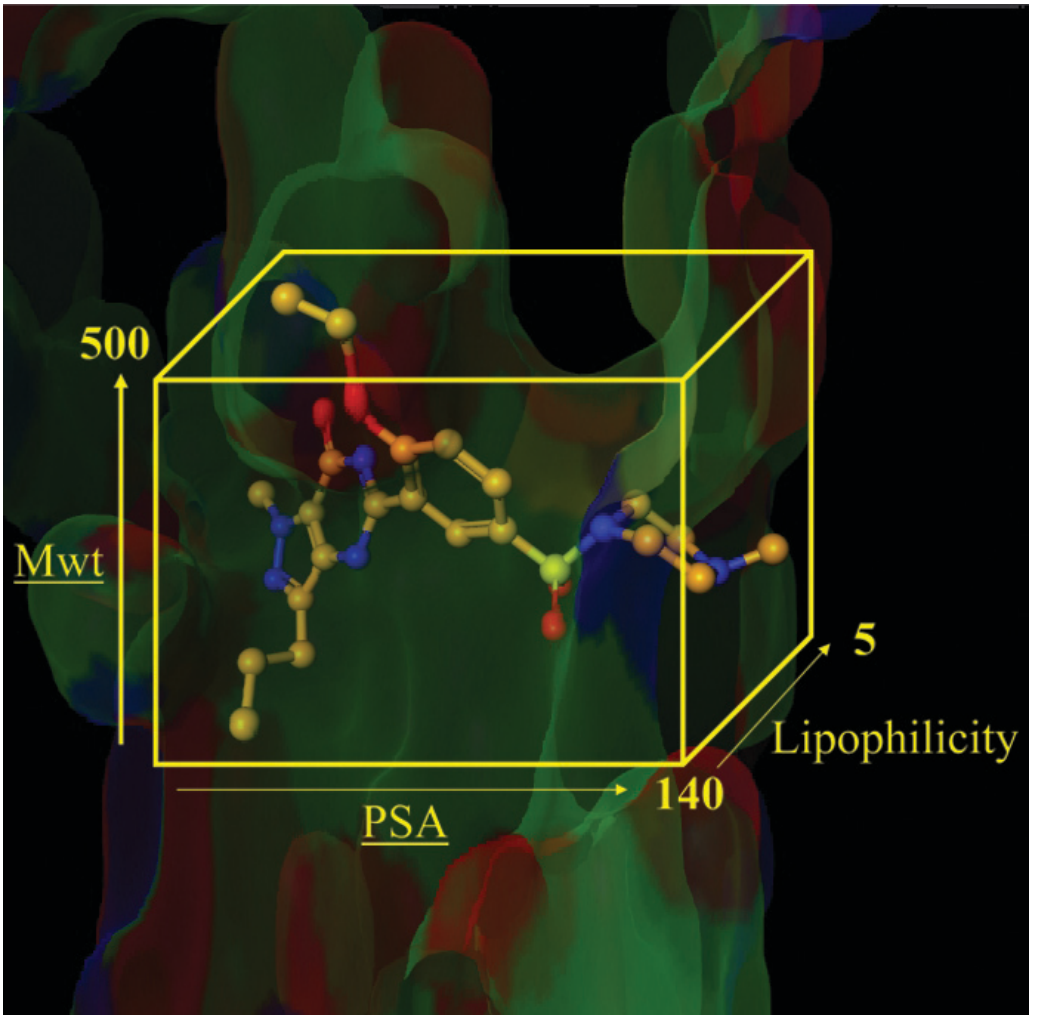
RSC Drug Discovery

Edited by Dennis Allen Smith

Metabolism, Pharmacokinetics and Toxicity of Functional Groups

Impact of Chemical Building Blocks on ADMET



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Metabolism, Pharmacokinetics and Toxicity of Functional Groups

Impact of Chemical Building Blocks on ADMET

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Preface

When it was suggested to me that the area of Drug Metabolism and Pharmacokinetics was lacking in works available for the Medicinal Chemist I was somewhat surprised. On reflection a study of many of the volumes revealed that although the scholarship was outstanding many had not been written to provide information to the medicinal chemist as they encountered new compound series.

So often the change of project and perhaps even the chance of chemical lead series takes the chemist into an area that he has not encountered before. The changes in absorption, distribution, metabolism and excretion properties (ADME) can be profound. For instance a chemist working on aminergic GPCR receptors will be used to compounds cleared by metabolism, with large volumes of distribution and often good access to the CNS. Results from screens often do not need to be re-intepreted to allow for protein binding effects. Perhaps a switch to a non-aminergic GPCR will cause the chemist now to work with acidic molecules. Now high intrinsic clearance can be disguised by high protein binding, metabolic clearance is augmented by significant drug transporter effects and some of the metabolic steps are reversible (e.g. acyl glucuronides).

This volume attempts to fill this void. Metabolism, Pharmacokinetics and Toxicity of Functional Groups tries to do what it says on the cover. Our definition of the key functional groups seemed right at the outset. As we have assembled the work it is pleasing to see the holistic nature of the concept. Many of the chapters build off concepts described in others but each can be viewed as a seperate entity and one we hope chemists find rewarding as they move into new chemical areas or perhaps revisit others.

The aim of all the authors was to impart around 300 years of collective knowledge and wisdom about the impact of ADME on Drug Discovery and

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hopefully aid all those active in this noble profession. We like to think that someone, somewhere, has used a concept in this volume that has led to a new medicine, even better if it is medicines in the plural. If this turns out to be true our time will have been well spent. All the authors are associated with Pfizer and we would like to thank the company for encouraging the production of this work.

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CHAPTER 1

Drugs and their Structural Motifs

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1.1 Introduction

The major focus of the research-based pharmaceutical industry is the discovery of safe, efficacious, new chemical entities (NCEs) for therapeutic targets. The pharmaceutical industry can look back at a history of successful innovations, indicated by the fact that there are currently just over 1400 unique drugs on the market.

The success of the industry can be measured in, for example, the increase in life expectancy in men and women over the last four decades. For instance, a child born in the United States in 2005 can expect to live nearly 78 years (77.9 years). The increase in life expectancy represents a continuation of a long-running trend. Life expectancy has increased from 75.8 years in 1995 and from 69.6 years in 1955. (www.cdc.gov/nchs/pressroom/07newsreleases/lifeexpectancy.htm). Although there are multiple factors which potentially contribute to the increase in life expectancy, like for example diet and life style, the development and availability of new drugs appear to have made a substantial contribution.

Equally impressive, the impact of the industry can also be highlighted by the increase in five-year-survival rates for cancer when diagnosed 1975–1977

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compared to when diagnosed in 2000 (www.phrma.org/files/PhRMA%-202009%20Profile%20FINAL.pdf). Between 1975 and 1979, the five-year survival rate for cancer was just 50%; by 2000, survival had risen to 67%. Survival is increasing dramatically for many forms of cancer. The rate of five-year survival went up 21% for breast cancer, 42% for prostate cancer, 28% for colon and rectum cancer, and 25% for lung and bronchial cancer.

Drug discovery is a complex multivariate process, but the basic requirements for orally administered NCEs include novelty and patentability, intrinsic potency, oral bioavailability, no toxicological effects in humans, and a significant advantage over existing accepted therapies (if applicable). A schematic representation of the drug discovery process in the United States is shown in Figure 1.1.

Although it is possible to predict, with varying accuracy, what a NCE will do when orally administered to humans, the full potential of a NCE is not known until it has been tested in clinical trials. Therefore, any investment made will not yield any return until the NCE is on the market, which could be in the region of ten years after patenting, and for the majority of compounds there will be no return at all to offset the enormous costs of drug discovery and development. Therefore, drug discovery is a high risk business with massive, long-term upfront investments aiming at discovering the few blockbusters that are on the market at any one time. In addition, the pharmaceutical industry is one of the most research-intensive industries; in the United States, an average of 16% of sales is spent on R&D, second only to the aerospace industry (www.nsf.gov/statistics). The global pharmaceutical market is worth $553.4 billion in the top ten markets alone (Table 1.1).

The top ten marketed drugs and their revenue between June 2007 and June 2008 are shown in Table 1.2; they account for a total of $67.4 billion,[1] which is only 12.2% of total sales in the top ten markets.

Among the top ten drugs, Pfizer's Lipitor is by far the biggest seller, $5.5 billion ahead of a cohort of three drugs, Plavix, Nexium and Serentide with sales of $8.3, $7.7 and $7.5 billion, respectively. The top ten therapies are shown

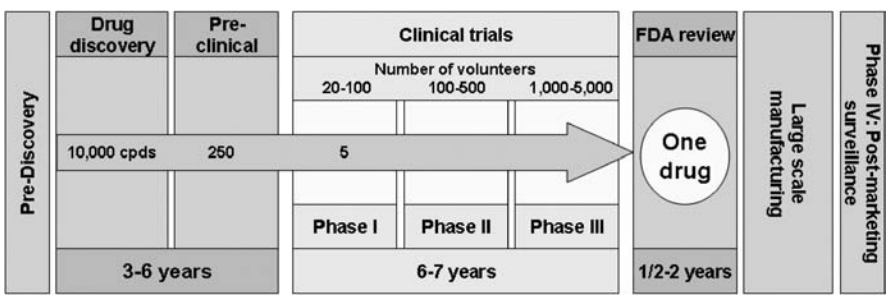


Figure 1.1 Schematic representation of the drug discovery process with typical time frames and attrition rates from drug discovery to FDA approval (adapted from www.phrma.org/files/PhRMA%202009%20Profile%20FINAL. pdf).

Table 1.1 Top ten pharmaceutical markets worldwide and their revenue for the period June 2007 to June 2008.

Country	*Sales June 2007–June 2008 ($ billions)*	*Share of global sales (%)*
USA	288.9	40.7
Japan	63.5	8.9
France	42.5	6.0
Germany	40.4	5.7
Italy	25.1	3.5
UK	23.5	3.3
Spain	21.6	3.0
Canada	19.1	2.7
China	16.8	2.4
Brazil	11.9	1.7
Total	**553.4**	**77.9**

Table 1.2 Top ten marketed drugs worldwide for the period June 2007 to June 2008.

Name	*Compound*	*Marketer*	*Indication*	*Sales June 2007–June 2008*
Lipitor	Atorvastatin	Pfizer	Hypercholesterolemia	13.8
Plavix	Clopidogrel	Bristol-Myers Squibb	Atherosclerotic events	8.3
Nexium	Esomeprazole	AstraZeneca	Acid reflux disease symptoms	7.7
Serentide	Fluticasone and salmeterol	GlaxoSmithKline	Asthma	7.5
Enbrel	Etanercept	Amgen	Rheumatoid arthritis	5.6
Seroquel	Quetiapine	AstraZeneca	Bipolar disorder, schizophrenia	5.1
Zyprexa	Olanzapine	Eli Lilly & Co.	Schizophrenia	5.1
Risperdal	Risperidone	Johnson & Johnson	Schizophrenia	5.0
Remicade	Infliximab	Centocor	Crohn's disease, rheumatoid arthritis	4.7
Singulair	Montelukast	Merck & Co.	Asthma, allergies	4.6
Top ten products				**67.4**

in Table 1.3 and account for 36.5% of global sales. The annual sales figures indicate that oncologics are by far the biggest revenue stream for the pharmaceutical industry, followed by lipid regulators. Interestingly, Pfizer's Lipitor alone accounts for almost half of lipid regulator sales.

Historically, big pharmaceutical companies delivered. The secret of their success was simple: pharmaceutical companies brought a huge number of innovative products to the market that genuinely helped sick people, and so

Table 1.3 Top ten drug therapies and their annual global sales for the period June 2007 to June 2008.

