including an extended half-life (36 min vs. 3 min for AG957). While adaphostin has been extensively studied in cell assays, it has not been tested in vivo in CML models or for activity against the imatinib resistant mutations [66–68].

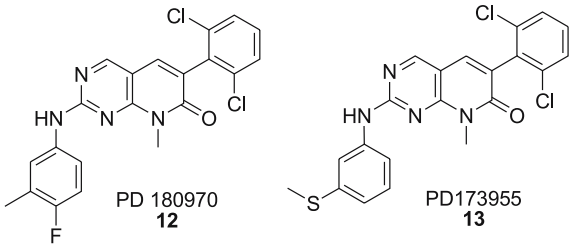
3.2.3 AG1024

Another tyrphostin, AG1024 (**11**), has also been examined for potential use in the treatment of CML [69] (Scheme 4). AG1024 inhibited the proliferation of both K562 cells and BaF3 cells transformed by Bcr-Abl. The IC_{50} values for AG1024 in these cell assays was about 5 μM. In a xenograft model employing wild-type Bcr-Abl transformed BaF3 cells, AG1024 reduced tumor growth when dosed ip for 10 days without any notable toxicity. All of these assays were indirect measurements of Abl activity with no data reported for the activity of AG1024 in an isolated Abl kinase assay or in a Bcr-Abl autophosphorylation assay.

AG1024 has been extensively studied as an IGFR inhibitor [70] and is a substrate competitive inhibitor of this kinase [71]. AG1024 also inhibits other kinases including c-Kit [72]. Additional studies will be needed, including a direct measurement of Abl activity and possible subsequent testing against the imatinib resistant Abl point mutations, to ascertain the possible therapeutic utility of AG1024.

4 Dual Inhibitors of Bcr-Abl and Src Kinases

Several of the new Bcr-Abl kinase inhibitors reported subsequent to imatinib also inhibit Src, a non-receptor tyrosine kinase. In 2000, it was reported that the known Src inhibitor PD180970 (**12**) also inhibited Abl kinase [73] (Scheme 5). This property was soon found to be shared by several other pyrido[2,3-*d*]pyrimidine Src inhibitors including PD173955 (**13**) [74] (Scheme 5). A crystal structure of PD173955 demonstrated that this compound could bind to both the active and inactive form of Abl [37]. While the conformation of active Src kinase is similar to that of active Abl, the conformations of the inactive kinases are quite different. Unlike PD173955, imatinib only binds the inactive form of Abl. The inability of imatinib to inhibit Src is



Scheme 5

likely a consequence of this conformational selectivity. So what is the benefit of a compound that inhibits both Abl and Src?

Over the years Src inhibitors have been widely studied as potential therapeutic agents for the treatment of several diseases including cancer [75]. Increased levels of Src activity often lead to increased tumor progression and metastasis [76–78]. Information on the current status of several Src inhibitors can be found in the work by Tomi Sawyer in this volume. More recently it was found that SFKs play a role in Bcr-Abl signaling and activation of two SFKs, namely Hck and Lyn, may lead to a more rapid progression of CML into blast phase [79–83]. One K562 cell line resistant to imatinib expresses high levels of activated Lyn [83]. Lyn was also over-expressed in cell lystates from some patients resistant to imatinib. Therefore, by inhibiting a second pathway, a dual Abl/ Src inhibitor may be more effective than a selective Abl inhibitor. In addition, since a major obstacle to the long-term effectiveness of imatinib is the acquired resistant Abl mutations, other compounds, perhaps especially those that bind to a different confirmation of Abl, may result in a lower number and/or a different pattern of resistant mutations.

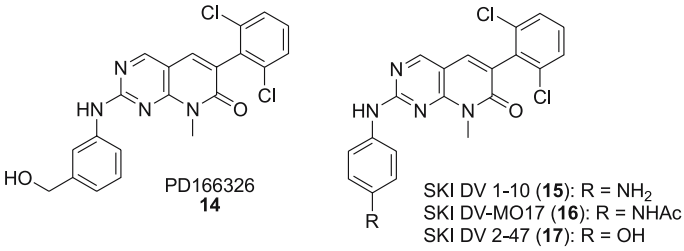
4.1 PD166326

Parke Davis first published on the SFK activity of a series of pyrido[2,3-*d*]pyrimidines in 1998 [84]. A more extensive evaluation of the SFK activity of some of these analogs, including PD173955 and PD180970, appeared in 2000 [85] and later that year the Abl kinase activity of PD180970 was disclosed [73]. PD180970 had an IC_{50} of 5 nM for the inhibition of Bcr-Abl autophosphorylation, and an IC_{50} of 2.2 nM in an isolated Abl kinase assay. This Abl inhibitory activity was confirmed by an independent group who obtained an IC_{50} of 1–2 nM in an Abl autophosphorylation assay [74]. A similar IC_{50} was obtained for PD 173955, and both these compounds were about 25–50-fold more potent than imatinib. However, other investigators observed comparable IC_{50}s for the inhibition of Abl autophosphorylation by PD180970 and imatinib of 45 and 25 nM, respectively [86]. This study also looked at the activity of PD180970 against some of the clinically relevant imatinib muta-

tions. While imatinib had IC_{50}s of greater than 1 μM for the inhibition of the autophosphorylation of the Y253F, E255K and T315I Abl mutants, PD180970 had IC_{50}s of 48 and 33 nM for Y253F and E255K but an IC_{50} of only 3.2 μM for the T315I mutation. Additional pyrido[2,3-*d*]pyrimidines were examined for both Abl kinase activity and activity in Bcr-Abl-dependent cell assays. All of these analogs had a 2,4-dichlorophenyl substituent at C-6, and a methyl group at C-8, so the effect of varying substituents at C-6 and C-8 on Abl activity is not known. One of these analogs, PD166326 (**14**) was found to be a more potent Abl inhibitor than the two earlier analogs [74] (Scheme 6). PD166326 had an IC_{50} of 0.1–0.2 nM in the Bcr-Abl autophosphorylation assay, where the IC_{50} of PD180970 was 1–2 nM. PD166326 also had the greatest potency for the inhibition of Bcr-Abl-dependent cell growth.

A second study of several pyrido[2,3-*d*]pyrimidines confirmed that PD166326 was the most potent Abl kinase inhibitor, having an IC_{50} of 8 nM [87]. These investigators reported an IC_{50} of 50 nM for imatinib in this assay. PD166326 potently inhibited the proliferation of K562 cells with an IC_{50} of 0.3 nM. In a K562 Bcr-Abl autophosphorylation assay, PD166326 had an IC_{50} of 1 nM and was also active in a similar assay with the E255K mutation. Autophosphorylation of the T315I mutant was not inhibited by PD166326, which was not surprising based on the previous results with the closely related PD180970. A study was also done to look at the activity of PD166326 in Bcr-Abl autophosphorylation assays with BaF3 cells transfected with several of the imatinib-resistant Abl mutants [88]. PD166326 inhibited the autophosphorylation of the H396P mutant with similar activity to that seen against the wild-type Bcr-Abl. Slightly reduced activity was seen against the Y253H, E255K and E255V mutants. Confirming what was observed earlier, the T315I mutant was resistant to PD166326.

Additional pyrido[2,3-*d*]pyrimidines were prepared at Memorial Sloan-Kettering Cancer Center, including SKI DV 1–10 (**15**), SKI DV-MO17 (**16**) and SKI DV 2–47 (**17**) [89] (Scheme 6). In proliferation assays with Bcr-Abl expressing MO7e cells, PD166326 had an IC_{50} of 0.4 nM and roughly comparable activity was seen with the other three analogs which had IC_{50}s of 0.6 to 1.5 nM. Once again the only structural changes with these analogs were the



Scheme 6

groups on the 2-phenylamino ring, so it remains to be determined how varying the substituents at other positions would influence the Abl activity of this series of compounds.

PD166326 and the three additional analogs were also examined in proliferation assays with BaF3 cells expressing Bcr-Abl [90]. PD166326 had an IC_{50} of 19 nM for the inhibition of proliferation of the wild-type line, while the other compounds were less potent having IC_{50}s from 46 to 120 nM, with the weakest activity observed with SKI DV-MO17. In a corresponding assay with the T315I mutated line, the compounds all showed greatly reduced activity, having IC_{50}s greater than 5 μM. However, these compounds did show activity in a proliferation assay with BaF3 cells that were resistant to imatinib due to over expression of Bcr-Abl. PD166326 had an IC_{50} of 140 nM in this assay and the IC_{50}s for the other three analogs ranged from 200 to 460 nM.

Since PD166326 does not make the same contacts with Abl as does imatinib, the two compounds may not select for the same Abl point mutations when administered to patients. Exposure of wild-type Bcr-Abl transformed BaF3 cells to imatinib produced a mutation pattern similar to that seen in the clinic [55]. Applying this technique to PD166326 led to a different mutation pattern than that seen with imatinib. While the most prevalent mutation was the T315I, the mutations in the P-loop were decreased with PD166326.

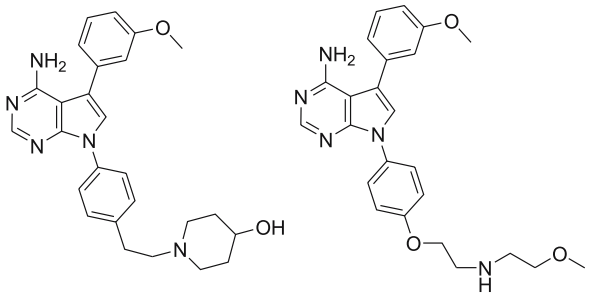
Although PD166326 had been extensively profiled in cell assays, the first report of activity in an animal model did not appear until 2005 [91]. In a preliminary pharmacokinetic study, PD166326 was orally administered to mice in a vehicle of 10% aqueous DMSO. The compound had a half-life of 8.4 h and the maximum tolerated dose of PD166326 was 50 mg/kg given twice a day. PD166326 was tested in a model where a CML-like disease was induced in mice, via a replication-defective Bcr-Abl retrovirus that targets bone marrow cells. The syngenic mice infected with this retrovirus were administered a twice daily dose of 50 mg/kg of PD166326. All the control animals died by day 26 and the treated animals were euthanized at days 33–37 and the spleens were harvested. Administration of PD166326 resulted in reduced spleen weight compared to the controls and the treated mice also had a 10 fold lower white blood cell count. Similar in vivo studies were done with mutated forms of Abl. Administration of PD166326 to H396P and M351T syngenic mice increased the life span of these animals but had no effect on T315I syngenic mice.

The initial pyrido[2,3-*d*]pyrimidines were first reported as Src inhibitors by Parke-Davis, now Pfizer, and the first disclosures of Abl activity were done in collaboration with academic researchers. Although the recent studies with these analogs were reported by academic groups, it has been stated that Sloan-Kettering is working with a pharmaceutical company to develop a compound from this class [92].

4.2 CGP76030

Several years ago, Novartis reported that a pyrrolo[2,3-*d*]pyridimine, CGP77675 (**18**), was a potent Src kinase inhibitor with activity in murine osteoporosis models [93] (Scheme 7). CGP77675 had an IC_{50} of 20 nM in a Src enzyme assay and was a weaker inhibitor of Abl, having an IC_{50} of 150 nM. For this program Novartis was interested in a selective Src inhibitor and optimized their SAR against this kinase. Interestingly, while Src knock-out mice have osteopetrosis, or greatly increased bone density, Abl knock-out mice have severe osteoporosis [94]. Additional analogs of CGP77675 were studied and most retained some degree of selectivity for Src over Abl [95–97]. One compound, CGP76030 (**19**), had IC_{50}s of 27 and 180 nM for the inhibition of Src and Abl, respectively, and also inhibited Lck (IC_{50} = 360 nM) [98] (Scheme 7).

CGP76030 was studied in assays with cell lines harboring wild-type Bcr-Abl and several of the clinically relevant imatinib-resistant Abl mutations [99]. CGP76030 was effective in inhibiting the autophosphorylation of wild-type Bcr-Abl but was ineffective against the T315I mutation. Some other additional Thr315 mutated lines were also resistant, but CGP76030 blocked autophosphorylation in the T315V line where the Thr residue was replaced by a comparably sized Val residue. CGP76030 also effectively blocked autophosphorylation and proliferation in cells harboring the A380C, A380T, A276S and G279S mutations. Interestingly, CGP76030 blocked the growth of cells expressing the additional T315 mutations, including T315I. Because of its lack of activity in the Abl autophosphorylation assays with these mutants, CGP76030 inhibited the proliferation of these cells by a mechanism independent of Abl inhibition, possibly a SFK-dependent pathway. Similar results were obtained in cell assays with an imatinib-resistant K562R line that expresses high levels of Lyn [83]. CGP76030 effectively inhibited SFKs in these cells at concentrations that had little effect on Bcr-Abl activity.