Therapy	*Sales June 2007–June 2008*	*Share of global sales(%)*
Oncologics	45.8	6.4
Lipid regulators	34.2	4.8
Respiratory agents	30.7	4.3
Acid pump inhibitors	26.7	3.8
Antidiabetics	26.0	3.7
Antipsychotics	22.4	3.1
Angiotensin-II antagonists	21.6	3.0
Antiepileptics	16.5	2.3
Autoimmune agents	14.8	2.1
Total	259.1	36.5

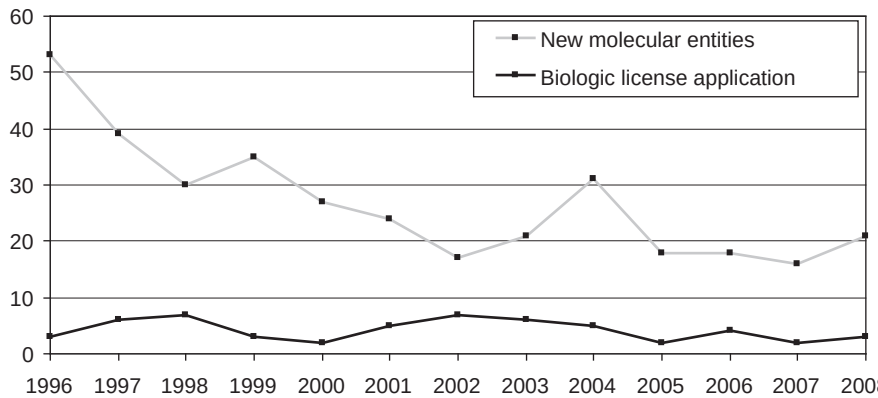


Figure 1.2 New molecular entities (NMEs) and biologic license applications approved by the US FDA's Center for Drug Evaluation and Research between 1996 and 2008.

were readily prescribed, which generated solid sales.[2] Even during times of economic hardship, drugs continued to be an essential purchase. During this flourishing period from the mid-1980s to the beginning of this decade, major drug companies routinely generated double-digit growth in sales year after year.

However, the pharmaceutical industry's investment in R&D has also risen steeply over the last 20 years, with R&D spending of $47.9 billion in 2007 compared with $26 billion in 2000 and $8.4 billion in 1990, and an average cost of $1.3 billion for bringing a new drug to market—an increase of 65% since 2000 (www.phrma.org/files/PhRMA%202009%20Profile%20FINAL.pdf).[3] Despite this increased investment in research and development, the number of new molecular entities (NMEs) has not increased in line with rising investment; in fact it declined between 1996 to 2008 from 54 to 21 (Figure 1.2).[4–15]

Figure 1.2 could suggest that there has been a significant decline in innovation rates in the pharmaceutical industry over the last decade. The reasons for this decline have been reviewed extensively[16] and several causes have been indicated as contributors to the R&D decline.[17] Among these are for example submaximal optimisation of resources and the inability to control costs as well as negative impact of mergers and acquisitions, which have been grouped together as factors internal to R&D. Alongside these, external reasons for the decline include evolving healthcare, regulatory burden, lack of regulatory harmonisation as well as changes in tolerance for risk.[17,18] Looking back over recent decades, total approvals by the US Food and Drug Administration (FDA) reached a record high of 381 entities in the decade between 1995 and 2004 compared with the two previous decades (241 in the decade 1985–1994 and 190 in the decade 1975–1984). Thus, it would appear that a myopic focus on near-term performance has given rise to a perception that bears very little relationship to the actual innovation rates of the pharmaceutical industry in the last decade.[19]

However, the issue of high attrition rates in drug discovery and development still remains, without which the innovation rates would be even higher and, potentially, would keep better track with the enormous increases in R&D investment. Only about 11% of compounds entering clinical development ever reach the market, being withdrawn for reasons such as efficacy (25%), toxicology (24%), clinical safety (12%), drug metabolism and pharmacokinetics (DMPK, 8%), formulation (1%) and portfolio-related and other reasons (30%).[20] Therefore, out of the 70% of failures caused by specific effects, the majority of 61% can be attributed to lack of efficacy, toxicology and clinical safety, whereas DMPK (physicochemical properties, or drug likeness, of the drug candidate itself) accounts for only 8% of attrition.[21] However, the actual proportion may be higher since some reported attrition, which was attributed to lack of efficacy, might be due at least in part to poor DMPK.[20] A similar proportion of 7% was discussed as having inappropriate absorption, distribution, metabolism and excretion (ADME) properties among NCEs between 1964 and 1985.[22] In addition, apparently only about 30% of marketed prescription drugs produce revenues that match or exceed average R&D costs.[22]

The apparent decrease in productivity in the entire pharmaceutical industry has put enormous financial pressures on individual companies and their share price—one of the measures of confidence of investors in future profitability.[23] Although the underlying reasons for this decline in productivity are complex, many factors have been suggested, such as for example increasing clinical development costs, FDA approval standards and political pressures on drug pricing.[20]

One of the key reasons for the decline in productivity is without doubt the high rate of attrition at all stages of the drug discovery process from failures in the early pre-clinical stages to the very expensive late stage failures in the clinic or even post-launch. Although exact figures on attrition in drug discovery are difficult to derive due to the sparseness of publicly available data, it is clear that success rates of discovery projects over the last decade, perhaps in part due to

the very high attrition rates, have not been able to match expectations in terms of productivity targets.

Therefore, attempts to reduce attrition early in the drug discovery process have been a major focus over the last decade. During that time, the application of guidelines linked to the concept of drug-likeness (in particular absorption) such as the 'rule of five'[24] (see Section 2.1.1 for details) has gained wide acceptance[25] as an approach to reducing attrition in drugs.[26,27] However, despite this acceptance, an analysis of recent trends revealed that the physical properties of molecules that are currently being synthesised in leading drug discovery companies differ significantly from those of recently discovered oral drugs and compounds in clinical development.[26] This was particularly notable for lipophilicity, where the consequences of a significant increase include a greater likelihood of lack of selectivity and attrition in drug development.[26] Physicochemical properties of molecules are completely under the control of medicinal chemists and can be easily calculated for very large numbers, in some cases for hundreds of thousands of designed structures prior to synthesis.

Close monitoring of physical properties during a drug discovery programme and compound series selection based on orthogonal attrition risks as indicated by compound properties and chemical scaffold may provide the medicinal chemist with opportunities to significantly reduce attrition rates, which are currently estimated at 93–96%.[28]

In this chapter, we focus on the relationship of molecular properties and functional groups of compounds on their interactions with biological targets, which can potentially impact on their pharmacological profile and their potential attrition risks.[29]

1.2 Launched Drugs

The relationship between chemistry, biology and medicine has been a remarkably productive one over the past century[30] since Paul Ehrlich pioneered the idea of systematically searching for drugs. By screening just over 600 synthetic compounds, Ehrlich discovered arsphenamine (Salvarsan)[31] in 1909 which, at the time, greatly improved the treatment of syphilis. Since then, there have been a large number of very significant breakthroughs, for example penicillin (1941), cortisone (1949), benzodiazepines (1960), beta blockers (pronethalol, 1967), anti-histamines (cimetidine, 1977), ACE inhibitors (captopril, 1981), insulin (1982), statins (lovastatin, 1987), HIV (zidovudine, AZT, 1987), COX-2 inhibitors (celecoxib, 1999) and kinase inhibitors (imatinib, 2001). Between 1983 and 2007, 907 different NCEs were approved as drugs.[32]

In their elegant analysis of drug targets, Overington *et al.* found the number of unique launched drugs to be 1357,[33] of which 1204 were considered to be 'small-molecule' drugs. Of those, 803 can be administered orally. The analysis included data up to the end of 2005; the number of small molecule drugs has since increased by 21 in 2006[11] and 19 in 2007[12], resulting in a total number of launched small molecule drugs of 1244. Of the 1204 drugs used in the 2005

Table 1.4 Molecular targets of FDA approved drugs.

Class of approved drugs	*Species*	*Number of molecular targets*
Targets of approved drugs	Pathogen and human	324
Human genome targets of approved drugs	Human	266
Targets of approved small-molecule drugs	Pathogen and human	248
Targets of approved small molecule drugs	Human	207
Targets of oral small molecule drugs	Human	186

analysis, 1065 were assigned protein molecule targets believed to be responsible for the efficacy of the drug.[33] The data is summarised in Table 1.4.

In the following we discuss the target space and chemical space of drugs separately, but it should be pointed out that these are not separate 'spaces' but are interlinked through common, complementary properties. These are, for example, the steric complementarity of a small molecule with a binding site—not only in terms of shape but also in terms of electrostatic interactions and physicochemical properties. This principle of complementarity of chemical and biological space has been discussed extensively elsewhere[34] and will not be expanded upon as part of this chapter.

1.2.1 Target Space of Launched Drugs

The first analysis of the draft sequence of the human genome resulted in an estimate of $\sim$31 000 protein-coding genes;[35] the current estimate has dropped to 22 287 genes.[36] It is generally estimated that 3000 of these are druggable.[37,38] The relationships of drugs and their targets has been studied extensively.[33,39] In this context, the term 'chemogenomics', described as 'the discovery and description of all possible drugs for all possible drug targets', has been coined.[40]

Chemogenomics has been identified as a new approach that can guide drug discovery based on integration of all information within a protein family, for example sequence, structure–activity relationship (SAR) data and protein structure. This allows very efficient cross-SAR analysis and exploration between targets that share small molecule inhibitors, leading to identification of new lead structures.[41] Chemogenomic approaches to drug discovery effectively explore the observation that similar receptors bind similar ligands and have shifted traditional receptor-specific studies towards a more cross-receptor view of pharmaceutical research.[42] Chemogenomic approaches have been exemplified recently for cardiovascular diseases[43] as well as for kinases.[44]

The druggable genome has been initially quantified as 483 small molecule drug targets,[45,46] with a later figure suggesting that number to be between 600 and 1500.[37,40] However, it has also been shown that out of these potential drug

targets, only a total of 324 drug targets account for all classes of approved therapeutic drugs. This number is reduced further to only 186 for targets of approved oral small molecule drugs. The gene family distribution of current drugs is shown in Figure 1.3.[33]

The concept of druggability, which has been used widely in recent years, postulates that since the binding sites on biological molecules are complementary with their ligands in terms of volume, topology and physicochemical properties, then only certain binding sites on putative drug targets will be compatible with high-affinity binding to compounds with drug-like properties.[47] The extension of this concept to the whole genome analysis led to the identification of the druggable genome. This is the expressed proteome predicted to be amenable to modulation by compounds with drug-like properties.[37] However, it needs to be noted that the meaning of the term druggability has broadened beyond its generally accepted definition to signify very different aspects along the discovery, development and clinical pipeline.[48]

A very useful categorisation of druggability has been published by Sugiyama[49] which differentiates between the druggable genome and druggable proteins, and the druggability of compounds in terms of their

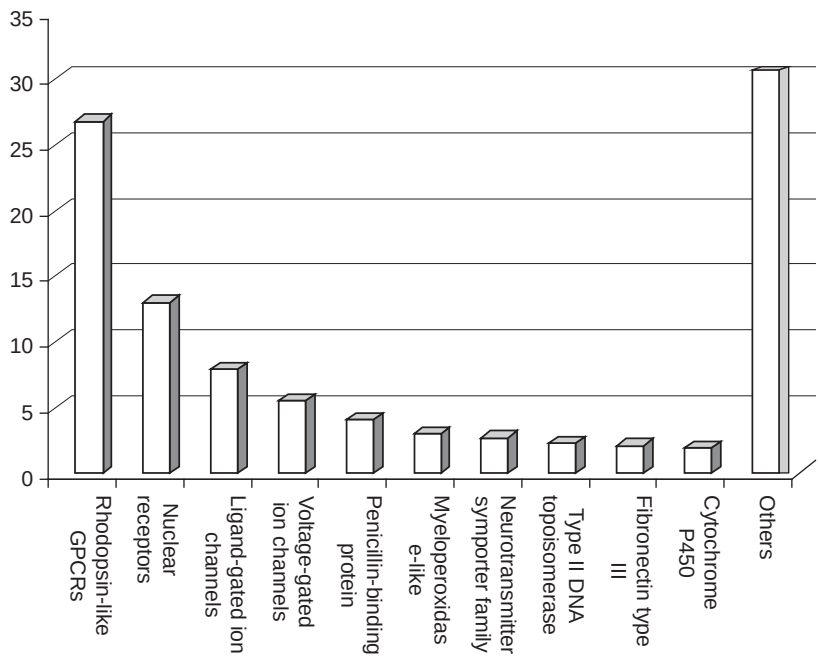


Figure 1.3 The gene family share as a percentage of all FDA-approved drugs for the top ten families. Beyond the ten most commonly drugged families, there are a further 120 domain families or singletons for which only a few drugs have been successfully launched. The data is based on 1357 dosed components from >20 000 approved products, FDA, December 2005.

molecular properties. The usefulness of the concept of druggability from a medicinal chemistry standpoint has been summarised by pointing out that the rule of five (Ro5) and its extensions have generated awareness about the importance of pharmacokinetic parameters for drug discovery and development. In addition, the concept of druggability has led to the realisation that there may be whole families of proteins for which it is either extremely challenging or impossible to design small molecules with acceptable oral bioavailability.[50]

Another concept related to druggability is ligand efficiency,[51,52] which generates a quantitative relationship between drugs and their biological targets, and is defined as the binding energy per non-hydrogen atom in a particular molecule. This concept can be very useful for lead selection by normalising binding energy for molecular weight, but also for differentiation between gene families that have a high or low probability of binding Ro5-compliant small molecules based on an analysis of experimentally determined ligand-binding energies for a particular target. These concepts of maximal affinity and ligand efficiency have been developed further into a computational approach to predict druggability.[53]

In the past ten years or so, expectations in the pharmaceutical industry have been raised as many companies have invested significantly in high-throughput technologies that would make use of information derived from the sequencing of the human genome.[54] Therefore, it would seem that companies are now well-placed to take advantage of the discovery of new targets that have appeared in the post-genomic era. However, there appears to be a reduced likelihood of delivering a preclinical drug development candidate against a new target, which could lead to a temptation to concentrate on more established targets to reduce risk in current development portfolios.[54] More recent *in silico* approaches such as high-throughput electronic biology may help in identifying, for example, previously unknown complex relationships between targets as well as compounds and targets in biological pathways on a large scale in order to support many parallel work streams in a drug discovery portfolio.[55]

1.2.2 Chemical Space of Launched Drugs

Chemical space, like target space and druggability, is another concept used frequently in the literature.[56,57] Defined as the number of synthesisable small molecule compounds, it is believed to be in the order of 10^{60} individual drug-like molecules. Analyses of chemical space have been published, for example, for natural products[58] and antibiotics.[59] Recently, drug–target interaction networks have been defined from the integration of chemical and genomic spaces for four target classes—enzymes, ion channels, G-protein coupled receptors (GPCRs) and nuclear receptors. The results indicate that there is significant correlation between drug structure similarity, target sequence similarity and the drug–target interaction network topology.[60] Below

we focus particularly on the molecular and pharmacological properties of drugs.