CGP77675
18

CGP76030
19

Scheme 7

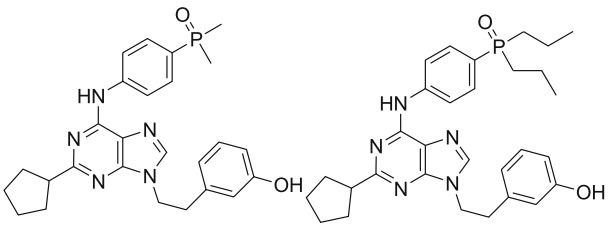
Although imatinib is efficacious in treating chronic phase CML it is less effective in treating Bcr-Abl positive B-cell acute lymphoblastic leukemia (B-ALL). A recent study showed this discrepancy results from the important role of SFKs in B-ALL [100]. It was determined that three SFKs, Lyn, Fgr and Hck, were activated to a greater extent in mouse B-ALL cells than in cells from control mice. Treatment of these B-ALL cells with CGP76030 led to a decrease in cell growth and survival as a result of inhibition of SFK activity. In an in vivo model of B-ALL, a combination of CGP76030, dosed orally twice daily at 50 mg/kg, and imatinib, dosed orally twice daily at 100 mg/kg, increased survival compared to either agent alone.

Novartis has not reported on the advancement of CGP76030 to clinical trials.

4.3 AP23464 and AP23848

ARIAD has published extensively on non-ATP competitive Src kinase inhibitors as potential agents for the treatment of osteoporosis [101]. These efforts were later expanded to ATP competitive Src kinase inhibitors that were based on a purine template. These compounds were designed to target bone via the incorporation of a bisphosphonate group [102]. AP23464 (**20**), which contains a dimethylphosphine oxide substituent, inhibited Src with an IC_{50} of 0.45 nM and inhibited Abl with an IC_{50} of 0.67 nM [103, 104] (Scheme 8). Although no SAR for Abl was reported for AP23464, removal of the dimethylphosphine oxide group decreased the Src inhibitory activity by about 80 fold [105]. While AP23464 inhibited c-Kit and PDGFR it was less potent against these two kinases than against Src and Abl [106]. Molecular modeling of AP23464 with the Abl kinase domain suggested that the compound bound to the active form of the kinase and that the Thr315 residue of Abl formed a key hydrogen bond with AP23464 [106].

BaF3 cell assays were used to study the effect of AP23464 against the most relevant Abl mutations. In a cell proliferation assay with BaF3 cells expressing



Scheme 8

wild-type Bcr-Abl, AP23464 had an IC_{50} of 14 nM while imatinib had an IC_{50} of 350 nM. AP23464 inhibited the proliferation of BaF3 cells harboring two P-loop mutations Q252H and Y253F, a C-terminal loop mutant M351T and an activation loop mutant H396P with IC_{50}s in the range of 8–26 nM. Decreased activity was seen with the P-loop mutation, E255K, where AP23464 had an IC_{50} of 94 nM. As expected based on the Abl binding model, the proliferation of BaF3 cells harboring the T315I mutation was not inhibited by AP23464.

In a cell free autophosphorylation assay, AP23464 had IC_{50}s of 31–61 nM against the four mutants (Q252H, Y253F, M351T and H396P) that were the most sensitive in the cell proliferation assays. Again in correlation with the cell proliferation assay, AP23464 was less effective in inhibiting the autophosphorylation of the E255K mutant, IC_{50} = 110 nM, and was not effective against the T315I mutant having an IC_{50} of greater than 5 μM. AP23464 also blocked cell cycle progression and caused apoptosis in K562 cells. Treatment of these cells with AP23464 reduced the phosphorylation of STAT5 and CrkL, two Bcr-Abl substrates.

A similar analog, AP23848, (**21**) was also reported to be an inhibitor of Bcr-Abl [107] (Scheme 8). In a proliferation assay with wild-type Bcr-Abl BaF3 cells, AP23848 had an IC_{50} of 18 nM. Activity was also seen against the M351I, Y253F and E255K mutations with IC_{50}s of 21–72 nM, while the T315I mutant did not respond (IC_{50} greater than 8 μM). These results were expected due to the structural similarity of AP23848 and AP23464.

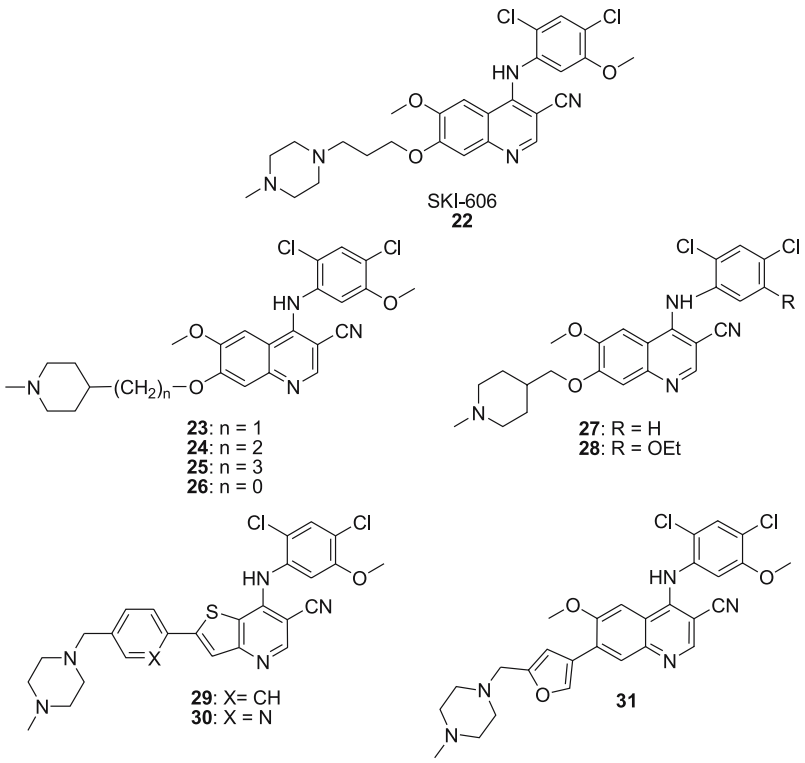
Although no studies in animal models of CML have been reported for these compounds, AP23848 was tested in a mouse model of c-Kit induced tumor growth. Both compounds are potent inhibitors of this kinase and are also active against the D816V mutant, the most commonly observed c-Kit mutation in the clinic after imatinib administration [108]. For the xenograft study AP23848 was selected over AP23464 due to its improved pharmacokinetics and metabolism profile although this comparative data was not reported. Oral administration of 100 mg/kg of AP23848 three times a day for three days inhibited c-Kit D816V mutant driven tumor growth. However, no decrease in tumor size was observed and it was not possible to extend the dosing time since weight loss was observed after three days of dosing. AP23464 may have sub-optimal solubility properties since the vehicle used was 15% dimethylacetamide, 14% vitamin E, 5% Tween-80, 26% PEG-400 and 40% water.

ARIAD recently disclosed the results of an in vitro mutagenesis study with AP23464 [109]. While AP23464 suppressed the formation of the P-loop mutations no effect was seen on the emergence of the T315I mutation even when AP23464 was combined with both PD166326 and imatinib. In this report it was stated that additional analogs of AP23464 were being characterized and it was predicted that some of these would be active against the T315I mutation. In an August 2004 press release, ARIAD announced that it had discontinued development of AP23464 but was investigating two classes of related compounds in order to identify a candidate with an improved metabolic profile [110].

4.4 SKI-606

SKI-606 (**22**), a potent Src inhibitor, was identified by Wyeth via optimization of a 4-anilino-3-quinolinecarbonitrile screening lead [111, 112] (Scheme 9). When tested against a panel of tumor cell lines it was observed that SKI-606 inhibited the proliferation of K562 and KU812 cells, with IC_{50}s of 5 and 20 nM, respectively [113]. SKI-606 reduced the phosphorylation of Bcr-Abl in these cells along with the phosphorylation of the SFKs Lyn and Hck. In both the K562 and KU812 lines, SKI-606 caused G1/S cell cycle arrest and increased apoptosis. SKI-606 inhibited the proliferation of v-Abl transformed rat fibroblasts with an IC_{50} of 90 nM and had an IC_{50} of 1 nM in an isolated Abl kinase assay. In assays for Src kinase activity and for the inhibition of the proliferation of Src-transformed fibroblasts, IC_{50}s of 1.2 and 100 nM were observed, making SKI-606 a potent dual inhibitor of Src and Abl.

To evaluate the in vivo efficacy of SKI-606, K562 tumors were implanted into nude mice and staged to 200–300 mgs. The animals were treated with a 75 mg/kg oral dose of SKI-606 twice a day for 10 days, resulting in tumor



Scheme 9

regression for two months. Once a day oral dosing at 100 and 150 mg/kg for five days resulted in animals that were tumor free for six weeks. When the tumors were staged to 800–900 mgs, oral dosing of SKI-606 at 100 mg/kg once a day for five days resulted in tumor free animals at the end of 40 days.

SKI-606 prevented both Bcr-Abl and Lyn phosphorylation in an imatinib-resistant K562 line that expresses high levels of Lyn [114]. SKI-606 also inhibited the proliferation of these cells and this activity correlated with decreased levels of SFK activation. In additional studies, SKI-606 inhibited phosphorylation of Bcr-Abl, Lyn and Hck in cells from blast-phase CML patients [115]. Furthermore, G1/S arrest and an increase in apoptosis was seen in cells from patients with the E255V, E255K, F359V and Y253H imatinib-resistant Abl mutations [116]. In an isolated kinase assay with T315I mutated Abl, SKI-606 had an IC_{50} of 344 nM [117]. The decrease in activity against this mutant was predicted based on the key role of the gate keeper Thr315 of Abl in the binding of the dual Src/Abl inhibitors.

To identify genes whose expression was affected by SKI-606, a transcriptional profiling study was done in K562 cells [118]. Oligonucleotide microarray analysis was used to compare cells treated with 10 nM SKI-606 to control cells. SKI-606 modified the expression of 121 genes, some of which are involved in transcriptional regulation, signal transduction and cell cycle regulation, including the down regulation of key apoptotic suppressor genes.

In a study of SKI-606 analogs, it was established that variation of the group at C-7 from a (1-methylpiperazin-4-yl)propoxy to a (1-methylpiperidine-4-yl)methoxy group retained much of the activity of the parent compound [119]. In a Lance format Src kinase assay, as opposed to the ELISA format used initially, SKI-606 and **23** had IC_{50}s of 3.8 and 7.0 nM, respectively. The IC_{50}s for Abl inhibition by SKI-606 and **23** were 1.1 and 2.9 nM, respectively (Scheme 9). While lengthening the alkoxy chain to two or three methylene groups also provided potent dual inhibitors, **24** and **25**, removing the methylene group was highly detrimental, with **26** having IC_{50}s of only 230 and 89 nM for the inhibition of Src and Abl kinases, respectively. Substituents on the 4-anilino group that resulted in decreased Src activity also resulted in decreased Abl activity. For example, removal of the 5-methoxy group of **23** led to **27** which had IC_{50}s for the inhibition of Src and Abl of 21 and 18 nM (Scheme 9). The most dramatic effect was seen with the 2,4-dichloro-5-ethoxy analog **28** which had IC_{50}s of only 1.4 and 1.5 μM for the inhibition of Src and Abl, respectively (Scheme 9).

Some related thieno[3,2-*b*]pyridines were also reported to be potent Src and Abl inhibitors [120, 121]. The C-2 phenyl analog **29**, had an IC_{50} of 13 nM for the inhibition of Src and was more potent against Abl, having an IC_{50} of 2.3 nM (Scheme 9). Very similar activity was seen with the C-2 pyridine analog **30** which had IC_{50}s of 13 and 1.3 nM for the inhibition of Src and Abl, respectively. While no information on the activity of **23–30** against the imatinib-resistant mutations or efficacy in an in vivo model

of CML was reported, these activities were recently disclosed for another 3-quinolinecarbonitrile derivative **31**, which contains a furan ring at C-7 of the quinoline core [117] (Scheme 9). In Src and Abl kinase assays, **31** had IC_{50}s of 0.78 and 0.35 nM, respectively, making it about a four-fold more potent inhibitor than SKI-606. In a kinase assay with the T315I Abl mutation, **31** was less active than against wild-type Abl, having an IC_{50} of 68 nM. A corresponding increase in activity compared to SKI-606 was observed in proliferation assays with K562 and KU812 cells, where **31** had IC_{50}s of 5.7 and 1.4 nM, respectively. Inhibition of STAT5 phosphorylation in both the K562 and KU812 cells was inhibited by **31** with IC_{50}s of 5.5 and less than 3 nM, respectively.

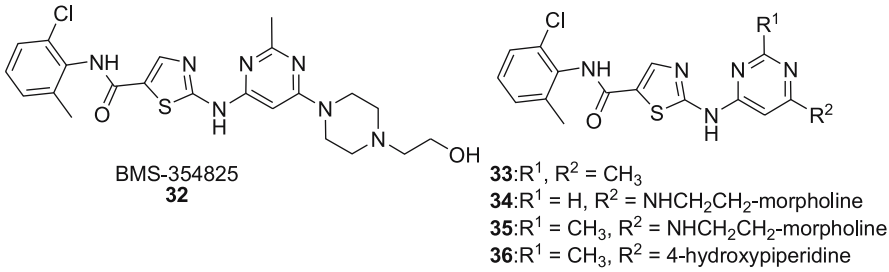
In a nude mouse xenograft model with K562 tumors staged to 300–400 mg, a daily oral dose of 50 mg/kg of **31** for five days resulted in survival of all 25 animals for 100 days. When **31** was dosed in this model at 5 mg/kg for five days, about half of the animals survived for 100 days. A second in vivo model was also used where the K562 tumors were staged to a much larger size, approximately 1.6 g. Treatment of these animals with a 50 mg/kg oral dose of **31** for five days resulted in complete tumor regression with no recurrence of the tumor observed over the next two months.

Of all the 4-anilino-3-quinolinecarbonitrile dual Src/Abl inhibitors, the most extensively profiled analog is SKI-606. Pharmacokinetics showed SKI-606 to have an oral bioavailability in nude mice of 18% and a half-life of 8.6 hours with a large volume of distribution [122]. SKI-606 was active in several colon tumor xenograft models when dosed orally at 25–150 mg/kg daily for 21 days with no weight loss or overt toxicity noted in the animals. On the basis of its pre-clinical properties, SKI-606 entered clinical trials in 2004 for the treatment of solid tumors and will soon be entering trials for the treatment of CML.

4.5 BMS-354825

Late in 2003 there were reports in CML patient newsletters about a new Src/Abl inhibitor from Bristol Myers Squibb (BMS) that was in clinical trials for the treatment of imatinib-resistant CML [123, 124]. At the 2004 AACR meeting, there were three presentations on this new compound, BMS-354825 (**32**), although the structure was not disclosed at this time [125–127]. It was reported that BMS-354825 was 500-fold more potent than imatinib at inhibiting Abl kinase activity and that it was also effective against 14 out of 15 of the imatinib-resistant Abl mutations, with the exception being the T315I mutant.

The first peer-reviewed report on BMS-354825 was published in Science soon after the meeting presentations [128]. BMS-354825 has a 2-aminothiazole core and is related to a series of Lck inhibitors from BMS based on this template [129] (Scheme 10). BMS-354825 potently inhibited the pro-



Scheme 10

liferation of BaF3 cells harboring wild-type Bcr-Abl with a low nanomolar IC_{50}. Similar efficacy was seen for the proliferation of BaF3 cells containing 14 imatinib-resistant mutations, including E255K/V, Y253F/H and M351T, three of the most clinically common mutations. Once again little inhibition was observed for cells with the T315I mutation. Parallel results were seen in autophosphorylation assays with these cells.

To test the in vivo effectiveness of BMS-354825, a SCID mouse model was employed. This model was similar to that used to assess the activity of AMN107 in that cells expressing both Bcr-Abl and luciferase were implanted into mice to allow for a non-invasive measure of efficacy. Three days after injection of these cells, the mice were treated for two weeks with a twice a day oral dose of 10 mg/kg of BMS-354825. The mice that received BMS-354825 had greatly decreased levels of bioluminescence, appeared healthy and had increased long-term survival. A similar result was seen in this model with BaF3 cells harboring the M351T mutation, while mice harboring the T315I mutation did not respond.

Shortly after this initial publication, there was a report describing the effect of varying the substituents on the pyrimidine ring of BMS-354825 [130]. The lead compound where R^1 and R^2 are methyl (**33**) was initially identified as a Lck inhibitor, and later found to have an IC_{50} of 96 pM for Src and an IC_{50} of less than 1 nM for Abl (Scheme 10). In an antiproliferation assay with K562 cells, **33** had an IC_{50} of about 2 nM. This cell assay was used to rank compounds as Abl inhibitors, not a kinase assay. Compounds were also tested in additional cell proliferation assays using prostate (PC3), breast (MDA-MB-231) and colon (WiDr) lines. Since **33** had poor pharmacokinetic properties after oral dosing in the mouse, analogs were prepared with solubilizing amine groups on the pyrimidine ring. Addition of a 2-morpholinoethylamino group provided **34**, which had excellent oral pharmacokinetics but showed reduced activity against the K562 and solid tumor lines (Scheme 10). Addition of a methyl group at C-2 of the pyrimidine ring of **34**, to provide **35**, decreased the pharmacokinetic properties but increased the activity in the proliferation assays (Scheme 10). The two compounds with the most favorable combination of

both cell activity and oral pharmacokinetic properties were BMS-354825 and **36** and it is not clear why **36** was not examined further (Scheme 10).

When evaluated in isolated kinase assays, BMS-354825 was a 500 pM inhibitor of Src and had an IC_{50} of less than 1 nM for Bcr-Abl. Similar activity was seen against the SFKs Lck and Yes, where BMS-354825 had IC_{50}s of 400 and 500 pM, respectively. Moderate activity was seen against c-Kit and PDGFRβ (IC_{50}s of 5 and 28 nM) while IC_{50}s of 100 nM or greater were observed against the other 13 kinases tested. Kinetic analysis confirmed that BMS-354825 was an ATP competitive inhibitor with K_is for Src and Bcr-Abl of 16 and 30 pM, respectively.

A crystal structure of BMS-354825 with Abl kinase revealed that the activation loop of the protein was in the active conformation. Two key hydrogen bonds were formed between the back-bone carbonyl and amide group of Met318 to the 2-amino hydrogen and the nitrogen of the thiazole ring of BMS-354825, respectively. As expected based on the activity profile observed with the imatinib-resistant Abl mutations, the crystal structure showed the presence of a hydrogen bond between Thr315 and the amide nitrogen of BMS-354825. On the basis of the crystal structure it was proposed that BMS-354825 could also bind the inactive conformation of Abl and this ability to bind both conformations could be the reason for the greater binding affinity of BMS-354825 compared to imatinib [131].

It was postulated that since BMS-354825 can bind both conformations of Abl, and since it makes less direct contacts with the protein, there may be fewer resistant mutations seen in the clinic. As a first step, a saturation mutagenesis study was done similar to that performed earlier with imatinib [132]. It was determined that only six amino acid residues were mutated and that four of these made contact with BMS-354825, namely Leu248, Val299, Thr315 and Phe317 [133]. These four residues accounted for 97.5% of the observed mutations and only Val299 had not been identified as a site of imatinib-resistant mutation. Some amino acids were mutated to more than one residue. For example, in addition to T315I, a T315A mutation was observed. In proliferation assays with BaF3 cells harboring the T315A mutation, the activity of BMS-354825 decreased 90 fold, while imatinib was only two-fold less active, highlighting that the Thr315 interaction is more critical for BMS-354825 than for imatinib. Mutations with the greatest resistance to BMS-354825, after T315I, were V299L, F317V and F317L. Interestingly, imatinib had less than a three-fold decrease in activity against these three mutations, raising the possibility that use of a combination of BMS-354825 and imatinib in the clinic could reduce the development of resistance.

In addition to the original report on the activity of BMS-354825 in a SCID mouse model using a Bcr-Abl transformed cell line, other in vivo studies were also carried out. In a K562 xenograft model of CML, tumors were allowed to grow to about 300 mg [130]. BMS-354825 was then dosed orally once a day at 5 and 50 mg/kg, for two weeks with a dosing regime of five days on and two

days off. Both doses resulted in elimination of the tumor with no observed toxicity. When BMS-354825 was administered twice daily, a 1.25 mg/kg dose was effective [134]. This K562 model was used to develop biomarkers for BMS-354825 [134]. In these tumors, the phosphorylation levels of Bcr-Abl and those of CrkL, a substrate protein for Bcr-Abl, correlated with the plasma levels of the compound.

BMS-354825 was also tested in vivo in a xenograft model with K562R cells that are resistant to imatinib due to the overexpression of SFKs [135]. Although imatinib was not effective in this model even at 150 mg/kg, doses of greater than 5 mg/kg of BMS-354825 were effective. These results reflect those seen in proliferation assays with these cells, where BMS-354825 was more than 1000-fold more potent than imatinib. BMS-354825 was also 29 fold more potent than AMN107, which, like imatinib, does not inhibit SFKs. While imatinib is not effective in a model of intracranial CML, most likely as a result of its lack of brain penetration [136], activity was seen with BMS-354825 [137]. K562 tumors were implanted intracranially into SCID mice and BMS-354825 was dosed orally twice a day for 40 days. Doses of both 5 and 15 mg/kg greatly increased the survival of these animals.

The activity of BMS-354825 in these preclinical studies led to its advancement to clinical trials in late 2003. The first reports of the Phase I dose escalation studies were presented at the 2004 ASH meeting [138, 139] and the 2005 ASCO meeting [140, 141] with additional details presented at the 2005 ASH meeting [142]. The enrolled patients were either imatinib-resistant or intolerant, with the majority being in the chronic phase. The trial also included patients in accelerated phase, myeloid or lymphoid blast crisis and those with Philadelphia chromosome positive ALL. For 40 patients with chronic-phase disease, who were treated for an average of 13 months, 88% showed a complete hematological response, 40% had a major cytogenetic response and 88% had a complete cytogenetic response [142]. For ten patients with advanced disease who were treated for an average of six to seven months, the major hematological response rates for those in accelerated phase and myeloid blast crisis were 50% and 18%, respectively. Patients in this study received doses as high as 240 mg twice a day. Since BMS-354825 is known to inhibit other SFKs such as Lyn and Lck, there is the possibility that the compound could have immunosuppressive properties. In studies of blood samples of 14 chronic-phase patients, treatment with BMS-354825 had no effect on T cell cytokine production, including IL-1, TNF-α and INF-γ [143].

BMS-354825, which has the generic name of dasatinib, entered Phase II clinical trials in December 2004 under the START (Src/Abl Tyrosine kinase inhibition Activity: Research Trial) program [144–147]. Dosing was initiated at 70 mg twice daily with the option of increasing the dose to 90 or 100 mg or reducing the dose to 40 or 50 mg, depending on low response or toxicity. As part of the Phase I and II trials, patients were assessed for the presence of Bcr-Abl mutations prior to treatment with BMS-354825. In a study at UCLA,

five patients with a pre-existing T315I mutation did not respond [148]. This mutation was also identified in samples from seven out of nine patients who developed resistance to BMS-354825. The other two patients were found to have T315A and F317I mutations, both of which were observed in the earlier mutagenesis study done to predict mutations that might arise in the clinic. Most interestingly, these two mutations were sensitive to imatinib [133]. As part of the clinical trials at M.D. Anderson, it was observed that of 12 patients who had P-loop mutations, 11 responded to BMS-354825 [149]. However, once again no response was seen with patients with a pre-existing T315I mutation, emphasizing the importance of identifying a compound with activity against this mutant.

The effect of BMS-354825 on cells from patients resistant to imatinib by mechanisms other than the presence of point mutations was also examined [150]. Treatment of these cells with concentrations of 1 μM imatinib had no effect on the regulation of SFK activity, while a 0.5 μM dose of BMS-354825 completely inhibited activation of two SFKs, namely Hck and Lyn, as measured by autophosphorylation. Both imatinib and BMS-354825 inhibited Bcr-Abl activity in these cells, as measured by the inhibition of CrkL phosphorylation. Therefore since BMS-354285 inhibits SFKs in addition to Abl, it may overcome clinical resistance to imatinib in those cases where resistance is due to the loss of Bcr-Abl mediated regulation of SFKs.