1.2.3 Molecular Properties of Launched Drugs

The physicochemical properties of molecules are important factors for pharmacological profiles and attrition risks. The distribution of the molecular properties of small-molecule launched drugs has changed little in the past 25 years, despite changes in the types of clinical indications for which drugs have been developed as well as in the range of targets.[61]

There have been a number of recent publications on the analysis of molecular properties of oral drugs,[62,63] as well as on how molecular properties influence oral drug-like behaviour.[64] A number of researchers have highlighted an upward trend in molecular weight (MW) and lipophilicity between older and newer drugs,[65,66] which has also been linked to attrition rates in clinical trials.[66]

The first systematic analysis of molecular properties relating to compound attrition resulted in the rule of five.[24] This states that a molecule is less likely to be absorbed if its molecular weight is above 500, its clogP is above 5, and the number of hydrogen bond acceptors and donors is more than ten and five, respectively. Although Ro5 parameters are interrelated, with cLogP being an additive property dependent on fragment values and therefore directly related to molecular weight, as well as hydrogen bond acceptor and donor functionalities of fragments, its simplicity and ease of calculation even for very large numbers of molecules has made it a very influential indicator for the likelihood of compound absorption, which is also often referred to as drug-likeness.[26]

It has been shown that the distribution of molecular weight and lipophilicity between marketed drugs and development phase I oral drugs is significantly different, with marketed drugs on average having a lower molecular weight compared with compounds in the discovery and development phases. It has also been shown that molecular properties of compounds vary significantly between gene families.[63] A graphical representation of the average molecular weight for oral drugs with respect to gene families is shown in Figure 1.4.[63]

Drugs in the protease and GPCR-peptidic families are characterised by significantly higher average molecular weight, while those in the ion channel family have lower average molecular weight. Drugs in the GPCR-lipid, GPCR-peptidic and nuclear hormone receptor (NHR) families have significantly higher cLogP. Drugs in the GPCR-peptidic and protease families have more acceptors, while those in NHR families have fewer acceptors. In only four families—CYP450, kinase, phosphodiesterase (PDE) and transporter—are the mean values of all four properties statistically similar to those of all oral drugs. Similar observations can be made while looking at the percentage of drugs in each family passing all four (or three of four) original Lipinski rules. GPCR-peptidic, GPCR-lipid and protease family targeted drugs have the lowest Ro5 compliance.[63]

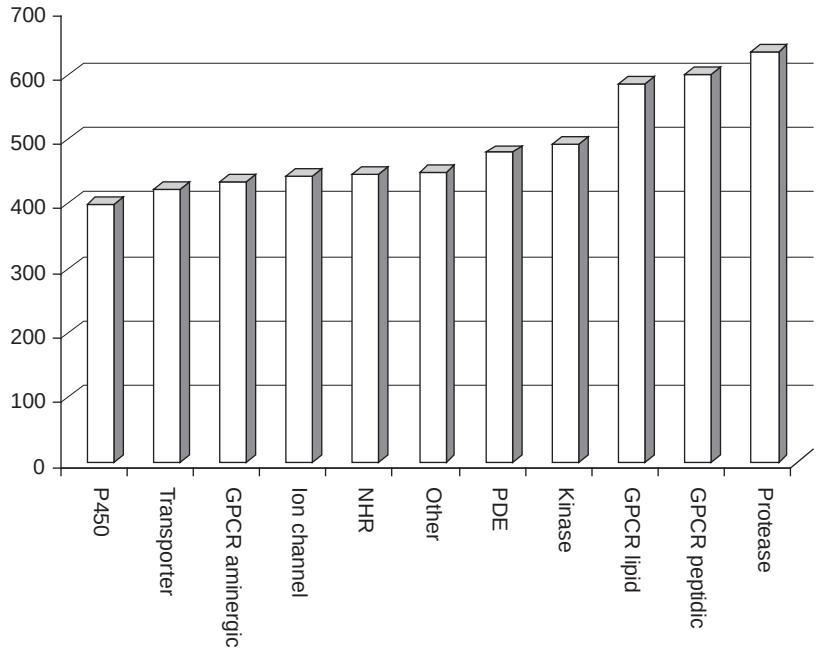


Figure 1.4 Average molecular weight of oral drugs for gene families.

Although the data seems to indicate that overall molecular weight of discovery and development compounds has increased over recent decades, it has been suggested that the upward trend can be explained largely by variations in the target portfolios of pharmaceutical companies. Most notably, a significant decrease of biogenic amine GPCR drugs in the recent decades (43% to 28%) and increases in protease and peptidic GPCR targeted drugs may explain much of the overall molecular weight trend. Variation in properties over time for a given family may result from varying pharmaceutical interest in its members (*e.g.* serine proteases, metalloproteases, *etc.*).[63]

The central assumption in the applicability of standard rules for drug likeness is that the target of interest requires molecular properties similar to those of the average drug. Since bioavailability results from the interactions of drugs with the same biological systems (*i.e.* membranes in the gastrointestinal tract as well as metabolic enzymes like P450s and transporters), it is possible that well-defined ranges of molecular properties can account for favourable interactions with those systems. For certain proteomic families, application of standard rules of drug likeness could bias the composition of corporate screening collections away from the molecular properties needed for achieving high affinity (*e.g.* for protein–protein interactions).[67] The need to balance bioavailability and affinity suggests that modified rules of drug likeness need to be adopted for certain target classes.[63]

The properties showing the clearest influence on the successful passage of a candidate drug through the different stages of development are molecular weight and lipophilicity. Statistical analysis shows that the mean molecular weight of orally administered drugs in development decreases on passing through each of the different clinical phases and gradually converges toward the mean molecular weight of marketed oral drugs. It is also clear that the most lipophilic compounds are being discontinued from development.[66] This work supports Lipinski's findings that there are limiting factors to the molecular weight and lipophilicity of a candidate drug that are reflected in the current physicochemical property profiles of the marketed oral drug data set. This study also suggests that these limiting values of physicochemical properties are not historical artefacts but are under physiological control.[66] In addition, an analysis of the difference between drugs and their original lead compounds shows that, for the majority of cases, only small structural changes are made in the lead to drug process.[68]

Therefore, it can only be advantageous if a screening collection already contains drug-like compounds with the right physical properties which carry a lower risk of attrition during drug development. This has been developed into the lead-like paradigm, which states that lead compounds need to be left-shifted in terms of molecular weight and lipophilicity compared to drugs in order to allow for the additional molecular weight and lipophilicity which has historically been added in the lead to drug process.[69] Recent studies have shown that molecular weight and log D are the most important factors in determining the permeability of drug candidates.[70] It has also been shown that the log D limits are dependent on molecular weight, and rules have been defined for log D limits required to achieve $>50\%$ chance of high permeability for a given MW band (Table 1.5).

Although both molecular weight and log D have been linked to permeability, log D may be the more important factor since it is an expression of multiple molecular properties such as hydrogen bond donors and acceptors, lipophilicity as well as dipole and polarisability, which are linked to physicochemical events like permeability and binding.

Table 1.5 Permeability rules defining LogD limits required to achieve $>50\%$ chance of high permeability for a given molecular weight band

Molecular weight	*AZLogD*
<300	>0.5
300–350	>1.1
350–400	>1.7
400–450	>3.1
450–500	>3.4
>500	>4.5

Differentiation between drugs and non-drugs on the basis of molecular properties has been reported in the literature, either based on statistical approaches[71] or analysis of structural features[72] with an accuracy of between 71 and 92%, respectively. However, these approaches are mostly limited to oral drugs and it has been shown that, for example, inhaled drugs reside in a region of molecular property space which is very different from that of oral drugs.[73]

In addition to predicting druggability for biological targets which can bind small molecules, there has been considerable interest in targeting protein–protein interactions with small molecules. Protein–protein interactions are highly attractive for drug discovery because they are involved in a large number of disease pathways where therapeutic intervention would bring widespread benefit. Recent successes have challenged the widely held belief that these targets are 'undruggable'.[74,75] Targeting interfaces between proteins has huge therapeutic potential, but discovering small-molecule drugs that disrupt protein–protein interactions is an enormous challenge, which is being faced with the support of for example bioinformatics approaches.[76] This vast new field of drug discovery has enormous potential but is outside the remit of this chapter; for further reading we refer you to recent successes reported in the literature.[67,74,75]

1.2.4 Polypharmacology

The understanding of pharmacological space is one of the fundamental aspects of drug discovery, relating to off-target activity and in turn to compound attrition. Pharmacological space has been mapped recently by Paolini *et al.* through large-scale data integration of proprietary and published screening data.[39] They have assigned 2876 targets to protein sequences from 55 organisms, with biologically active chemical tools for 1306 proteins. After removing redundancies in the mammalian genes due to orthologs among species, 836 genes could be unambiguously identified in the human genome for which small-molecule chemical tools with biological activity of $IC_{50} < 10\,\mu M$ have been discovered. This number drops to 529 when a perhaps more realistic threshold of 100 nM is applied (Table 1.6).

Of the pharmacological targets selected, 158 human proteins have been identified as the primary modes-of-action for approved small-molecule drug targets, with oral small-molecule drugs primarily targeting only 141 human proteins.

A key question in global pharmacological space is how extensive is promiscuity, which is defined as the specific binding of a chemical to more than one target. Considering each pair of targets in turn, if two proteins both bind to the same ligand, they can be considered as interacting in chemical space even if they have no other interaction in physical space or similarity in sequence space. The concept of 'target-hopping', where chemical matter for one target can be considered as the basis for leads or tools for another target, has historically

Table 1.6 Pharmacological target space.[39]

Gene family	*Human targets with* $< 10\,\mu M$ *binding affinity*	*Human targets with* $< 100\,nM$ *binding affinity*
Protein kinases	105	83
Peptide GPCRs	63	42
Transferases	49	24
Aminergic GPCRs	35	35
GPCRs (class A and others)	44	32
Oxidoreductases	40	25
Metalloproteinases	44	35
Hydrolases	36	21
Ion channels (ligand-gated)	29	22
Nuclear hormone receptors	24	19
Serine proteases	30	21
Ion channels (others)	18	11
Phosphodiesterases	19	18
Cysteine proteases	16	13
GPCRs (class C)	10	6
Kinases (others)	12	5
GPCRs (class B)	7	3
Aspartyl proteases	7	4
Miscellaneous	139	63
Enzymes (others)	109	47
Total	**836**	**529**

been an extremely fruitful method of drug discovery.[77,78] Of all the 276 122 active compounds found in the database used by Paolini *et al.*,[39] 65% have recorded activity for one target, whereas 35% are reported to hit more than one target.

The observed polypharmacology interaction network for human proteins was mapped to navigate polypharmacology relationships between targets. The entire protein interaction network consists of 700 proteins connected by 12 119 interactions for all compounds below the affinity threshold of IC_{50} 10 μM, and with a difference in affinity of up to three orders of magnitude between two targets. Promiscuity can be considered from the perspective of both the compound and the pharmacological target, to measure compound selectivity and target overlap.[79,80] Table 1.7 shows the top ten promiscuous targets taken from ref. 39.