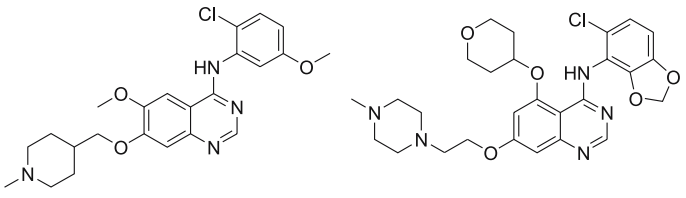
In addition to in vivo studies in models of CML, BMS-354825 has also been studied in models of solid tumors. BMS-354825 was efficacious in head and neck squamous cell carcinoma and non-small cell lung cancer animal models [151]. Based on this activity, BMS-354825 has been advanced into clinical trials for the treatment of solid tumors.

Note added in proof: On June 29, 2006 the Food and Drug Administration (FDA) granted approval for dasatinib (Sprycel) [152].

4.6 AZD0530

In 2004 Astra Zeneca published on a series of 4-anilinoquinazolines as potent Src inhibitors [153]. One analog, AZM475271 (**37**), inhibited Src kinase activity with an IC_{50} of 10 nM, and had in vivo efficacy in a mouse model of pancreatic cancer [154] (Scheme 11).

While there was no information reported on the activity of AZM475271 against Abl kinase, it was disclosed at the 2005 AACR meeting that a related 4-anilinoquinazoline, AZD0530 (**38**), was a potent inhibitor of Src having an IC_{50} of 2.7 nM and also inhibited Abl with an IC_{50} of 30 nM [155] (Scheme 11). AZD0530 inhibited Yes and Fyn with IC_{50}s similar to that for Src. Modest activity was seen against c-Kit (IC_{50} = 200 nM) with all the other kinases tested having IC_{50}s of greater than 800 nM. No information was presented on the activity of AZD0530 against the imatinib-resistant Abl mutations.



Scheme 11

While no in vivo activity has been reported in an Abl-dependent CML model, AZD0530 was active in a xenograft model using Src-transformed NIH 3T3 cells and in an orthotopic model of pancreatic cancer, two Src-dependent models. The results of a Phase I trial in normal volunteers have been disclosed and AZD0530 was tolerated when administered at doses of up to 250 mg [156, 157]. While it appears that the initial efficacy trial for AZD0530 will be as an anti-invasive agent for the treatment of metastatic bone disease, this compound probably also has potential for use in CML.

5
Key Issues

5.1
Direct Comparison of AMN107 and BMS-354825

In a key study, the two new Bcr-Abl inhibitors currently in the clinic, AMN107 and BMS-354825, were tested side-by-side to directly compare their activity against some of the imatinib resistant Abl mutations [158]. In a kinase assay with wild-type Bcr-Abl, imatinib had an IC_{50} of 280 nM, while the IC_{50}s for AMN107 and BMS-354825 were 15 and 0.6 nM, respectively. BMS-354825 also had IC_{50}s of 0.8 and 2.8 nM for Src and Lyn while imatinib and AMN107 were not active against these SFKs. In a cell-free Abl autophosphorylation assay the IC_{50}s for imatinib, AMN107 and BMS-354825 were 300, 7.0 and 0.8 nM, respectively, in good correlation with the enzyme IC_{50}s. In Bcr-Abl transformed BaF3 cell proliferation assays the IC_{50}s for imatinib, AMN107 and BMS were 260, 13 and 0.8 nM respectively, while in BaF3 cell autophosphorylation assays the IC_{50}s for these three compounds were 280, 10 and less than 10 nM, respectively. All three compounds were ineffective in assays with the T315I mutant, corresponding to what was reported previously. Of the 15 additional imatinib-resistant mutations, those least affected by AMN107 were Y253H, E255V and F359V, with a larger effect seen in the cell assays. In proliferation assays with the Y253H and E255V mutant, AMN107 had IC_{50}s of only 450 and 430 nM compared to 13 nM against wild-type Bcr-Abl. The activ-

ity of BMS-354825 against the Y253H and E255V mutants did not change as dramatically with this compound having IC_{50}s of 1.3 and 11 nM. Similar results were seen in the autophosphorylation assay where AMN107 had IC_{50}s of 160 and 250 nM against the Y253H and E255V mutants, and the IC_{50}s for BMS-354825 were less than 10 nM.

AMN107 and BMS-354825 were also tested side-by-side with imatinib in an in-vitro mutagenesis study [159]. BaF3 cells expressing wild-type Bcr-Abl were exposed to a minimal cytotoxic dose of N-ethyl-N-nitrosourea to accelerate mutagenesis. Cells were then treated with the three Abl inhibitors at doses that were twice that of the IC_{90} for the inhibition of cell proliferation. A 2 μM dose of imatinib produced 18 mutations, a 50 nM dose of AMN107 produced eight mutations and a 5 nM dose of BMS-354825 produced four mutations. At higher doses of AMN107 (2 μM) and BMS-354825 (25 nM) only the T315I mutant was observed. Once again, this study showed the importance of identifying a compound with activity against the T315I mutation.

5.2 Combinations of Imatinib and New Bcr-Abl Inhibitors

One area of interest is the potential use of imatinib in combination with another Bcr-Abl inhibitor, including the dual Src/Abl inhibitors, since all of the imatinib-resistant Abl mutants, with the exception of the T315I mutant, are sensitive to these agents. In addition, in cell mutagenesis studies these compounds all generated a different mutation pattern from that of imatinib [56, 57, 109, 141, 159]. In the case of BMS-354825 some of these mutants were sensitive to imatinib [133]. Co-administration of imatinib and a second Bcr-Abl inhibitor might reduce the number of resistant mutations. The success of a combinatory approach depends on imatinib not preventing the additional inhibitor from binding to the same region of the kinase.

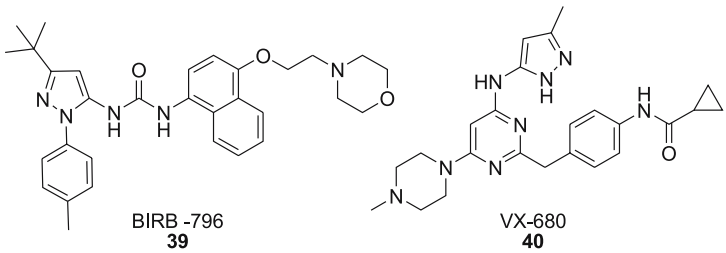
To test the viability of this strategy, cell assays were performed wherein BMS-354825 and AP23848 were tested in combination with imatinib [107]. Both wild-type Bcr-Abl BaF3 cells and BaF3 cells transformed by four of the most clinically relevant Abl mutations were employed. In proliferation assays with the wild-type cells, BMS-354825 and AP23848 had IC_{50}s of 1.5 and 18 nM, respectively. Adding increasing concentrations of imatinib did not decrease the activity of either AP23848 or BMS-354825, with the IC_{50}s actually being about two-fold lower in the presence of a 150 nM concentration of imatinib. A similar effect was seen in the M351T line that is moderately resistant to imatinib where higher concentrations of imatinib were used. For the Y253F and E255K mutations in the P-loop, which have a greater level of resistance to imatinib than the M351T mutant, even higher concentrations of imatinib were used. A concentration of 3 μM imatinib did not prevent either compound from binding to the Y253F mutant line as shown by the IC_{50}s of 0.85 and 4.0 nM for BMS-354825 and AP23848, respectively.

There is also a preliminary report on combination studies with imatinib and AMN107 [160]. Additive or synergistic effects were seen in assays with several Bcr-Abl-dependent cell lines. In a bioluminescent in vivo murine model employing 32D cells transformed by Bcr-Abl that also express luciferase, treatment with both AMN107 at 15 mg/kg and imatinib at 75 mg/kg improved efficacy compared to either compound alone.

5.3 Overcoming the T315I Mutation

The major issue with the new Bcr-Abl kinase inhibitors that bind in the ATP pocket is that none of them can overcome the T315I mutation. A study of 20 known kinase inhibitors measured their binding affinity for 119 kinases, including wild-type Abl and some of the clinically relevant imatinib-resistant mutated forms of Abl [161]. BIRB-796 (**39**), a p38 inhibitor, was found to have a 40 nM binding constant for the T315I Abl mutant (Scheme 12). Interestingly, reduced activity was seen against wild-type Abl (binding constant of 1.5 μM) and also against three other imatinib-resistant mutations, M351T, Q252H and Y253F (binding constants of 2.2, 4.2 and 2.3 μM, respectively). Imatinib had binding constants of 2.2 nM and 6.2 μM for wild-type Abl and the T315I mutant in these assays. Some other kinase inhibitors were found to bind to wild-type Abl and the mutant variants with similar affinity.

A follow-up publication reported that BIRB-796 had weak binding affinity for two additional Abl mutations, F359V and T315N, further demonstrating the specificity of this compound for the T315I mutation [162]. However, when tested in an isolated kinase assay BIRB-796 inhibited the T315I mutant with an IC_{50} of only 4 μM, much lower than what was expected based on the binding constant of 40 nM. A similar result was seen in proliferation assays in BaF3 cells transformed with either wild-type Bcr-Abl or the T315I mutant. BIRB-796 had an IC_{50} of greater than 10 μM for the wild-type cells and an IC_{50} of only 2–3 μM for inhibition of the growth of the T315I mutated line. A separate group confirmed the poor activity of BIRB-796 in a kinase assay with the T315I Abl mutant, obtaining an IC_{50} of 5.3 μM, which was identi-



Scheme 12

cal to the IC_{50} they obtained with wild-type Abl [163]. In proliferation assays with BaF3 cells with both wild-type Abl or with four Abl mutants, treatment with up to 5 μM BIRB-796 did not inhibit the growth of the cells, including those with the T315I mutation. These results point out the importance of follow-up assays due to possible discrepancies between binding studies and inhibition assays. This finding was a major disappointment to those who hoped that it might be possible to use BIRB-796 in combination with Abl inhibitors that are not effective against the T315I mutation.

Additional kinase inhibitors were also profiled for their affinity to wild type and eight mutated forms of Abl. VX-680 (40), an Aurora kinase inhibitor, was found to have a binding constant of 20 nM for wild-type Abl and of 5 nM for the T315I mutant [162] (Scheme 12). The binding affinities for the other Abl mutants were in the range of 7–50 nM, with the exception being the T315N mutant for which VX-680 had an affinity of 100 nM. In enzyme assays, VX-680 had IC_{50}s of 10 and 30 nM for wild-type Abl and the T315I mutant, respectively. It should be noted that this potent activity for VX-680 was observed in the same report where BIRB-796 had an IC_{50} of only 4 μM. In an Abl autophosphorylation assay in BaF3 cells harboring the T315I mutant, VX-680 was only weakly active having an IC_{50} of about 5 μM. Additional studies will be needed to understand this discrepancy between the isolated kinase and cell assays.

VX-680 is being developed by Vertex in collaboration with Merck. In June 2005 it was announced that a Phase I trial of VX-680 in hematological cancers had started [164]. This trial will include CML patients in blast crisis. These trials are in addition to the initial clinical trials in solid tumors.

The search for compounds with activity against the T315I mutation continues. At the 2005 ASH meeting there was a report of a new Abl inhibitor, SGX-70430, that also inhibited the activity of the T315I mutant [165]. In proliferation assays with BaF3 cells harboring wild-type Bcr-Abl and the T315I mutant, SGX-70430 had EC_{50}s of 11 and 21 nM, respectively. The EC_{50}s for imatinib, AMN107 and BMS-354825 in the wild-type Bcr-Abl-dependent proliferation assay were 790, 33 and 12 nM with all three compounds having EC_{50}s of greater than 10 μM against the T315I mutant. While the structure of SGX-70430 was not revealed, SGX Pharmaceuticals has stated on their corporate website that they plan to file an IND in late 2006 for a compound from this series for the treatment of imatinib-resistant CML [166].

6 Present Status and Future Outlook

This account has presented an overview of the new Bcr-Abl inhibitors in both preclinical and clinical development. While AMN107 and BMS-354825 have advanced to Phase II trials for the treatment of CML, it is frustrating from

a medicinal chemistry perspective that only limited SAR was reported for these compounds. Novartis has not yet published on the SAR of AMN107 analogs for Abl inhibition, except to state that replacement of the trifluoromethyl group by a methyl group decreased the Abl activity. The SAR data available on the BMS-354825 analogs only covers one portion of the molecule. In addition, this SAR is in tumor cell proliferation assays, not isolated Src and Abl assays. Interestingly, when BMS-354825 was screened against a panel of 148 kinases at a dose of 10 μM, affinity constants of less than 200 nM were reported for 47 kinases [162]. This pan kinase activity could explain the potent anti-proliferative activity of BMS-354825 in the solid tumor lines.

As noted previously, the first public mention of BMS-354825 was in newsletters that are available to anyone with Internet access. These websites contain a large amount of information concerning the new therapies for CML including the "UnOfficial Gleevec (STI-571) Site" started by Jerry Mayfield, a CML patient who began imatinib treatment in 2000 and switched to BMS-354825 in 2003 [167]. The site contains a section entitled "Jerry's Diary" which details his personal experiences on these therapies. The site also has areas for the discussion of CML related topics. One of these topics is the resistance of the T315I mutation, illustrating the increasing knowledge of CML patients and their growing involvement in decisions concerning the management of their disease.

While the rapid approval of imatinib made it appear to be an overnight success story, many years of prior research were needed to sort out the fundamental biology of CML and Bcr-Abl. It now appears that many additional years of research will be needed to overcome the "Achilles heel" of imatinib: the resistance of the T315I mutation. It was initially thought that a compound such as AMN107 that was a more potent Bcr-Abl inhibitor than imatinib, or a compound such as BMS-354825 that was not only more potent but also bound to the active conformation of the kinase, could overcome the appearance of this mutation, but unfortunately, the clinical trials with these two new agents showed this not to be the case. Since the T315I mutant is resistant to Bcr-Abl kinase inhibitors that bind in the kinase domain, it may be necessary to use these inhibitors in combination with agents that have an alternative mechanism of action. Preliminary clinical studies on overcoming imatinib resistance using combinations of imatinib with farnesyl transferase inhibitors have been reported [168, 169].

The development of resistant mutations is not unique to imatinib. A mutation in the kinase domain of EGFR was observed upon treatment of patients with the EGFR inhibitors gefitinib and erlotinib [170, 171]. This T790M mutation is that of the gatekeeper Thr residue of EGFR that corresponds to Thr 315 of Abl. Interestingly, it was reported that some irreversible inhibitors of EGFR and ErbB2, that inactivate the enzyme by binding to a Cys residue in the active site, could overcome resistance to the T790M mutation in vitro [162, 172]. It is not yet known if this finding will translate into the clinic.

The link between CML and Bcr-Abl allowed for the development of imatinib, the first successful small molecule kinase targeted therapy. Unfortunately, the clinical success of imatinib was diminished by the development of resistance. While progress has been made and new Bcr-Abl inhibitors are showing clinical efficacy, the problem of the T315I mutation still exists. Hopefully, this issue will soon be resolved and a new drug will be approved that will result in the same excitement that was experienced just a few years ago with the launch of imatinib. Furthermore, the insights gained in the treatment of CML with imatinib will undoubtedly provide a useful roadmap to drive the development of similar treatment paradigms with other signal transduction agents in patients with solid tumors.

Acknowledgements I thank Frank Boschelli, Janis Upeslacis and Dennis Powell for their critical review of this manuscript.

References

1. Goldman JM, Melo JV (2003) N Eng J Med 349:1451
2. Thijsen SFT, Schuurhuis GJ, Van Oostveen JW, Ossenkoppele GJ (1999) Leukemia 13:1646
3. Kalidas M, Kantarjian H, Talpaz M (2001) JAMA 286:895
4. Goldman JM, Druker BJ (2001) Blood 98:2039
5. Salesse S, Verfaillie CM (2002) Oncogene 21:8547
6. Faderl S, Talpaz M, Estrov Z, O'Brien S, Kurzrock R, Kantarjian HM (1999) N Engl J Med 341:164
7. Ribeiro R, Abromowitch M, Raimondi S, Murphy S, Behm F, Williams D (1987) Blood 70:948
8. Bloomfield C, Goldman A, Alimena G, Berger R, Borgstrom G, Brandt L, Catovsky D, de la Chapelle A, Dewald G, Garson (1986) Blood 67:415
9. Westbrook C, Hooberman A, Spino C, Dodge R, Larson R, Davey F, Wurster-Hill D, Sobol R, Schiffer C, Bloomfield C (1992) Blood 80:2983
10. Faderl S, Jeha S, M. KH (2003) Cancer 98:1337
11. Buchdunger E, Zimmermann J, Mett H, Meyer T, Mueller M, Druker BJ, Lydon NB (1996) Cancer Res 56:100
12. Zimmermann J, Buchdunger E, Mett H, Meyer T, Lydon NB (1997) Bioorg Med Chem Lett 7:187
13. Buchdunger E, Cioffi CL, Law N, Stover D, Ohno-Jones S, Druker BJ, Lydon NB (2000) J Pharmacol Exp Ther 295:139
14. Boggon TJ, Eck MJ (2004) Oncogene 23:7918
15. Superti-Furga G, Courtneidge SA (1995) BioEssays 17:321
16. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB (1996) Nat Med 2:561
17. Carroll M, Ohno-Jones S, Tamura S, Buchdunger E, Zimmermann J, Lydon NB, Gilliland DG, Druker BJ (1997) Blood 90:4947
18. Gambacorti-Passerini C, Le Coutre P, Mologni L, Fanelli M, Bertazzoli C, Marchesi E, Di Nicola M, Biondi A, Corneo GM, Belotti D, Pogliani E, Lydon NB (1997) Blood Cells Mol Dis 23:380

19. Deininger MWN, Goldman JM, Lydon N, Melo JV (1997) Blood 90:3691
20. Le Coutre P, Mologni L, Cleris L, Marchesi E, Buchdunger E, Giardini R, Formelli F, Gambacorti-Passerini C (1999) J Natl Cancer Inst 91:163
21. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL (2001) N Engl J Med 344:1031
22. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R, Talpaz M (2001) N Engl J Med 344:1038
23. Lydon NB, Druker BJ (2004) Leukemia Res 28:S29
24. Cowan-Jacob SW, Guez V, Fendrich G, Griffin JD, Fabbro D, Furet P, Liebetanz J, Mestan J, Manley PW (2004) Mini-Reviews Med Chem 4:285
25. Crossman LC, O'Brien S (2004) Leukemia Res 28:S3
26. Deininger M, Buchdunger E, Druker BJ (2005) Blood 105:2640
27. Capdeville R, Buchdunger E, Zimmermann J, Matter A (2002) Nat Rev Drug Disc 1:493
28. Vasella D, Slater R (2003) Magic Cancer Bullet. Harper Business, New York
29. Tuveson DA, Willis NA, Jacks T, Griffin JD, Singer S, Fletcher CDM, Fletcher JA, Demetri GD (2001) Oncogene 20:5054
30. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, Heinrich MC, Tuveson DA, Singer S, Janicek M, Fletcher JA, Silverman SG, Silberman SL, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker BJ, Corless C, Fletcher CD, Joensuu H (2002) N Engl J Med 347:472
31. Wiedmann MW, Caca K (2005) Curr Cancer Drug Targets 5:171
32. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, Sawyers CL (2001) Science 293:876
33. Shah N, Sawyers CL (2003) Oncogene 22:7389
34. Corbin AS, La Rosee P, Stoffregen EP, Druker BJ, Deininger MW (2003) Blood 101:4611
35. von Bubnoff N, Peschel C, Duyster J (2003) Leukemia 17:829
36. Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J (2000) Science 289:1938
37. Nagar B, Bornmann WG, Pellicena P, Schindler T, Veach DR, Miller WT, Clarkson B, Kuriyan J (2002) Cancer Res 62:4236
38. Manley PW, Breitenstein W, Brueggen J, Cowan-Jacob SW, Furet P, Mestan J, Meyer T (2004) Bioorg Med Chem Lett 14:5793
39. Mestan J, Ellen W, Sandra C-J, Doriano F, Pascal F, Gabriele F, Gerard G, Dominique K, Markus G, James GD, Manley PW (2004) Annu Meeting Am Soc Hematol, Abstract 1978
40. Weisberg E, Manley PW, Breitenstein W, Brueggen J, Cowan-Jacob SW, Ray A, Huntly B, Fabbro D, Fendrich G, Hall-Meyers E, Kung AL, Mestan J, Daley GQ, Callahan L, Catley L, Cavazza C, Mohammed A, Neuberg D, Wright RD, Gilliland DG, Griffin JD (2005) Cancer Cell 7:129
41. Manley P, Cowan-Jacob SW, Fendrich G, Mestan J (2005) Annu Meeting Am Soc Hematol, Abstract 3365
42. Golemovic M, Verstovsek S, Giles F, Cortes J, Manshouri T, Manley PW, Mestan J, Dugan M, Alland L, Griffin JD, Arlinghaus RB, Sun T, Kantarjian H, Beran M (2005) Clin Cancer Res 11:4941
43. Martinelli G, Martelli AM, Grafone T, Mantovani I, Cappellini A, Tazzari PL, Ottaviani E, Soverini S, Amabile M, Renzulli M, Terragna C, Colarossi S, Iacobucci I, Poerio A, Baccarani M (2005) Annu Meeting Am Soc Hematol, Abstract 2004

44. Martinelli G, Martelli AM, Grafone T, Rosti G, Mantovani I, Cappellini A, Tazzari PL, Ottaviani E, Renzulli M, Amabile M, Poerio A, Soverini S, Terragna C, Taccioli C, Bassi S, Baccarani M (2004) Annu Meeting Am Soc Hematol, Abstract 4687
45. Ma G, Lu D, Wu Y, Liu J, Arlinghaus RB (1997) Oncogene 14:2367
46. Van Etten RA (2004) Leukemia Res 28:S21
47. Ottmann OG, Druker BJ, Sawyers CL, Goldman JM, Reiffers J, Silver RT, Tura S, Fischer T, Deininger MW, Schiffer CA, Baccarani M, Gratwohl A, Hochhaus A, Hoelzer D, Fernandes-Reese S, Gathmann I, Capdeville R, O'Brien SG (2002) Blood 100:1965
48. Verstovsek S, Golemovic M, Kantarjian H, Manshouri T, Estrov Z, Manley P, Sun T, Arlinghaus RB, Alland L, Dugan M, Cortes J, Giles F, Beran M (2005) Cancer 104: 1230
49. Giles F, Kantarjian H, Wassmann B, Cortes J, O'Brien S, Tanaka C, Rae P, Mietlowski W, Romano A, Alland L, Dugan M, Albitar M, Ottmann O (2004) Annu Meeting Am Soc Hematol, Abstract 22
50. Ottman O, Giles F, Wassman B, Hochhaus A, Rae P, Beran M, Albitar M, Dugan M, Kantarjian H (2005) Annu Meeting Am Soc Clin Oncol, Abstract 3015
51. Kantarjian H, Ottman O, Cortes J, Wassman B, Hochhaus A, Alland L, Dugan M, Albitar M, Giles F (2005) Annu Meeting Am Soc Clin Oncol, Abstract 3014A
52. Giles F, Ottman O, Cortes J, Wassman B, Wunderle S, O'Brien S, Jones D, Hochhaus A, Verstovsek S, Rae P, Rose K, Alland L, Dugan M, Manley P, Beran M, Albitar M, Kantarjian H (2005) Annu Meeting Am Assoc Cancer Res, Abstract 3971
53. Kantarjian H, M., Ottman O, Cortes J, Wassman M, Wunderle S, Bhalla K, Jones D, Hochhaus A, Rae P, Alland L, Dugan M, Albitar M, Giles F (2005) Annu Meeting Am Soc Hematol, Abstract 37
54. La Rosee P, Holm-Eriksen S, Ernst T, Konig H, Erben P, Mueller M, Schenk Y, Emig M, Binckebanck A, Wunderle S, Alland L, Schuld P, Hehlmann R, Ottman OG, Hochhaus A (2005) Annu Meeting Am Soc Hematol, Abstract 497
55. Von Bubnoff N, Veach DR, Van der Kuip H, Aulitzky WE, Saenger J, Seipel P, Bornmann WG, Peschel C, Clarkson B, Duyster J (2005) Blood 105:1652
56. von Bubnoff N, Saenger J, Manley PW, Mestan J, Peschel C, Duyster J (2005) Annu Meeting Am Soc Hematol, Abstract 1983
57. Ray A, Cowan-Jacob S, Manley PW, Mestan J, Griffin JD (2005) Annu Meeting Am Soc Hematol, Abstract 494
58. Kimura S, Naito H, Segawa H, Kuroda J, Yuasa T, Sato K, Yokota A, Kamitsuji Y, Kawata E, Ashihara E, Nakaya Y, Naruoka H, Wakayama T, Nasu K, Asaki T, Niwa T, Hirabayashi K, Maekawa T (2005) Blood 106:3948
59. Naito H, Kimura S, Nakaya Y, Naruoka H, Wakayama T, Kimura S, Ito S, Ashihara E, Maekawa T, Hirabayashi K (2005) Annu Meeting Am Soc Hematol, Abstract 1518
60. Asaki T, Sugiyama Y, Hamamoto T, Higashioka M, Umehara M, Naito H, Niwa T (2006) Bioorg Med Chem Lett 16:1421
61. Gumireddy K, Baker SJ, Cosenza SC, John P, Kang AD, Robell KA, Reddy MVR, Reddy EP (2005) Proc Natl Acad Sci USA 102:1992
62. http://www.onconova.com, (Last visited on: Jan 05, 2006)
63. Gumireddy K, Reddy MVR, Cosenza SC, Nathan RB, Baker SJ, Papathi N, Jiang J, Holland J, Reddy EP (2005) Cancer Cell 7:275
64. Kaur G, Gazit A, Levitzki A, Stowe E, Cooney DA, Sausville EA (1994) Anti-Cancer Drug 5:213
65. Kaur G, Narayanan VL, Risbood PA, Hollingshead MG, Stinson SF, Varma RK, Sausville EA (2005) Bioorg Med Chem 13:1749