Although different definitions of promiscuity result in different rankings, the same target classes (aminergic GPCRs, cytochrome P450s and protein kinases) appear at the top regardless of the method used in the analysis (Table 1.6). Aminergic GPCRs and protein kinases exhibit the greatest intra- as well as inter-gene family promiscuity. The data set used for this work is a sparse matrix, since activity data for each compound is mostly limited to only a few targets. There are indications that molecular properties and the potentially

Table 1.7 Top ten most promiscuous targets.[39]

Number	*Protein*	*Gene family*
1	Cytochrome P450 1A2	Enzyme
2	5-hydroxytryptamine 2C receptor	Aminergic GPCR
3	Cytochrome P450 3A4	Enzyme
4	D2 dopamine receptor	Aminergic GPCR
5	SRC kinase	Protein kinase
6	5-hydroxytryptamine 1A receptor (5HT1A)	Aminergic GPCR
7	5-hydroxytryptamine 2A receptor (5HT2A)	Aminergic GPCR
8	D4 dopamine receptor	Aminergic GPCR
9	Alpha-1A adrenergic receptor	Aminergic GPCR
10	5-hydroxytryptamine 7 receptor (5HT7)	Aminergic GPCR

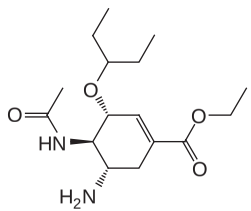
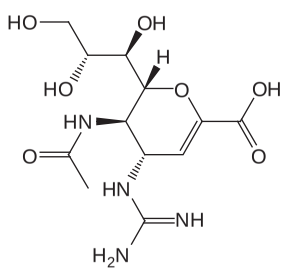
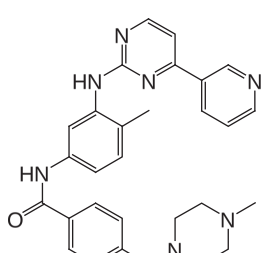
resulting promiscuity play an important role associated with *in vivo* toxicological outcomes.[81] It appears that the statistical odds for toxicity are significantly higher for compounds with a clogP >3 and a topological polar surface area (TPSA) of less than 75Å^2.[81] This indicates that lipophilicity is potentially linked to toxicity, which is in agreement with the perception that lipophilic binding is non-specific, whereas polar binding is related to specificity and therefore selectivity. This is in contrast to molecular weight, where low molecular weight and complexity will increase promiscuity and lead to lower selectivity.[69]

An alternative way to assess polypharmacology has been introduced by Fliri *et al*. with the concept of 'biological spectra' based on the BioPrint® data set.[82] This enables the comparison of compound similarity at the biological level rather than at the level of chemical structure similarity. The underlying idea is that compounds with similar bio spectra are by definition similar, even though their scaffold similarity might be very low. The relevance of this approach has been demonstrated with examples that some pharmacology is associated with *in vivo* clinical effects.[83] *In vitro–in vivo* associations have been established from experimental and predicted data between M_1 activity and tachycardia, H_1 activity and somnolence, as well as D_1 activity and tremor. Further evidence is provided by examples from corticosteriods, adrenergics, sedatives[84] as well as ligands of the dopamine receptors D_2–D_4.[85]

1.3 Drugs Bound to their Targets

In order to understand the interactions at a molecular level of drugs with their targets, we have analysed examples of experimental protein–ligand structures of some marketed drugs. Experimental structures for a large number of drugs in their pharmaceutically relevant targets (see Table 1.8) are available in the Protein Data Bank (PDB) (www.rcsb.org/pdb/home/home.do). These structures are a manifestation of the increasing value and application of structural biology in drug discovery.[86]

Table 1.8 Experimental protein–ligand structures for marketed drugs and their targets available in the Protein Data Bank

Drug name	*Structure*	*Target*	*Indication*	*PDB code*
Oseltamivir (Tamiflu, prodrug)		Neuraminidase	Influenza	1l7f
Zanamivir (Relenza)		Neuraminidase	Influenza	3b7e
Imatinib (Gleevec)		Bcr-abl kinase	Cancer	3gvu

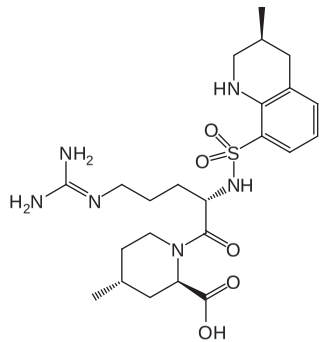
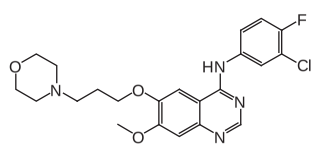
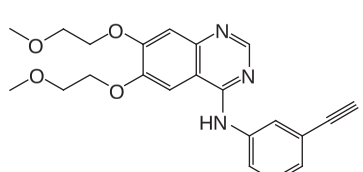
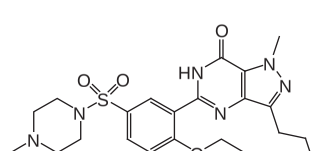
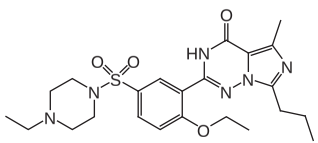
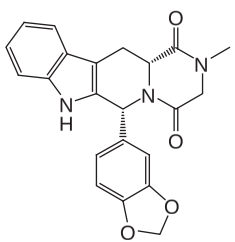
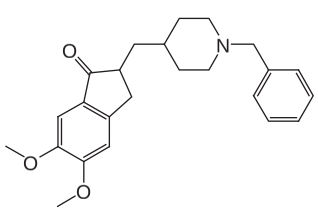
Argatroban		Thrombin	Anti-coagulant	1dwc
Gefitinib (Iressa)		EGFR kinase	Cancer	2ity
Erlotinib (Tarceva)		EGFR kinase	Cancer	1m17
Sildenafil (Viagra)		Phosphodiesterase 5 (PDE5)	Erectile dysfunction	1udt

Table 1.8 (*continued*)

Drug name	*Structure*	*Target*	*Indication*	*PDB code*
Vardenafil (Levitra)		Phosphodiesterase 5 (PDE5)	Erectile dysfunction	3b2r
Tadalafil (Cialis)		Phosphodiesterase 5 (PDE5)	Erectile dysfunction	1udu
Donepezil (Aricept)		Acetylcholinesterase	Alzheimers	1eve

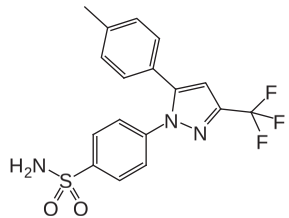
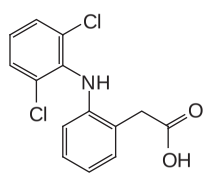
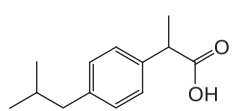
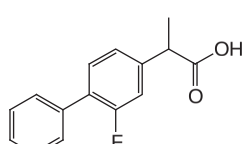
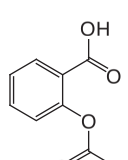
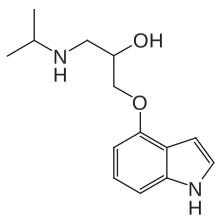
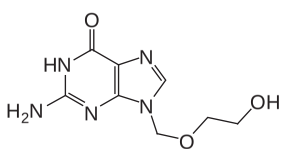
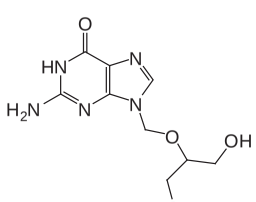
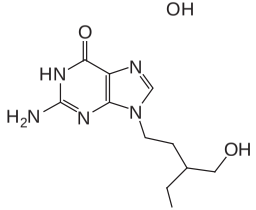
Celecoxib (Celebrex) (structure of analogue SC-544)		Cyclooxygenase 2	Inflammation, pain, arthritis	6cox
Diclofenac (Voltaren)		Cyclooxygenases	Inflammation, pain, arthritis	1pxx
Ibuprofen		Cyclooxygenases	Inflammation, pain, arthritis	1eqg
Flurbiprofen (Ansaid)		Cyclooxygenases	Inflammation, pain, arthritis	1eqh
Aspirin (structure of acetyl-salicylic acid)		Cyclooxygenases	Inflammation, pain	1pth

Table 1.8 (*continued*)

Drug name	*Structure*	*Target*	*Indication*	*PDB code*
Pindolol (structure of analogue Cyanopindolol)		b-adrenergic receptor	Hypertension	2vt4
Aciclovir (Zovirax)		Herpes simplex type-1 thymidine kinase	Herpes	2ki5
Ganciclovir		Herpes simplex type-1 thymidine kinase	Herpes	1ki2
Penciclovir		Herpes simplex type-1 thymidine kinase	Herpes	1ki3

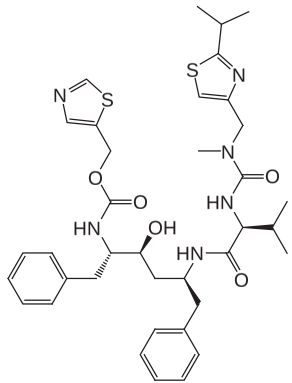
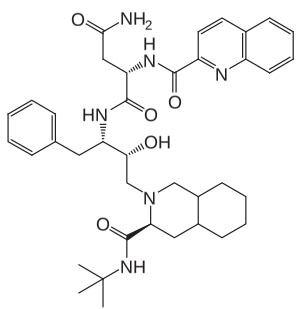
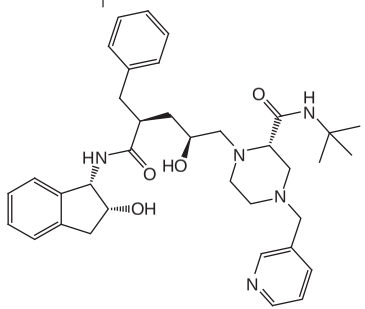
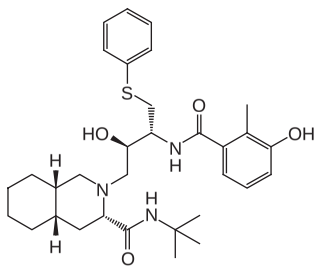
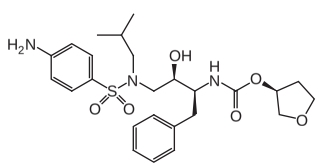
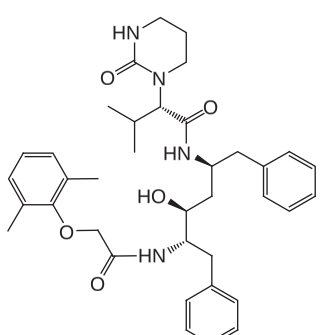
Ritonavir		HIV-1 protease	HIV	1rl8
Saquinavir		HIV-1 protease	HIV	2nmw
Indinavir		HIV-1 protease	HIV	1hsg

Table 1.8 (*continued*)

Drug name	*Structure*	*Target*	*Indication*	*PDB code*
Nelfinavir (Viracept)		HIV-1 protease	HIV	1ohr
Amprenavir (Agenerase)		HIV-1 protease	HIV	1t7j
Lopinavir		HIV-1 protease	HIV	1mui

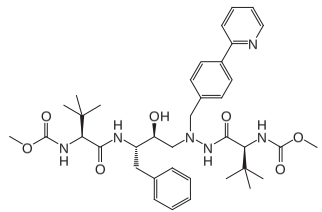
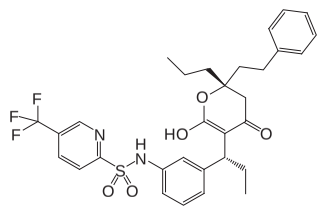
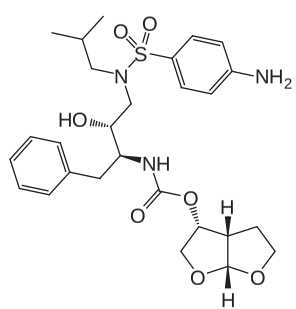
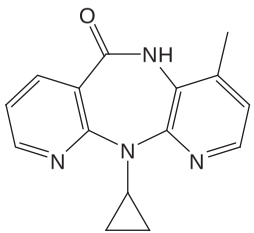
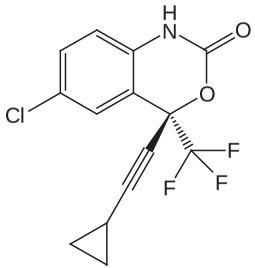
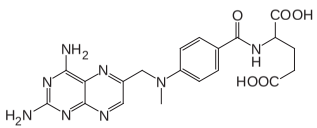
Atazanavir (Reyataz)		HIV-1 protease	HIV	2aqu
Tipranavir (Aptivus)		HIV-1 protease	HIV	2o4l
Darunavir		HIV-1 protease	HIV	3d1z

Table 1.8 (*continued*)

Drug name	*Structure*	*Target*	*Indication*	*PDB code*
Nevirapine (Viramune)		HIV reverse transcriptase	HIV	1s1u
Efavirenz (Sustiva)		HIV reverse transcriptase	HIV	1fk9
Methotrexate		Dihydrofolate reductase	Cancer	4dfr

1.3.1 Comparison of Binding Sites of Drugs Bound in their Biological Targets

One of the concepts discussed above is druggability, which relates to the likelihood of success for discovery of a drug based on the molecular properties of a small molecule ligand and the binding site properties of the receptor. The druggability concept is also based on the assumption that there are particular features of a binding site which enable it to bind small molecules with sufficient potency in order to meet requirements for a drug.

Rules have been derived in order to quantify druggability[87] and make predictions based on analysis of the receptor structure as to whether a target is likely to be druggable.[53] Some of the parameters linked to druggability are:[53]

- the degree to which the binding site is buried inside the protein;
- the curvature of the binding pocket;
- the topology of multiple pockets and their relative positions in the binding site;
- lipophilicity;
- polarity;
- the ability to form hydrogen bonds.

Below is the comparison of three marketed drugs, Tamiflu, Sildenafil and Iressa, bound to their respective receptors, neuraminidase, phosphodiesterase 5 and EGFR kinase (Figure 1.5).

In the binding site of neuraminidase, the whole of the ligand oseltamivir can be seen in the view from solvent into the binding site. In contrast, the binding site of PDE5 is deeper and much narrower, and it also encloses most of the ligand. The binding site of EGFR kinase binding Iressa forms a relatively narrow cleft compared to the binding sites of PDE-5 and neuraminidase, and the ligand structure is flat in comparison to Sildenafil and oseltamivir with quinazoline as the central template. Following the concept of druggability, it would be fair to say that for example PDE-5 and epidermal growth factor receptor (EGFR) kinase are druggable targets, whereas it would appear to be more difficult to develop a small molecule drug for neuraminidase. This example also highlights the difficulty with predicting druggability, since oseltamivir (Tamiflu) is a marketed drug acting on neuraminidase, as is Relenza. Therefore, druggability as a concept is perhaps not very useful as a filter but rather for pointing out potential opportunities for discovery.

1.3.2 Phosphodiesterase 5 (PDE5) Drugs

Figure 1.6 indicates how three drugs marketed against erectile dysfunction (Sildenafil, Vardenafil and Tadalafil) bind to their target phosphodiesterase 5. Sildenafil and Vardenafil are structurally very similar—in fact they only differ by two atoms and have a very similar binding mode—whereas Tadalafil has a molecular structure which is very different from the other two drugs but still is

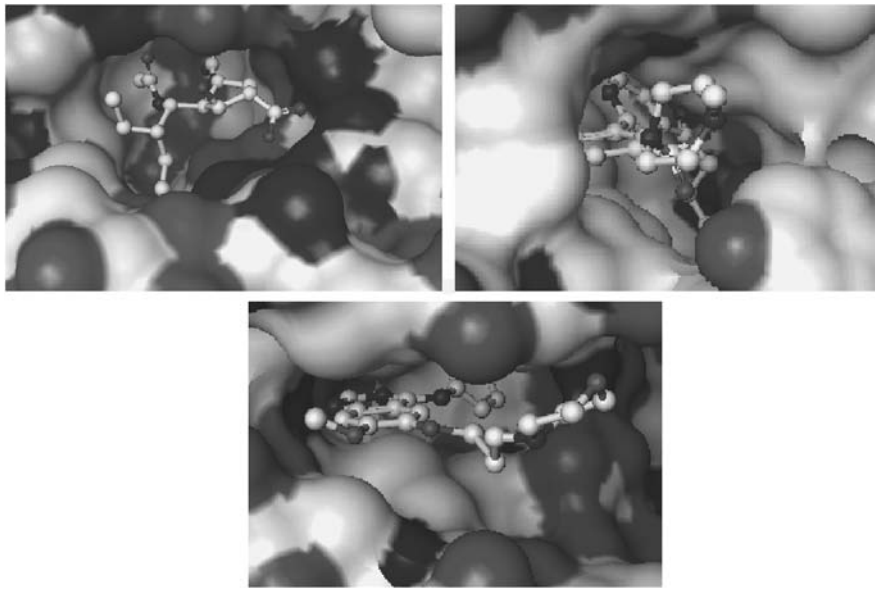


Figure 1.5 Tamiflu bound to neuraminidase (PDB 1l7f, left), Sildenafil bound to phosphodiesterase 5 (PDB 1udt, right) and Iressa bound to EGFR kinase (PDB 2ity, bottom). Carbon atoms are yellow, light blue and purple, respectively; oxygen atoms are red, nitrogen atoms are blue and sulfur atoms are yellow. Surfaces are coloured by atom type: carbon = green, nitrogen = blue, oxygen = red, sulfur = yellow.

able to bind to the same binding site, occupying significantly different areas in the receptor (*e.g.* such as at the bottom and to the right of Figure 1.6). Therefore, it appears that although sildenafil and vardenafil may contain substructures which bind strongly to phosphodiesterase, they are two very different interpretations of the phosphodiesterase pharmacophore which can both show significant levels of activity in the receptor sufficient for the desired pharmacological effect.

This ability to bind diverse substructures which are expressing a similar pharmacophore does not seem to be specific to PDE5, but is observed perhaps in an even more striking example of cyclooxygenase as highlighted below.

1.3.3 Cyclooxygenase (Cox) Drugs

Cyclooxygenase binds a variety of ligands, a number of them with acidic groups, but also neutral compounds. The overlay of ibuprofen, flurbiprofen, diclofenac and the celecoxib derivative SC-544 shown in Figure 1.7 highlights the structural diversity of Cox inhibitors. Interestingly, the acid groups of ibuprofen and diclofenac bind in very different areas of the binding pocket, so that the compounds show very different pharmacophores.

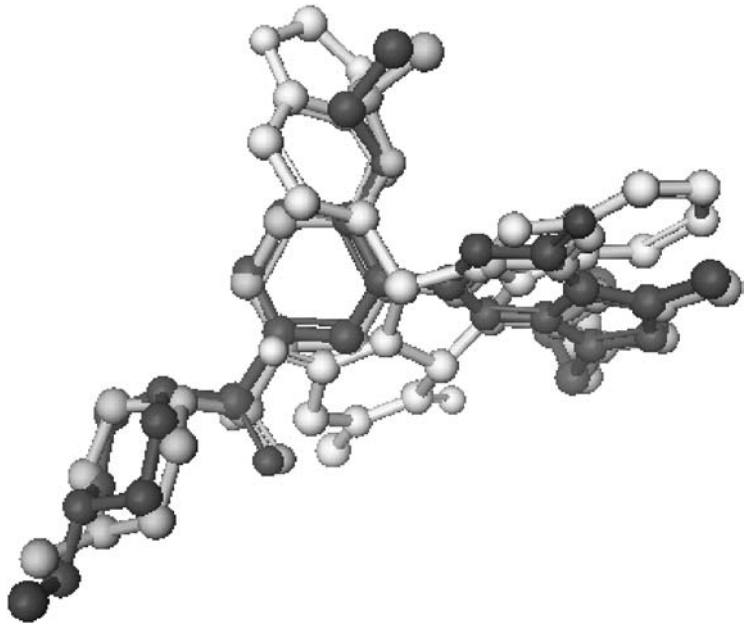


Figure 1.6 Overlay of experimental binding orientations for three marketed drugs for erectile dysfunction: sildenafil (light grey), vardenafil (dark grey) and tadalafil (white). Compound structures are shown in Table 1.8.

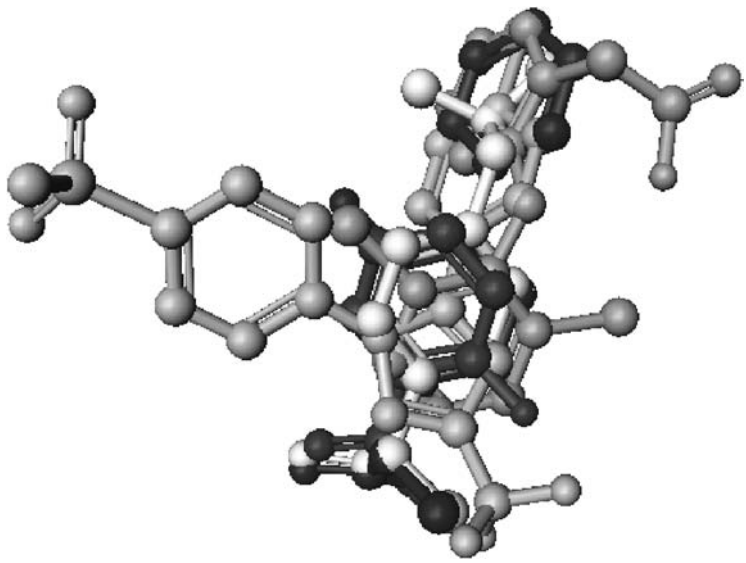


Figure 1.7 Overlay of ibuprofen (white), flurbiprofen (black), diclofenac (medium grey), celecoxib analogue SC544 (light grey) and salicylic acid (dark grey). The acid groups of diclofenac (top right) and ibuprofen, flurbiprofen and salicylic acid (bottom) are 9.1Å apart. Comound structures are shown in Table 1.8.

Although a CF_3 group is considered to be a bioisostere for an acid group, in this case the CF_3 group overlaps with the methyl groups of ibuprofen and flurbiprofen, rather than the acid groups, and is therefore a bioisostere for a lipophilic rather than a polar group. A more detailed analysis of the binding of Cox inhibitors (particularly Cox-2 inhibitors) is presented in Chapter 5.1.2.

1.3.4 Classes of Drugs with High Structural Similarity

The two examples of the drug targets PDE5 and Cox discussed above raise a number of questions relating to the ability of targets to accommodate and bind a relatively diverse set of structural features in ligands. For example, ibuprofen and flurbiprofen are structurally closely related, and were discovered during the same screening programme in the 1960s when many analogues of phenylacetic and phenoxyacetic acid were made in an attempt to identify potential herbicides. Thus the similarity of ibuprofen and flurbiprofen can be explained by the composition of the screening collection used rather than through a strong receptor preference for a particular structure. Similarly, sildenafil (UK patent 1993, FDA approval 1998) and vardenafil (FDA approval 2003) are both structural analogues of cyclic AMP, the natural substrate of PDE5.

In principle there are two compelling reasons why drugs have similar structures. First, screening files contain structurally similar compounds due to the fact that very often drug discovery programmes are driven by synthetic accessibility rather than structural diversity, yielding similar chemical matter through preparation of closely related analogues to study structure–activity relationships. Secondly, many drugs are structurally related to the natural substrate or inhibitor of the biological target receptor. Even though natural substrates or inhibitors have been developed through millions of years of evolution, their ability to bind to a certain receptor is not exclusive to a closely related series of compounds, as has been shown above. This is the case particularly in the gene family of kinases, where a large number of scaffolds are able to mimic adenosine triphosphate (ATP) and adenosine diphosphate (ADP), which bind in the hinge region of the catalytic domain of kinases.

1.4 Privileged Substructures in Drugs

The concept that similar molecules act in a similar manner is a fundamental principle of medicinal chemistry. The earliest reference in SciFinder to structure–activity relationship (SAR) was in 1899, describing the diuretic action in relation to osmotic properties of sugars.[88] The concept of SAR forms the basis of analogue-based discovery[89] and has been validated through decades of empirical observations. However, as our ability to evaluate small molecules on a relatively large number of targets *in vitro* expanded—a process called 'profiling' or 'secondary pharmacology'—there was a realisation that similar molecules can have very different overall profiles, making the prediction of secondary pharmacology very difficult. This observation of large changes in

pharmacology caused by rather small changes in molecular structure has also been referred to as 'activity cliffs'.[90] Therefore, the similarity principle is a good approximation for near-neighbours for a range of properties, but is not a reliable concept even within the same chemical scaffold.