66. Svingen PA, Tefferi A, Kottke TJ, Kaur G, Narayanan VL, Sausville EA, Kaufmann SH (2000) Clin Cancer Res 6:237
67. Mow BMF, Chandra J, Svingen PA, Hallgren CG, Weisberg E, Kottke TJ, Narayanan VL, Litzow MR, Griffin JD, Sausville EA, Tefferi A, Kaufmann SH (2002) Blood 99:664
68. Avramis IA, Laug WE, Sausville EA, Avramis VI (2003) Cancer Chemother Pharmacol 52:307
69. Deutsch E, Maggiorella L, Wen B, Bonnet ML, Khanfir K, Frascogna V, Turhan AG, Bourhis J (2004) Br J Cancer 91:1735
70. Camirand A, Zakikhani M, Young F, Pollak M (2005) Breast Cancer Res 7:R465
71. Blum G, Gazit A, Levitzki A (2000) Biochemistry 39:15705
72. Camirand A, Pollak M (2004) Br J Cancer 90:1825
73. Dorsey JF, Jove R, Kraker AJ, Wu J (2000) Cancer Res 60:3127
74. Wisniewski D, Lambek CL, Liu C, Strife A, Veach DR, Nagar B, Young MA, Schindler T, Bornmann WG, Bertino JR, Kuriyan J, Clarkson B (2002) Cancer Res 62: 4244
75. Courtneidge SA (2002) Biochem Soc Trans 30:11
76. Ito H, Gardner-Thorpe J, Zinner MJ, Ashley SW, Whang EE (2003) Surgery 134:221
77. Wiener JR, Windham TC, Estrella VC, Parikh NU, Thall PF, Deavers MT, Bast RC, Mills GB, Gallick GE (2003) Gynecol Oncol 88:73
78. Myoui A, Nishimura R, Williams PJ, Hiraga T, Tamura D, Michigami T, Mundy GR, Yoneda T (2003) Cancer Res 63:5028
79. Danhauser-Riedl S, Warmuth M, Druker BJ, Emmerich B, Hallek M (1996) Cancer Res 56:3589
80. Warmuth M, Bergmann M, Priess A, Hauslmann K, Emmerich B, Hallek M (1997) J Biol Chem 272:33260
81. Roginskaya V, Zuo S, Caudell E, Nambudiri G, Kraker AJ, Corey SJ (1999) Leukemia 13:855
82. Lionberger JM, Wilson MB, Smithgall TE (2000) J Biol Chem 275:18581
83. Donato NJ, Wu JY, Stapley J, Gallick G, Lin H, Arlinghaus R, Talpaz M (2003) Blood 101:690
84. Klutchko SR, Hamby JM, Boschelli DH, Wu Z, Kraker AJ, Amar AM, Hartl BG, Shen C, Klohs WD, Steinkampf RW, Driscoll DL, Nelson JM, Elliott WL, Roberts BJ, Stoner CL, Vincent PW, Dykes DJ, Panek RL, Lu GH, Major TC, Dahring TK, Hallak H, Bradford LA, Showalter HDH, Doherty AM (1998) J Med Chem 41:3276
85. Kraker AJ, Hartl BG, Amar AM, Barvian MR, Showalter HDH, Moore CW (2000) Biochem Pharmacol 60:885
86. La Rosee P, Corbin AS, Stoffregen EP, Deininger MW, Druker BJ (2002) Cancer Res 62:7149
87. Huron DR, Gorre ME, Kraker AJ, Sawyers CL, Rosen N, Moasser MM (2003) Clin Cancer Res 9:1267
88. von Bubnoff N, Veach DR, Miller WT, Li W, Saenger J, Peschel C, Bornmann WG, Clarkson B, Duyster J (2003) Cancer Res 63:6395
89. Veach DR, Lambek C, Liu C-Y, Duyster J, Beresten T, Li W, Wolff NC, Ilaria RL, Gelovani J, Miller WT, von Bubnoff N, Bornmann WG, Clarkson BD (2003) 226th Nat Meeting Am Chem Soc, Abstract MEDI-067
90. Tipping AJ, Baluch S, Barnes DJ, Veach DR, Clarkson BM, Bornmann WG, Mahon FX, Goldman JM, Melo JV (2004) Leukemia 18:1352
91. Wolff NC, Veach DR, Tong WP, Bornmann WG, Clarkson B, Ilaria RL Jr (2005) Blood 105:3995

92. http://www.medicalnewstoday.com, (Last visited on: Jan 05, 2006)
93. Missbach M, Jeschke M, Feyen J, Muller K, Glatt M, Green J, Susa M (1999) Bone 24:437
94. Li B, Boast S, De los Santos K, Schieren I, Quiroz M, Teitelbaum SL, Tondravi MM, Goff SP (2000) Nat Genet 24:304
95. Missbach M, Altmann E, Widler L, Susa M, Buchdunger E, Mett H, Meyer T, Green J (2000) Bioorg Med Chem Lett 10:945
96. Widler L, Green J, Missbach M, Susa M, Altmann E (2001) Bioorg Med Chem Lett 11:849
97. Altmann E, Missbach M, Green J, Susa M, Wagenknecht HA, Widler L (2001) Bioorg Med Chem Lett 11:853
98. Altmann E, Widler L, Missbach M (2002) Mini-Reviews Med Chem 2:201
99. Warmuth M, Simon N, Mitina O, Mathes R, Fabbro D, Manley PW, Buchdunger E, Forster K, Moarefi I, Hallek M (2003) Blood 101:664
100. Hu Y, Liu Y, Pelletier S, Buchdunger E, Warmuth M, Fabbro D, Hallek M, Van Etten RA, Li S (2004) Nat Genet 36:453
101. Shakespeare WC, Metcalf CA III, Wang Y, Sundaramoorthi R, Keenan T, Weigele M, Bohacek RS, Dalgarno DC, Sawyer TK (2003) Curr Opin Drug Dis Dev 6:729
102. Wang Y, Metcalf CA III, Shakespeare WC, Sundaramoorthi R, Keenan TP, Bohacek RS, Van Schravendijk MR, Violette SM, Narula SS, Dalgarno DC, Haraldson C, Keats J, Liou S, Mani U, Pradeepan S, Ram M, Adams S, Weigele M, Sawyer TK (2003) Bioorg Med Chem Lett 13:3067
103. Metcalf C III, Wang Y, Shakespeare W, Sundaramoorthi R, Keenan T, Dalgarno D, Bohacek R, Burns K, Roses J, van Schravendijk MR, Ram M, Keats J, Liou S, Adams S, Snodgrass J, Rivera V, Weigele M, Iuliucci J, Clackson T, Frame M, Brunton V, Sawyer T (2003) 226th Nat Meeting Am Chem Soc, Abstract COMP-079
104. Dalgarno D, Bohacek R, Stehle T, Narula S, Metcalf C III, Shakespeare W, Sundaramoorthi R, Keenan T, Wang Y, Keats J, Ram M, Adams S, Van Schravandijk MR, Weigele M, Sawyer T (2003) AACR-NCI-EORTC Int Conf Mol Targets Cancer Ther, Abstract A5
105. Dalgarno D, Stehle T, Narula S, Schelling P, Van Schravandijk MR, Adams S, Andrade L, Keats J, Ram M, Jin L, Grossman T, MacNeil I, Metcalf C III, Shakespeare W, Wang Y, Keenan T, Sundaramoorthi R, Bohacek R, Weigele M, Sawyer T (2006) Chem Biol Drug Des 67:46
106. O'Hare T, Pollock R, Stoffregen EP, Keats JA, Abdullah OM, Moseson EM, Rivera VM, Tang H, Metcalf CA III, Bohacek RS, Wang Y, Sundaramoorthi R, Shakespeare WC, Dalgarno D, Clackson T, Sawyer TK, Deininger MW, Druker BJ (2004) Blood 104:2532
107. O'Hare T, Walters DK, Stoffregen EP, Sherbenou DW, Heinrich MC, Deininger MWN, Druker BJ (2005) Clin Cancer Res 11:6987
108. Corbin AS, Demehri S, Griswold IJ, Wang Y, Metcalf CA III, Sundaramoorthi R, Shakespeare WC, Snodgrass J, Wardwell S, Dalgarno D, Iuliucci J, Sawyer TK, Heinrich MC, Druker BJ, Deininger MWN (2005) Blood 106:227
109. Azam M, Nardi V, Shakespeare WC, Latek RR, Veach DR, Clarckson B, Bohacek R, Sawyer TK, Daley GQ (2005) Annu Meeting Am Soc Hematol, Abstract 1996
110. http://www.ariad.com, (Last visited on: Jan 05, 2006)
111. Boschelli DH, Wang YD, Ye F, Wu B, Zhang N, Dutia M, Powell DW, Wissner A, Arndt K, Weber JM, Boschelli F (2001) J Med Chem 44:822
112. Boschelli DH, Ye F, Wang YD, Dutia M, Johnson SL, Wu B, Miller K, Powell DW, Yaczko D, Young M, Tischler M, Arndt K, Discafani C, Etienne C, Gibbons J, Grod J, Lucas J, Weber JM, Boschelli F (2001) J Med Chem 44:3965

113. Golas JM, Arndt K, Etienne C, Lucas J, Nardin D, Gibbons J, Frost P, Ye F, Boschelli DH, Boschelli F (2003) Cancer Res 63:375
114. Donato NJ, Wu JY, Talpaz M, Dutia M, Ye F, Boschelli D, Boschelli F (2002) Annu Meeting Am Soc Hematol, Abstract 1434
115. Grafone T, Mancini M, Ottaviani E, Renzulli M, Boschelli F, Ferraretti L, Santucci MA, Amabile M, Soverini S, Terragna C, Poerio A, Rosti G, Taccioli C, Baccarani M, Martinelli G (2004) Annu Meeting Am Soc Hematol, Abstract 1991
116. Grafone T, Mancini M, Ottaviani E, Renzulli M, Boschelli F, Ferraretti L, Santucci MA, Soverini S, Amabile M, Terragna C, Poerio A, Rosti G, Taccioli C, Palmerini E, Baccarani M, Martinelli G (2005) Annu Meeting Am Assoc Cancer Res, Abstract 5984
117. Boschelli DH, Boschelli F, Wu B, Ye F, Wang Y, Golas JM, Young T, Lucas J (2005) Haematologica Rep 1(8):28
118. Renzulli M, Grafone T, Taccioli C, Terragna C, Boschelli F, Mancini M, Creo P, Ottaviani E, Colarossi S, Amabile M, Soverini S, Poerio A, Iacobucci I, Baccarani M, Martinelli G (2005) Annu Meeting Am Soc Hematol, Abstract 4870
119. Boschelli DH, Wang YD, Johnson S, Wu B, Ye F, Barrios Sosa AC, Golas JM, Boschelli F (2004) J Med Chem 47:1599
120. Boschelli DH, Wu B, Barrios Sosa AC, Durutlic H, Ye F, Raifeld Y, Golas JM, Boschelli F (2004) J Med Chem 47:6666
121. Boschelli DH, Wu B, Barrios Sosa AC, Chen JJ, Golas JM, Boschelli F (2005) Bioorg Med Chem Lett 15:4681
122. Golas JM, Lucas J, Etienne C, Golas J, Discafani C, Sridharan L, Boghaert E, Arndt K, Ye F, Boschelli DH, Li F, Titsch C, Huselton C, Chaudhary I, Boschelli F (2005) Cancer Res 65:5358
123. http://www.cmlsupport.com/cml/newletters.html (2003) CML Update 2:1, (Last visited on: Jan 05, 2006)
124. MD Anderson (2003) Cancer Center Leukemia Insights Newsletter 8(2), http://www.mdanderson.org/publications/insights, (Last visited on: Jan 05, 2006)
125. Lee FY, Lombardo L, Borzilleri R, Camuso A, Castaneda S, Donato N, Fager K, Flefleh C, Gray h, Inigo I, Kan D, Luo R, Pang S, Wen M-L, Wild R, Wong TW, Talpaz M, Kramer R (2004) Annu Meeting Am Assoc Cancer Res, Abstract 3987
126. Shah NP, Tran C, Lee FY, Sawyers CL (2004) Annu Meeting Am Assoc Cancer Res, Abstract 5624
127. Wu JY, Donato NJ, Hong DS, Lee FY, Talpaz M (2004) Annu Meeting Am Assoc Cancer Res, Abstract 3850
128. Shah NP, Tran C, Lee FY, Chen P, Norris D, Sawyers CL (2004) Science 305:399
129. Wityak J, Das J, Moquin RV, Shen Z, Lin J, Chen P, Doweyko AM, Pitt S, Pang S, Shen DR, Fang Q, de Fex HF, Schieven GL, Kanner SB, Barrish JC (2003) Bioorg Med Chem Lett 13:4007
130. Lombardo LJ, Lee FY, Chen P, Norris D, Barrish JC, Behnia K, Castaneda S, Cornelius LAM, Das J, Doweyko AM, Fairchild C, Hunt JT, Inigo I, Johnston K, Kamath A, Kan D, Klei H, Marathe P, Pang S, Peterson R, Pitt S, Schieven GL, Schmidt RJ, Tokarski J, Wen M-L, Wityak J, Borzilleri RM (2004) J Med Chem 47:6658
131. Tokarski JS, Newitt J, Lee FY, Lombardo L, Borzilleri R, Kish K, Xie D, Zhang Y, Cheng JD, Kiefer SE, Chang CYJ, Klei HE (2004) Annu Meeting Am Soc Hematol, Abstract 553
132. Azam M, Latek RR, Daley GQ (2003) Cell 112:831
133. Burgess MR, Skaggs BJ, Shah NP, Lee FY, Sawyers CL (2005) Proc Natl Acad Sci USA 102:3395