The concept of privileged structures corresponds to the smallest structural subunit that has been encountered in several drugs or lead compounds which is able to provide ligands for multiple families of drug targets. In the late 1970s, Ariens *et al.* observed that many biogenic amine antagonists contain hydrophobic double ring systems as key structural elements.[91] Other groups noted that recurring molecular cores could exist across diverse drug targets.[92] However, the term 'privileged structure' was first used by Evans *et al.* in reference to their work with benzodiazepine ligands.[93–95] Numerous other authors have since identified other substructures that have also been observed in numerous drug programmes. Notably work by Patchett and Nargund advanced the discussion around understanding the inherent reasoning behind some groups appearing privileged by identifying properties in the substructures that facilitate their interactions with biomolecules, often via distinctly different interactions to the respective endogenous ligands.[96] These observations raised the possibility that there existed preferred molecular scaffolds that have an inherent tendency towards biological activity and that these groups could be modified to provide ligands for a range of biological targets. In agreement with this hypothesis, an analysis of all known drugs by Murko *et al.* in 1996 revealed that the 5120 compounds in the Comprehensive Medicinal Chemistry (CMC) database contain 1179 different frameworks; however 32 (3%) of those frameworks accounted for 50% of all drugs.[97] The analysis was purely based on size and shape did not fully take into account atom types or bond order. However, the existence of preferred molecular frameworks is still evident. When atom type and bond order are included, a larger diversity of frameworks results but again a large proportion of drug molecules (24%) are based on a small number of molecular frameworks.[92]

Other groups have modified the definition of privileged structure to encompass commonly occurring fragments within ligands that are promiscuous within only a single target family.[98] The motivation to identify such substructures is derived from the need to avoid indiscriminate off-target activity. More recently, however, this family specific concept has been re-categorised as distinct from the original definition of a privileged structure and been termed 'target family-directed masterkeys' by Mueller.[98]

Recent discussions by Schnur *et al.* have reverted back to the original definition of privileged structures whereby the motif is observed across numerous target families but by more distinctly defining differences between potentially hazardous 'frequent hit' motifs that systematically appear in high throughput screening (HTS) from those groups that occupy good 'drug-like' physicochemical space where the desired selectivity profile can be modulated via peripheral structural modification.[99,100]

The concept of privileged structures has been applied to the planning of new chemical libraries and has been associated with the application of

computational methods and pharmacophore models.[98–99,101] Furthermore, fragmentation of bioactive molecules and drugs has permitted a more thorough identification of relevant structural patterns that represent authentic biophores, providing frameworks for the generation of new compound databases. Numerous investigations have focussed on the identification of desirable privileged structural elements.

Two early studies examined methods to fragment compounds into core structures with peripheral modification. Both studies identified substructures with the intention of using the results to focus chemistry efforts for file enrichment strategies, via chemically enabled libraries.[97,102,103] In an alternative approach, Fesik *et al*. investigated a new experimentally based method to identified privileged structures by NMR-based screening of 10 000 selected fragments across 11 diverse target proteins, and identified 12 privileged substructures that appeared to bind to a range of targets with higher than average probability.[104] Naturally this work was only able to cover a fairly narrow collection of core fragments and target proteins. In addition, no clear rationale was obvious as to the reasoning for why the fragments bound to a particular target. Siegel and Vieth studied a set of marketed drugs and pointed out that there are a large number of drugs contained in other drugs either in their entirety or as substructures.[105] More recently, Sutherland *et al*. investigated the relevance of chemical fragments as foundations for understanding target space and activity prediction by decomposition of molecules into fragments and comparing the similarity of those fragments and their relationship in target space in an attempt to better understand cross-target activities.[106] In addition, the construction and use of small molecule libraries for fragment-based primary screening based on privileged substructures has been discussed recently.[107]

1.4.1 Examples of Privileged Substructures

The concept of privileged substructures is highlighted below with examples of marketed drugs for a variety of indications. Over the past two decades, numerous structures and functionalities have been labelled as privileged.[92,98,100,101,108,109] For illustrative purposes, some key examples and highlights from these analyses are discussed below covering classes of benzodiazepines, sulphonamide antibacterials, chemokine receptor 5 (CCR5) antagonists, 3-hydroxy-3-methylglutaryl-coenzyme A CoA (HMG-CoA) reductase inhibitors, cyclooxygenase inhibitors as well as kinase inhibitors.

1.4.2 Benzodiazepines

In the late 1980s, Evans and co-workers first defined the concept of privileged structures.[95] They worked on the development of novel non-peptidic cholecystokinin (CKK) receptor antagonists for the treatment of gastrointestinal disorders (*e.g*. pancreatitis and gastroesophageal reflux) based on analogues of the natural product asperlicin via structural modification of anxiolytic

benzodiazepine drugs such as diazepam.[110] This work resulted in the development of devazepide (MK-329) as the first non-peptidic benzodiazepine antagonist, highly selective for cholecystokinin-1 (CCK-1) (IC_{50} 0.8 nM).[111–113,93–95] Interestingly, this work involved key elements of structural planning and molecular simplification from the natural product asperlicin. It was suggested that both the benzodiazepine and tryptophan subunits of asperlicin are key elements of the pharmacophore for molecular recognition be the CCK-1 receptors. Natural product guided development of CCK-1 antagonists is shown in Figure 1.8.

Evans *et al*. recognised that this was not the first successful incorporation of benzodiazepines into bioactive molecules. In fact the benzodiazepine motif constitutes a broad class of neuroactive compounds acting as ligands to ion channels and GPCRs. Notable examples of anxiolytic drugs of this class are diazepam and lorazepam, which are ligands of central nervous system (CNS) gabaergic receptors.[114] In addition, there are the extensive numbers of CCK-1 diazepine containing ligands and numerous other applications as ligands to other GPCRs such as k-opioid agonists like tifluadom[115] for the treatment of visceral pain, antithrombitic platelet activation factor (PAF) antagonists,[116] analgesic and anti-inflammatory neurokinine (NK-1) receptor antagonists,[117] and GPIIbIIIa receptor antagonists with antithrombitic profiles.[118] In addition, multiple classes of enzyme inhibitors have been developed that contain the benzodiazepine unit; for example, HIV reverse transcriptase inhibitors such as nevirapine,[119] RAS-farnesyltransferase inhibitors for the treatment of cancer (*e.g*. BMS-214662[120]). This diversity of bioactivity for benzodiazepines across a broad range of classes of drug target led Evans to define this group as privileged. A group of representative diazepine-containing drugs is shown in Figure 1.9.

Examining reasons for benzodiazepines to be privileged in this manner has led to them being identified as β-turn peptidomimetics.[121–123,92] The presence of such structural motifs that are complementary to an array of primary and secondary structural elements in proteins offers a potential explanation for the promiscuous nature of the binding of many recurring scaffolds.

1.4.3 Arylsulfonamides and Drugs Derived from them

The following section describes the pivotal role of derivatives of sulphonamide drugs in opening up new areas of pharmacology. These are also reviewed from an ADMET perspective in Sections 5.1.1, 5.1.2 and 5.1.4 of Chapter 5.

1.4.3.1 Arylsulfonamides as Antibacterial Drugs

One of the best known classes of drugs are the antibacterial sulfonamides, which were first patented in 1932,[124] and were originally derived from azo dyes, whose antibacterial properties were discovered in the early 20th century.[125] The first patent featured a red dye, sulfamidochrosoidine (Prontosil Rubrum®) (Figure 1.10), which made medical history by treating streptococcal infections,

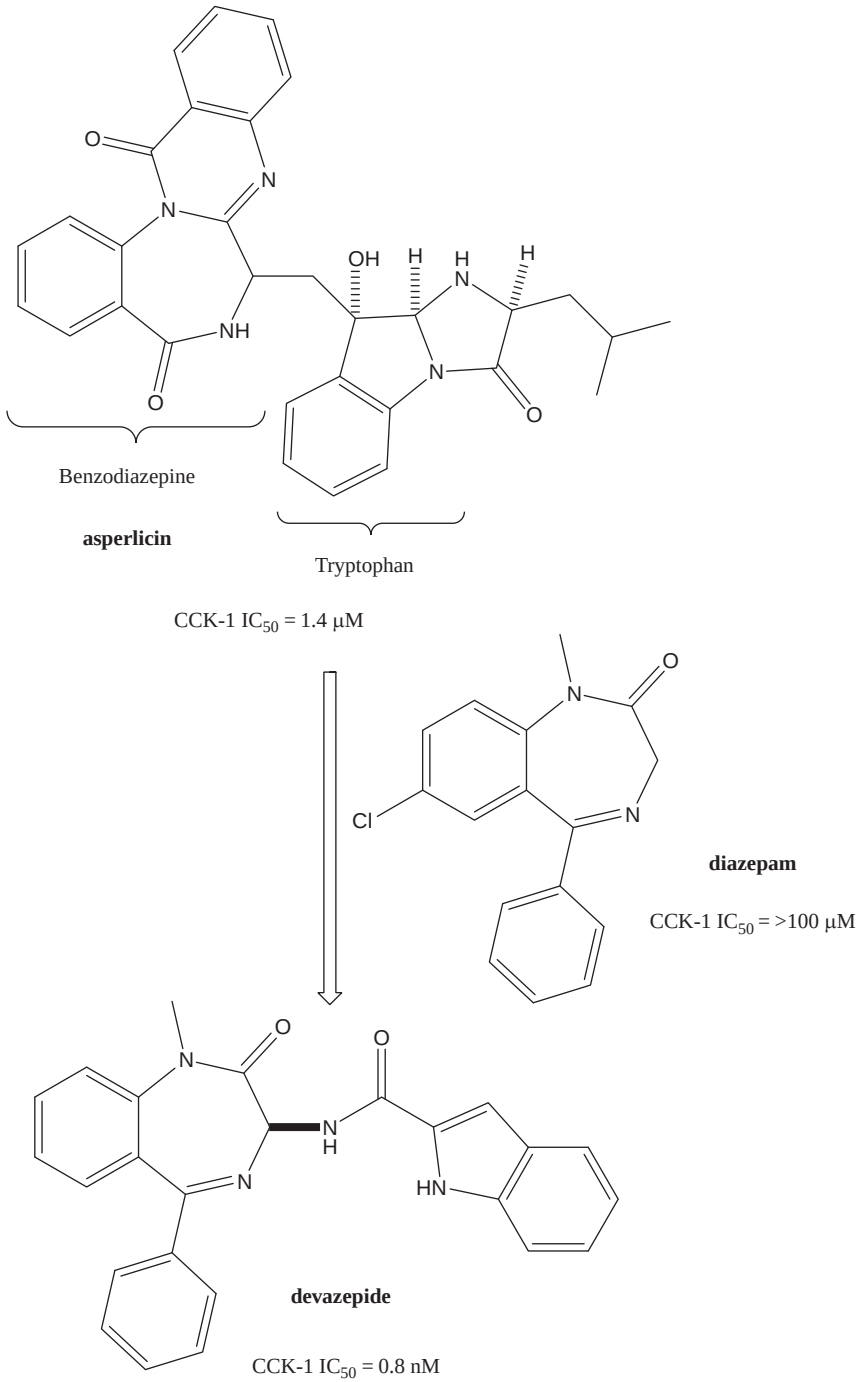


Figure 1.8 Natural product guided development of CCK-1 antagonists.

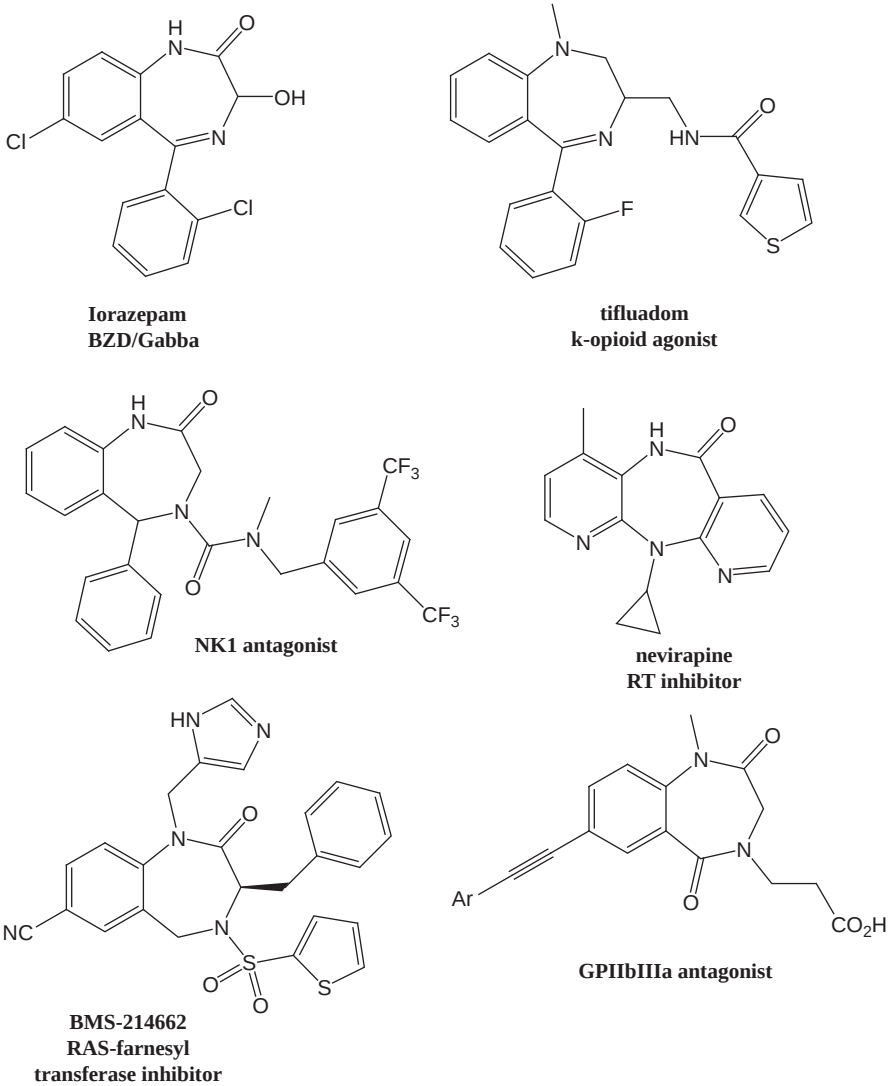


Figure 1.9 Representative diazepine-containing drugs.

including pneumonia and septicaemia, that were considered to be largely fatal. For this extremely significant discovery, Gerhard Domagk, the director of the IG Farbenindustrie laboratories at Elberfeld in Germany, was awarded the Nobel Prize for Medicine in 1939. It was later discovered that it was indeed the active metabolite sulfanilamide that was responsible for the antibacterial activity. The first of the sulfanilamide analogues, sulfacefamide (Figure 1.11) was marketed in 1938 and was used for many years to treat urinary infections.