134. Luo FR, Yang Z, Camuso A, Smykla R, Castenada S, Flefleh C, Fager K, Inigo I, Kan D, McGlinchey K, Wen M-L, Wild R, Borzilleri R, Lombardo L, Kramer R, Lee FY (2004) Annu Meeting Am Soc Hematol, Abstract 1987
135. Lee FY, Wen M-L, Bhide R, Camuso A, Castaneda S, Fager K, Flefleh C, Inigo I, Kan D, Luo R, McGlinchey K, Smykla R, Wild R, Borzilleri R, Lombardo L (2005) Annu Meeting Am Soc Hematol, Abstract 1994
136. Dai H, Marbach P, Lemaire M, Hayes M, Elmquist WF (2003) J Pharmacol Exp Ther 304:1085
137. Wild R, Castaneda S, Flefleh C, Fager K, Inigo I, Kan D, Smykla R, Camuso A, McGlinchey K, Wen M-L, Luo FR, Borzilleri R, Lombardo L, Kramer R, Lee FY (2004) Annu Meeting Am Soc Hematol, Abstract 1988
138. Sawyers CL, Shah NP, Kantarjian HM, Donato N, Nicoll J, Bai SA, Huang F, Clark E, DeCillis AP, Talpaz M (2004) Annu Meeting Am Soc Hematol, Abstract 1
139. Talpaz M, Kantarjian H, Shah NP, Donato N, Nicoll J, Bai SA, Huang F, Clark E, DeCillis AP, Sawyers C (2004) Annu Meeting Am Soc Hematol, Abstract 20
140. Talpaz M, Kantarjian HM, Paquette R, Shah N, Cortes J, Nicoll J, Bai SA, Huang F, Decillis AP, Sawyers CL (2005) Annu Meeting Am Soc Clin Oncol, Abstract 6519
141. Sawyers CL, Shah NP, Kantarjian HM, Cortes J, Paquette R, Nicoll J, Bai SA, Clark E, Decillis AP, Talpaz M (2005) Annu Meeting Am Soc Clin Oncol, Abstract 6520
142. Sawyers CL, Kantarjian H, Shah N, Cortes J, Paquette R, Donato N, Nicoll J, Bleickardt E, Chen T-T, Talpaz M (2005) Annu Meeting Am Soc Hematol, Abstract 38
143. Gao H, Talpaz M, Lee BN, Donato N, Cortes JE, Kantarjian HM, Reuben JM (2005) Annu Meeting Am Soc Clin Oncol, Abstract 6619
144. Guilhot F, Apperley JF, Shah N, Kim DW, Grigg A, Cheng S, Iyer M, Cortes J (2005) Annu Meeting Am Soc Hematol, Abstract 39
145. Talpaz M, Rousselot P, Kim DW, Guilhot F, Corm S, Bleickardt E, Zink R, Rosti G, Coutre S, Sawyers C (2005) Annu Meeting Am Soc Hematol, Abstract 40
146. Hochhaus A, Baccarani M, Sawyers C, Nagler A, Facon T, Goldberg SL, Cervantes F, Larson RA, Voi M, Ezzeddine R, Kantarjian H (2005) Annu Meeting Am Soc Hematol, Abstract 41
147. Ottman O, Martinelli G, Dombret H, Kantarjian H, Hochhaus A, Simonsson B, Aloe A, Apanovitch A, Shah N (2005) Annu Meeting Am Soc Hematol, Abstract 42
148. Shah N, Nicoll J, Branford S, Hughes TP, Paquette RL, Talpaz M, Nicaise C, Huang F, Sawyers CL (2005) Annu Meeting Am Soc Hematol, Abstract 1093
149. Jabbour E, Cortes J, Talpaz M, Jones D, O'Brien S, Nicaise C, Kantarjian H (2005) Annu Meeting Am Soc Hematol, Abstract 1091
150. Donato N, Wu J, Kong LY, Meng F, Lee F, Talpaz M (2005) Annu Meeting Am Soc Hematol, Abstract 1087
151. Johnson FM, Saigal B, Talpaz M, Donato NJ (2005) Clin Cancer Res 11:6924
152. http://www.fda.gov/bbs/topics/NEWS/2006/NEW01400.html, (Last visited on: Jan 05, 2006)
153. Ple PA, Green TP, Hennequin LF, Curwen J, Fennell M, Allen J, Lambert-van der Brempt C, Costello G (2004) J Med Chem 47:871
154. Yezhelyev MV, Koehl G, Guba M, Brabletz T, Jauch KW, Ryan A, Barge A, Green T, Fennell M, Bruns CJ (2004) Clin Cancer Res 10:8028
155. Hennequin LF, Allen J, Costello GF, Fennell M, Green TP, Jacobs V, Morgentin R, Olivier A, Ple PA (2005) Annu Meeting Am Assoc Cancer Res, Abstract 2537
156. Lockton JA, Smethurst D, Macpherson M, Tootell R, Marshall AL, Clack G, Gallagher NJ (2005) Annu Meeting Am Soc Clin Oncol, Abstract 3125

157. Gallagher NJ, Lockton AJ, Macpherson M, Marshall A, Clack G (2005) Annu Meeting Am Assoc for Cancer Res, Abstract 3972
158. O'Hare T, Walters DK, Stoffregen EP, Jia T, Manley PW, Mestan J, Cowan-Jacob SW, Lee FY, Heinrich MC, Deininger MWN, Druker BJ (2005) Cancer Res 65:4500
159. Deininger MWN, Bradeen H, Jia T, O'Hare T, Willis SG, Lee F, Druker BJ (2005) Annu Meeting Am Soc Hematol, Abstract 691
160. Griffin JD, Weisberg EL (2005) Annu Meeting Am Soc Hematol, Abstract 694
161. Fabian MA, Biggs WH, Treiber DK, Atteridge CE, Azimioara MD, Benedetti MG, Carter TA, Ciceri P, Edeen PT, Floyd M, Ford JM, Galvin M, Gerlach JL, Grotzfeld RM, Herrgard S, Insko DE, Insko MA, Lai AG, Lelias J-M, Mehta SA, Milanov ZV, Velasco AM, Wodicka LM, Patel HK, Zarrinkar PP, Lockhart DJ (2005) Nat Biotechnol 23:329
162. Carter TA, Wodicka LM, Shah NP, Velasco AM, Fabian MA, Treiber DK, Milanov ZV, Atteridge CE, Biggs WH III, Edeen PT, Floyd M, Ford JM, Grotzfeld RM, Herrgard S, Insko DE, Mehta SA, Patel HK, Pao W, Sawyers CL, Varmus H, Zarrinkar PP, Lockhart DJ (2005) Proc Natl Acad Sci USA 102:11011
163. O'Hare T, Druker BJ (2005) Nat Biotechnol 23:1209
164. http://www.vrtx.com, (Last visited on: Jan 05, 2006)
165. Burley SK (2005) Annu Meeting Am Soc Hematol, Abstract 698
166. http://www.sgxpharma.com, (Last visited on: Jan 05, 2006)
167. http://www.newcmldrug.com, (Last visited on: Jan 05, 2006)
168. Cortes J, Garcia-Manero G, O'Brien S, Hernandez I, Rackoff W, Faderl S, Thomas D, Ferrajoli A, Talpaz M, Kantarjian H (2004) Annu Meeting Am Soc Hematol, Abstract 1011
169. Cortes J, O'Brien S, Verstovsek S, Daley G, Koller C, Ferrajoli A, Pate O, Faderl S, Ravandi-Kashani F, Talpaz M, Zhu Y, Statkevich P, Sugrue M, Kantarjian H (2004) Annu Meeting Am Soc Hematol, Abstract 1009
170. Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG, Halmos B (2005) N Engl J Med 352:786
171. Pao W, Miller VA, Politi KA, Riely GJ, Somwar R, Zakowski MF, Kris MG, Varmus H (2005) PLoS Med 2:e73, http://www.plosmedicine.org, (Last visited on: Jan 05, 2006)
172. Kwak EL, Sordella R, Bell DW, Godin-Heymann N, Okimoto RA, Brannigan BW, Harris PL, Driscoll DR, Fidias P, Lynch TJ, Rabindran SK, McGinnis JP, Wissner A, Sharma SV, Isselbacher KJ, Settleman J, Haber DA (2005) Proc Natl Acad Sci USA 102:7665

Author Index Volume 1

The volume numbers are printed in italics

Angibaud P, see End DW (2007) *1*: 115–150
Arts J, see ten Holte P (2007) *1*: 273–311

Blake JF, see Wallace EM (2007) *1*: 65–114
de Bono JS, see ten Holte P (2007) *1*: 273–311
Boschelli DH (2007) Bcr-Abl Kinase Inhibitors. *1*: 387–424
Bradbury RH (2007) Overview. *1*: 1–17

Van Emelen K, see ten Holte P (2007) *1*: 273–311
End DW, Mevellec L, Angibaud P (2007) Farnesyl Protein Transferase Inhibitors: Medicinal Chemistry, Molecular Mechanisms, and Progress in the Clinic. *1*: 115–150

Fong PC, see ten Holte P (2007) *1*: 273–311

Galemmo Jr RA, see Moriarty KJ (2007) *1*: 189–271
Garcia-Echeverria C (2007) Survival Signaling. *1*: 151–188

Hoffmann J, Sommer A (2007) Anti-hormone Therapy: Principles of Endocrine Therapy of Cancer. *1*: 1–64
ten Holte P, Van Emelen K, Janicot M, Fong PC, de Bono JS, Arts J (2007) HDAC Inhibition in Cancer Therapy: an Increasingly Intriguing Tale of Chemistry, Biology and Clinical Benefit. *1*: 273–311

Janicot M, see ten Holte P (2007) *1*: 273–311
Johnson DL, see Moriarty KJ (2007) *1*: 189–271

Koblish H, see Moriarty KJ (2007) *1*: 189–271

Laird ER, see Wallace EM (2007) *1*: 65–114
Lyssikatos J, see Wallace EM (2007) *1*: 65–114

Mevellec L, see End DW (2007) *1*: 115–150
Moriarty KJ, Koblish H, Johnson DL, Galemmo Jr RA (2007) Progress in the Development of Agents to Control the Cell Cycle. *1*: 189–271

Paz K, Zhu Z (2007) Development of Angiogenesis Inhibitors to Vascular Endothelial Growth Factor Receptor 2 for Cancer Therapy. *1*: 313–362

Sawyer TK (2007) Novel Small-Molecule Inhibitors of Src Kinase for Cancer Therapy. *1*: 363–385
Sommer A, see Hoffmann J (2007) *1*: 1–64