Various derivatives of sulfanilamide were later marketed as drugs, their main differentiation being around variation of the pKa of the sulfonamide to reduce

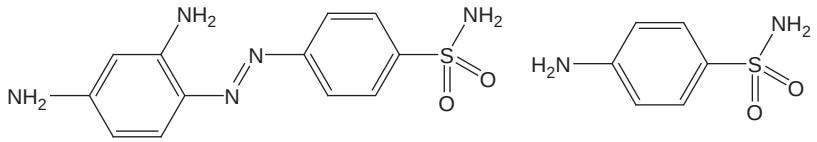


Figure 1.10 Structures of sulfamidochrosoidine (Prontosil Rubrum®, left), and its active metabolite sulfanilamide.

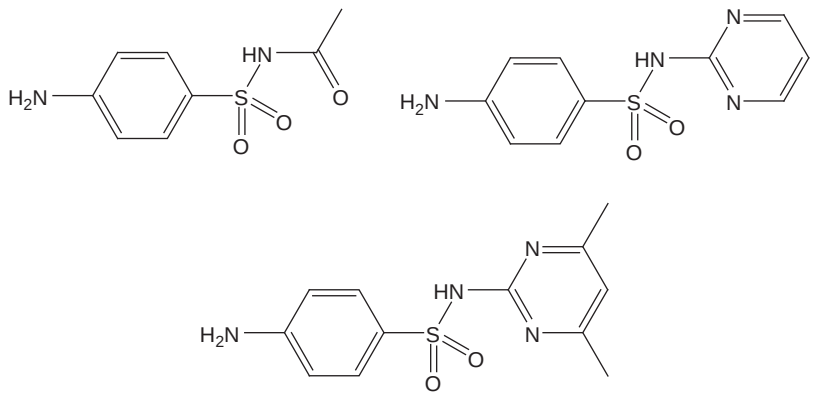


Figure 1.11 Structures of sulfacetamide (top left), sulfadiazine (top right) and sulfadimidine.

the observed deposition of crystals in the kidneys. This problem of crystals depositing in the kidneys was finally overcome by the introduction of sulfonamides that were more acidic and therefore more highly ionised in the urine. The ideal sulfonamides required a pKa in the range of 6.5–7.5 in order to balance the risk of kidney damage against rapid excretion. Two drugs, sulfadiazine and sulfadimidine (Figure 1.11), were in this range and both remain in use for the treatment of meningitis.[125]

Since sulfonamides could be easily synthesised from commercially available 4-aminobenzenesulfonylchloride, many companies were researching in this area resulting in the discovery of several classes of novel chemotherapeutic agents. Although their side effects caused many problems in the clinic, they were successfully exploited to provide oral antidiabetic drugs and valuable diuretics as highlighted below.[125]

1.4.3.2 Arylsulfonamides as Antileprotic Drugs

A further discovery related to new indications for sulfonamides was made when analogues of sulphanilamide were investigated for antibacterial properties. One of the analogues, 4,4′-diaminodiphenylsulfone (Dapsone), was found to be 30 times more potent than sulphanilamide against streptococci; however, it was

also significantly more toxic.[126] Therefore, several derivatives of Dapsone were synthesised and analysed, and the close analogue glucosulfone was found to be active against *Mycobacterium tuberculosis*. Since another mycobacterium, *M. leprae*, is responsible for leprosy, both compounds were tested and found to be the first effective treatments of the disease.[127] Dapsone was also found to be effective against leprosy and it is still in use as the standard antileprotic sulfone.[125]

1.4.3.3 Arylsulfonamides as Diuretics

One of the effects of some sulfonamides was a reduction in carbon dioxide binding power of the blood. This effect was discovered in 1940 and was associated with the inhibition of the enzyme carbonic anhydrase,[128] which reversibly converts carbon dioxide into carbonate. However, only those sulfonamides in which both hydrogen atoms on the sulfonamide function were unsubstituted were enzyme inhibitors. These included sulfanilamide and seven sulfonamides that lacked antibacterial activity. A derivative of these, acetazolamide, which was far more potent than sulfanilamide, was subsequently used as an orally active diuretic. Inhibition of carbonic anhydrase was also turned to advantage in the treatment of glaucoma. By acting in the eye in the same way as in the kidneys by reducing bicarbonate levels and the water secreted with it, the build-up of pressure from excess fluid was overcome. Acetazolamide still remains in use for this treatment today.[125]

A further potential use of sulfonamides was for the lowering of the concentrations of sodium and chloride in the body. It was believed that such a drug would have the added bonus of being useful as an antihypertensive agent, since clinicians in the 1950s were already beginning to believe that low salt diets were effective in treating high blood pressure.[125] The first carbonic anhydrase inhibitor that increased chloride excretion was 4-sulfonamidebenzoic acid, receiving the approved name carzenide. This scaffold led to the discoveries of a number of diuretics (Figure 1.12).

Chlorothiazide was one of the first of many thiazide diuretics. It effectively made mercurial diuretics obsolete for the treatment of cardiac oedema associated with congestive heart failure. Thiazide diuretics are still in use today for the treatment of hypertension,[125] and one of the analogues, bendrofluazide, which had a longer duration of action, remains widely used in patients with either mild heart failure or hypertension. The realisation that a second acidic group in dichlorphenamide may be replaced with a carboxyl group, as long as an appropriate substituent is present on the amino group, led to the introduction of frusemide in 1962. It had a quicker onset of activity, which was more intense and of shorter duration than that of other diuretics. Despite thiazides being indicated for most patients requiring a diuretic, frusemide is widely prescribed. Bumetanide is a more potent loop diuretic introduced ten years after frusemide.[129] Hoechst introduced the analogue piretanide when its patent on frusemide expired.[125]

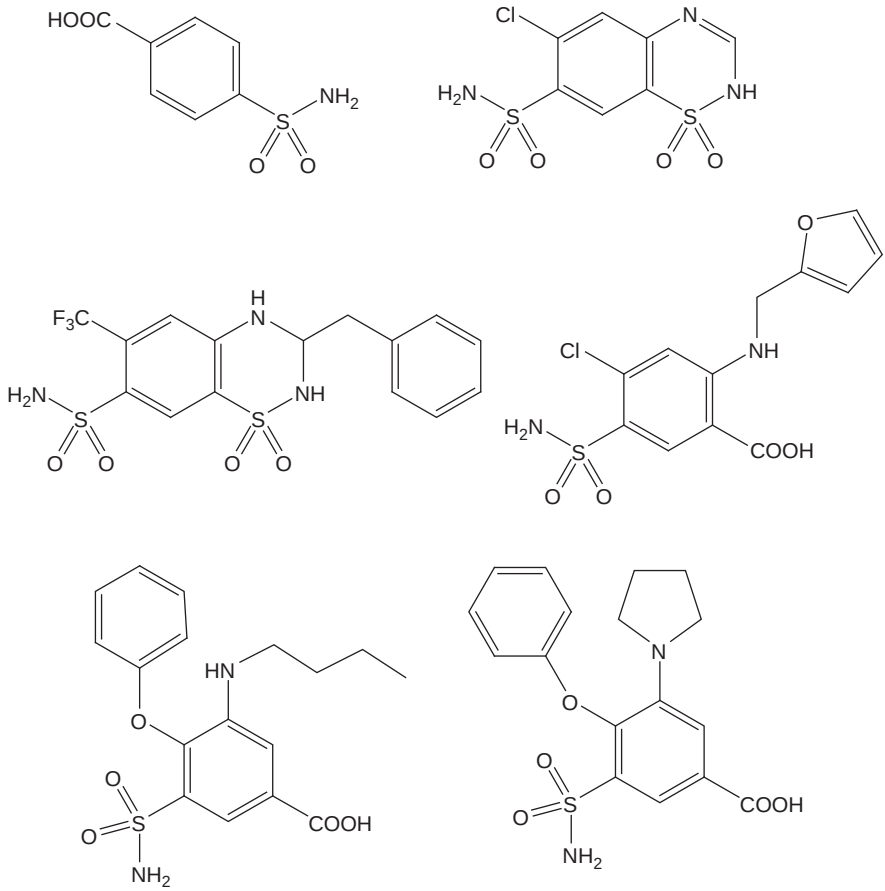


Figure 1.12 Structures of diuretics: carzenide (top left), chlorothiazide (top right), bendrofluazide (middle left), frusemide (middle right), bumetanide (bottom left) and piretanide (bottom right).

1.4.3.4 Arylsulfonamides and Arylsulfonylureas as Antidiabetics

In the early 1940s, a clinical trial with an experimental sulfonamide, 2254 RP, showed severe side effects which were linked to hypoglycaemia. This discovery was confirmed in the mid-1950s with another experimental sulfonamide developed by C. H. Boehringer Company, which also induced hypoglycaemia.[125] This compound was introduced as an oral hypoglycaemic agent under the name carbutamide. However, the compound showed unacceptable side effects in clinical trials in the United States, even though the drug was already being used in Europe. Meanwhile, Upjohn conducted a trial of Hoechst's closely related compound tolbutamide,[130] which then received approval from the FDA for the treatment of type 2 (non-insulin dependent) diabetes. Unlike carbutamide, it did not have any antibacterial properties and therefore eliminated the risk of inducing

resistant bacteria. However, it had to be taken three times a day due to rapid metabolism to the carboxylic acid. Soon after the introduction of tolbutamide, Pfizer marketed chlorpropamide which did not have the metabolically sensitive methyl group of tolbutamide and was about twice as potent.[131] Other long-acting sulfonylureas (structures are shown in Figure 1.13) have been marketed, including highly potent agents such as glibenclamide (glyburide) and glipizide.[125]

All the compounds discussed above for various indications all contain the common privileged substructure phenylsulfonamide. Although the reasons are not completely understood, it can therefore be concluded that this substructure appears to have physicochemical as well as binding properties which are favourable in pharmacologically active compounds. One reason could be that the sulfonamide group introduces polarity, which can place compounds in a favourable region of chemical space with regards to drug properties like for example for clearance. Therefore, despite the fact that phenylsulfonamide is a substructure which is common in drugs, it is by no means specific to a particular gene family. It can therefore be concluded that the reasons for its prevalence is more related to ease of synthesis and the often favourable contribution to the overall molecular properties such as polarity and hydrogen bonding potential; these are related to specificity of binding and therefore increased selectivity, which can so often determine the success or failure of a drug candidate. In contrast, in the case of carbonic anhydrase inhibitors, the sulfonamide acts as a zinc-binding group most likely in its deprotonated form, leading to a strong electrostatic interaction which is probably, in a large part, responsible for the good potency of the phenylsulfonamide substructure. It can only be speculated as to which role the sulfonamide is playing in the other indications mentioned above, since conclusive evidence as to its specific function in compounds acting as antibacterials, diuretics or antidiabetics is still lacking. The role of the sulfonamide group in ADME is reviewed extensively in Chapter 5.