Wallace EM, Yeh TC, Laird ER, Blake JF, Lyssikatos J (2007) Inhibition of Growth Factor Signaling by Small-Molecule Inhibitors of ErbB, Raf, and MEK. *1*: 65–114

Yeh TC, see Wallace EM (2007) *1*: 65–114

Zhu Z, see Paz K (2007) *1*: 313–362

Subject Index

Abiraterone 50
Ableson (Abl) kinase 407
Absorption, variable 1, 11
Aclobifene 53
Acute lymphoblastic leukemia (ALL) 3, 408
Acute myeloid leukemia 133
N-Acyl-/aryl-2-aminothiazoles 248
Adaphostin 417
Adrenocorticotropic hormone (ACTH) 22
AEE788 109, 359
AG1024 418
Aminoglutethimide 35
Aminoimidazo[1,2-*a*]pyridines 240
3-Aminopyrazoles 225
AMN107 411
Anastrozole 5, 35
Androgen receptor 20, 28
Angiogenesis 333
Angiogenesis inhibitor 333
Anti-androgens 64
Anti-apoptotic pathway 171
Anti-estrogens 51
Anti-hormones, targeted drugs 5, 21
Antibodies 333, 339
AP23451 397
AP23464 397, 423
AP23588 397
AP23846 397
AP23848 397, 423
AP23994 397
Arimidex–tamoxifen, alone or in combination 38
Aromatase inhibitor 5, 34
ARRY-142886 (AZD6244) 117
ARRY-334543 106
Asoprisnil 64
Aurora kinases 207, 251
–, inhibitors 256
Aurora-A 252
Aurora-B 255
Axitinib (AG13736) 357
Azasteroides 47
AZD0530 431

BAY57-9352 362
Bazedoxifene 56
Bcr-Abl 407
Bcr-Abl kinase inhibitors 411
Benign prostate hyperplasia 40
2-(Benzimidazol-1-yl)thiazole 277
5-(Benzimidazol-1-yl)thiophene 277
Benzopyranones 182
Benzothiazoles 277
5-Benzoyl-2,4 diaminothiazoles 249
Bevacizumab 9
BIBF1120 360
BIBW 2992 107
Bicalutamide 67
Biochanin A 37
Biomarkers 121, 146
BMS-354825 (Dasatinib) 393, 427
BMS-582664 366
BMS-599626 105
Bortezomib 9
B-Raf 92

CAAX peptide competitive heterocyclic Inhibitors 149
CAAX peptidomimetics 147
cAMP-activating compounds 65
Camptothecin 189
Canertinib 106
Cardiac arrhythmia potential 1, 11
CDKs 209
– inhibitors 212
CDP791 341
Cediranib (AZD2171) 349

Celecoxib 185
CEP7055 364
CGP-76030/CGP-76775 396, 422
CHIR258 363
Chronic myelogenous leukemia (CML) 408
CI-994 (Tacedinaline) 323
Cisplatin 4
CML 407
Co-factors 29
Complete androgen insensitivity syndrome (CAIS) 29
COX-2 41, 185
CP-724714 101
CPA 68
CREB-binding protein (CBP) 30
Cyclic peptides 303
Cyclin-dependent kinases 207, 209
Cyclooxygenase 2 41
– inhibitor 185
Cyclophosphamide 3
CYP-17 inhibitors 50
Cyproterone acetate (CPA) 19, 66
Cytochrome P450 liability 1, 11
Cytotoxic drugs 3

Dasatinib 393
DC101 339
Dehydro-epiandrosterone sulfate (DHEAS) 43
Depsipeptide (FR901228/FK-228) 322
DHT 46, 50
Diaminotriazoles 247
Diaryl ureas 231
Diethylstilbestrol (DES) 69
Doxorubicin 5, 189
Dual aromatase-sulfatase inhibiton (DASI) 37
Dual inhibitors, Bcr-Abl/Src kinases 418
Dutasteride 47-49

Effectors, downstream 138
EGF signalling pathway, inhibitor 9
EGFR 89
–/ErbB2 dual inhibitors 102
– inhibitor 83
EMATE 44
Endocrine feedback cycle 22
Endocrine therapy 34
Epidermal growth factor (EGF) 65
Epidermal growth factor receptor (EGFR) 173, 387
ErbB family 95
–, selective inhibitors 83, 96
ERK 83
Erlotinib 9, 96
Estrogen biosynthesis, inhibitor 5
Estrogen receptors, ERα/ERβ 20, 24
Estrogens 69
Estrone-3-*O*-sulfamate (EMATE) 44
Estrone sulfate (E1S) 43
Estrone-sulfatase (STS) 43
Ethinyl estradiol 69
3-Ethylthiazolidinone 280

Fadrozole 35
Farnesyl protein transferase 136
Farnesyl transferase inhibitors 133, 147
Finasteride 47
Flavonoids 37, 271
5-Fluorouracil 3
Flutamide 6, 68
fMEK, inhibitors 117
Focal adhesion kinase (FAK) 388
Follicle stimulating hormone (FSH) 22, 70
FPP competitive compounds 148
FTase knockout mice 146
Fulvestrant 55, 58
Furopyrazoles 260

G-protein-coupled receptors (GPCRs) 182
G2/M growth delay, nuclear proteins 142
Gefitinib 9, 99
Glucocorticoid receptor (GR) 51
Gonadotropin-releasing hormone (GnRH) 22, 70
Goserelin 6

HDACs 296
–, inhibitors 293, 300, 314
Heat-shock protein inhibitors 195
Histone acetyltransferases (HATs) 26, 30, 296
Histone deacetylases (HDACs) 26, 30, 293, 296
HKI-272 107
Hormone antagonists 21, 51
Hormone deprivation 21, 34
Hormone interference 21, 69
Hormone release, inhibitors 70

Hormone response elements (HREs) 28
Hsp90 169
Human placenta estradiol dehydrogenase 41
Hydroxamates 318
Hydroxamic acid 293
– replacements 305
17β-Hydroxysteroid dehydrogenases 41
– inhibitors 41

IGF-IR 169, 172
–, kinase inhibitors 174
Imatinib, Bcr-Abl kinase inhibitor, CML 9, 390, 407, 409
IMC-1121B 341
IMC-1C11 340
Imidazopyrazines 262
Imidazopyridines 262
Imidazopyrimidines 181
Imidazolopyrimidines 262
Imidazotriazines 279
Indazoles 260
Indazoles, 3,5-substituted 222
Indeno[1,2-*c*]pyrazol-4-ones 222
Indolinone template-based inhibitor, SU-6656 396
Indolopyrimidines 267
Inositol polyphosphates 169, 194
Insulin-like growth factor (IGF-1) 65
Insulin-like growth factor I receptor 172
Interleukin-6 receptor (IL6-R) 387
Isoform selective 293

KDR 333, 337, 342
–, target for antiangiogenesis therapy 338
Keratinocyte growth factor (KGF) 65
Kinase inhibitor 83
Kinase insert domain receptor (KDR) 333, 337, 342
Kinase selectivity 383
Kinases 207
–, dual specificity 385

L21649 366
Lapatinib 102
Lasofoxifene 53
Letrozole 35
Luteinising hormone (LH) 22, 70
Luteinising hormone-releasing hormone (LHRH) 71

Mechanism-based design 383
MEK1/MEK2 93
MEK inhibitor 83
Metformin 181
Methotrexate 3
N-Methyl piperazine 11
Mifepristone 61
Mineralocorticoid receptor (MR) 23, 51
MS-275 322
mTor 169
Multi-kinase inhibitors 109

Neovastat (Ae941) 354
Neuropilin (NRP) 336
Nilutamide 68
Nitrogen mustard 3
NR boxes, leucine-rich motifs (LXXLL) 29
NS-187 415
Nuclear proteins, G2/M growth delay 142

ofmTOR, inhibitors 190
ON012380 416
Onapristone 62
Ospemifene 56
Ovarian estrogen, inhibitor 6
Oxindoles 244, 267

Paclitaxel 5
Paullones 219
Pazopanib (GW786034) 355
PD0325901 120
PD166326 419
PDK1 169
–, kinase inhibitors 184
Peptides, cyclic 303
2-Phenylaminopyrimidines 409, 411
Phenylureas 279
Phosphatidylinositol 169
Phosphatidylinositol analogues 192
Phosphatidylinositol-3-kinases 169, 178
3-Phosphoinositide-dependent protein kinase-1 183
Phospholipids 169, 194
PI3K 169
–, kinase inhibitors 179
PKB 169
–, kinase inhibitors 187
Placenta growth factor (PlGF) 336
Platinum 4

Picropodophyllin 176
Polo-like kinases 207, 269
–, inhibitors 271
PP1/PP2 395
Pregnenolone 50
Progesterone receptor 26
Progesterone receptor antagonists (PRAs) 60
Progestin megestrol acetate (MGA) 70
Progestins 69
Proliferating cell nuclear antigen (PCNA) 57
Prostate Cancer Prevention Trial (PCPT) 49
Protein kinases 8, 169, 186
Protein prenylation 133
Protein tyrosine kinases 385
Purine template-based inhibitors 397
Purvalanol 271, 390
Pyrazoles 256
Pyrazolo[1,5-*a*]pyridines and Pyrazolo[1,5-*a*]pyrimidines 242
1*H*-Pyrazolo[3,4-*b*]pyridine 220
Pyrazolones 256
Pyrazolopyrimidine template-based inhibitors 395
Pyridofuropyrimidines 181
Pyrido[2,3-*d*]pyrimidin-7-one 227
Pyridopyrimidinone template-based inhibitors 395
3-(Pyrimidin-4-yl)imidazo[1,2-*a*]pyridine 237
3-(Pyrimidin-4-yl)imidazo[1,2-*b*]pyridazines 237
Pyrimidines 233, 274
5*H*-Pyrimidino[5,4-*d*]benzazepines 268
Pyrimidinopyrazines 274
Pyrimidinylaminothiazole template-based inhibitor 393
Pyrrolocarbazoles 216
Pyrrolopyrazoles 259
Pyrrolopyrimidine template-based inhibitors CGP-76030/CGP-76775 396
Pyrrolopyrimidines 174, 262

Quinazoline template-based inhibitors 394
Quinazolines 182, 229, 263
Quinoline 266
Quinoline template-based inhibitor SKI-606 (Bosutinib) 394
Quinox-2(1*H*)-ones 232

Raf, inhibitors 83, 113
Raloxifene 56
Rapamycin 190
Ras proteins 133, 138
Receptor tyrosine kinases 8
5α-Reductase inhibitors 46
Rheb 144
Rho proteins 141
Rituximab 8
RNA interference (RNAi) therapies 65
RU38486 61

Scytonemin 271
Selective androgen receptor modulators (SARMs) 67
Selective estrogen receptor modulators (SERMs) 66
Selective progesterone receptor modulators (SPRMs) 61
Semaxanib (SU5416) 342
Serine/threonine kinases 11, 385
Sex hormone binding globulin (SHBG) 57
Signal transducer and activator of transcription-3 (STAT-3) 387
SKI-606 (Bosutinib) 394, 425
Src 383
Src kinases 383, 386
–, inhibitor 399, 409
Staurosporine 187, 271
Steroid hormone biosynthesis, inhibition 34
Steroid hormone receptors 23
–, blockade 51
–, ligand-independent activation 31
Steroid sulfatase inhibitors 43
Steroids, genomic effect 31
Structure-based design 383
SU-6656 342, 396
Sulphur mustard gas 2
Sunitinib (SU11248) 342

T315I 407
Tamoxifen 5, 19, 26, 53, 55
Targeted drugs 6
Template-based inhibitors 388
Testicular androgen, inhibitor 6

Testosterone 47, 50
Thiazolopyrimidines 262
Thienopyrazoles 260
Toluidides 66
Toremifene 55
Transactivation function, nuclear hormone receptors 29
Transcription factors 23
Transforming growth factor β (TGF-β) receptor 144
Trastuzumab 8
Triazoles 35
Trichostatin A (TSA) 297, 300
Tyrosine kinase 10
Tyrosine kinase inhibitors 333, 342

Vandetanib (ZD6474) 349
Vascular endothelial growth factor (VEGF) 9, 333
Vascular endothelial growth factor receptor (VEGFR) 333, 388
Vascular permeability factor (VPF) 335
Vatalanib (PTK787) 347
VEGF 9, 333
VEGFR 333, 388
VEGFR2 334
–, neutralizing antibodies 339
Vorinostat (SAHA) 319
Vorozole 35
VPF 335

Wortmannin 179

XL647 111, 356
XL999 356

ZD4190 349
ZK304709 365