1.4.4 Chemokine Receptor 5 (CCR5)

The GPCR gene family is one of the most important drug target classes. Therefore, an understanding of what features are contained in active GPCR ligands and drugs has been of significant interest to the pharmaceutical industry over recent decades. A recent analysis of over 17 000 GPCR ligands revealed well-known motifs and also new substructural features such as the imidazole-like substructure common for the histamine binding receptor ligands, as well as the indole-like substructure which is common for serotonin receptor ligands.[132] The chemokine receptor CCR5, a member of the family of GPCRs, is a target for anti-HIV therapy, which is being targeted by the recently launched antagonist maraviroc.[133]

All these CCR-5 antagonists have a common phenylpropylpiperidine substructure (Figure 1.14), effectively a basic centre with a lipophilic group linked by three carbon atoms. However, this substructure is also contained in a number of other GPCR inhibitors, and is therefore not indicative of CCR-5 but rather a large proportion of the GPCR gene family.

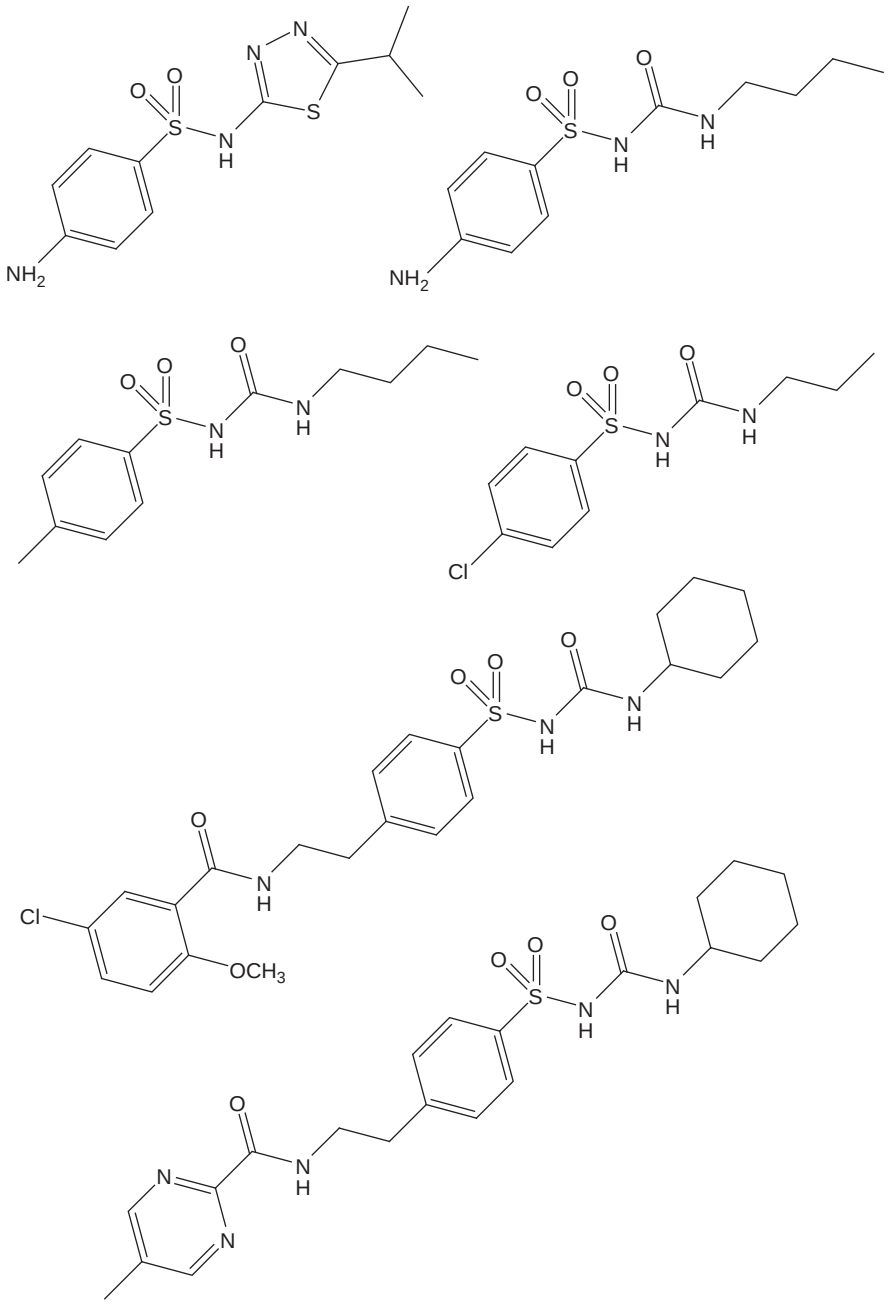


Figure 1.13 Structures of antidiabetic sulfonamides and sulfonylureas: 2254 RP (top left), carbutamide (top right), tolbutamide (second row left), chlorpropamide (second row right), glibenclamide (second from bottom) and glipizide (bottom).

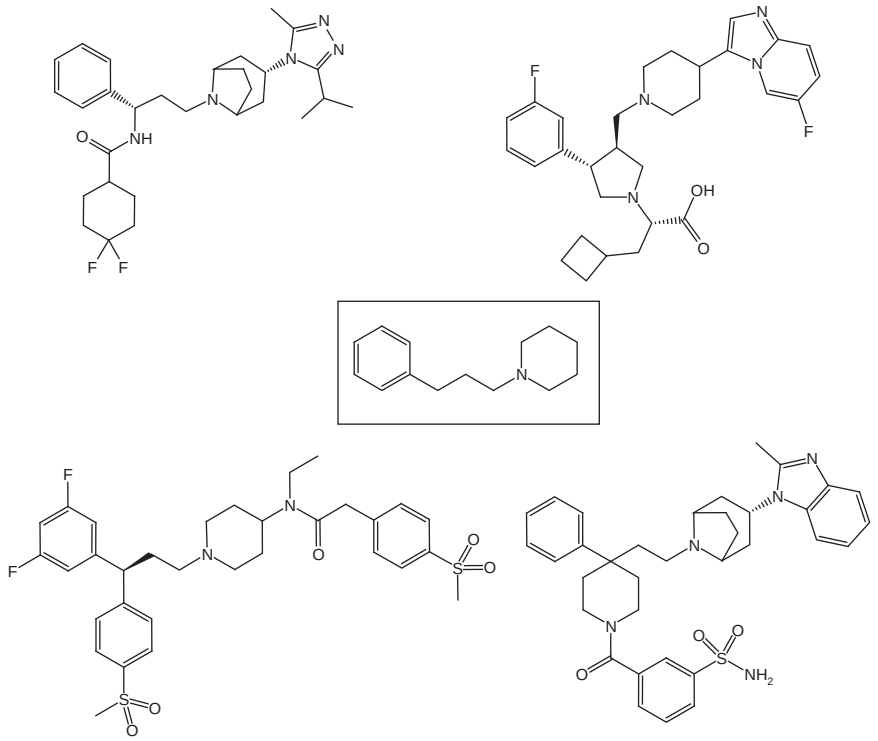


Figure 1.14 Representative structures of published CCR-5 antagonists from Pfizer (Maraviroc, Celsentri™, top left), Merck (top right), AstraZeneca (bottom left) and GlaxoSmithKline (bottom right).

Therefore, privileged substructures can be indicative of a preference of a receptor for a certain molecular structure but are not necessarily sufficient to describe the minimum pharmacophoric requirements for activity at a particular receptor. This is particularly the case for large gene families like GPCRs and also kinases (discussed in more detail below). Both recognise binding motifs which, although indicative of activity potentially against a whole gene family, will not enable differentiation between its members for achieving selectivity—particularly against closely related targets. Many GPCRs require a basic function which is normally a primary, secondary or tertiary amine and the ADME influences of these groupings are reviewed in Chapter 4.

1.4.5 Diaryl Heterocycles such as Cyclooxygenase Inhibitors (COX-2)

A five-membered heterocycle with a conserved vicinal 1,2-diphenyl substitution pattern has been observed in numerous medicinal chemistry programmes. Probably the most notable application of this group is among the second

generation non-steroidal anti-inflammatory (NSAID) agents that act via selective COX-2 inhibition such as celecoxib[134] and rofecoxib.[135] However this motif has been orthogonally optimised in other programmes to produce ligands for multiple targets including P38 MAP kinase,[136] adenosine A3 and phosphodiesterase-4 (PDE4).[98] Besides anti-inflammatory targets, diarylheterocycle derivatives have also been optimised as CB1 receptor agonists like Rimonabant[98] and dopamine transporter inhibitors.[137] By definition the broad spectrum application of this group in selective compounds for a range of target families renders this a privileged subunit. A group of representative structures of published diaryl heterocycle compounds is shown in Figure 1.15.

Although protein–ligand X-ray structures are known for a variety of these compounds, a structural interpretation of the privileged status of the common fragment motif is not clear.[98] Interestingly, the nature of the five-membered heterocycle is quite diverse and helps define different reactivity space. From a basic level, this 1,2-diphenyl heterocycle offers a rigid, chemically enabled

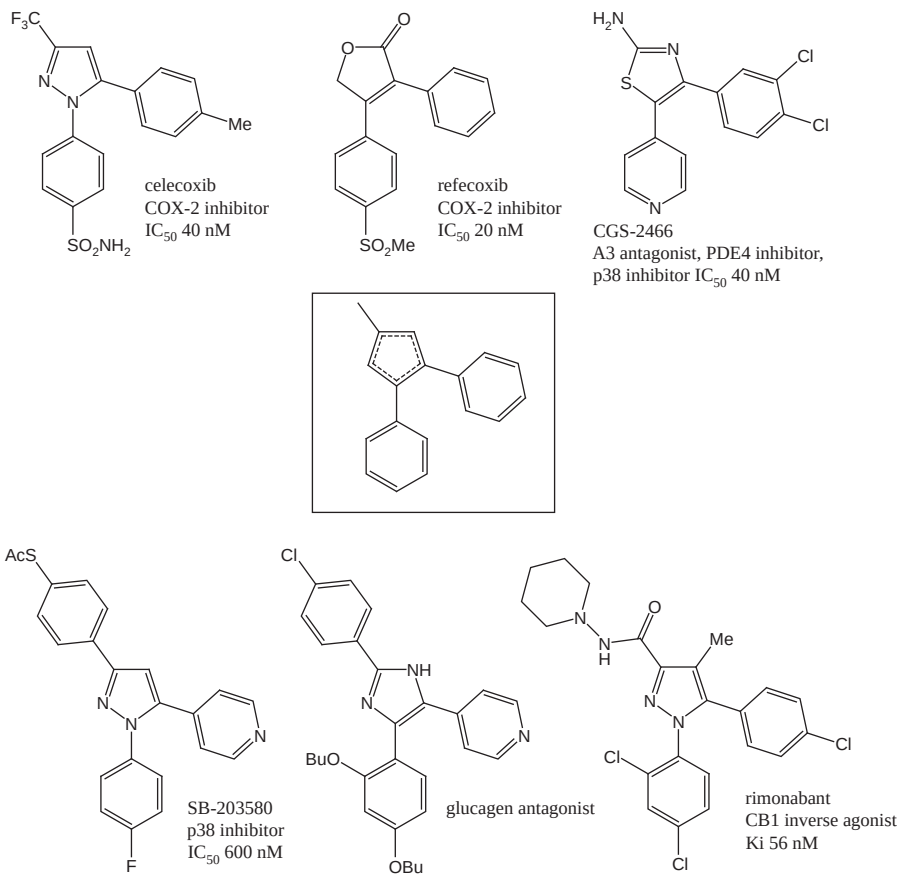


Figure 1.15 Representative structures of published diaryl heterocycle compounds.

template from which a phenyl ring can be positioned into pockets in an active site of a protein. As phenyl rings are rigid lipophilic groups that are able to occupy lipophilic sites or interact directly with other aromatic groups and side chains, it can be argued that it is not surprising that this simple motif is able to display activity for a range of proteins. Furthermore the option to add differential polarity and H-bonding capacity to both the core and periphery in a chemically enabled manner provides optimal opportunity to build in target selectivity. In accordance with this, multiple companies have opted to enrich their compound collections with this template for high throughput screening, thereby maximising probability of discovering new pharmacologies for this group and cementing its place among identified privileged substructures.

1.4.6 Aminoheterocycles as Kinases Inhibitors

The purine scaffold is a key component of DNA and an important recognition element in endogenous signalling molecules such as ATP and guanosine triphosphate GTP.[92] Consequently a large number of proteins have evolved to recognise the purine structure and related mimics. Such molecules have been identified as adenosine receptor ligands,[138] gamma-aminobutyric (GABA) receptor ligands,[139] kinase enzyme inhibitors,[109,140] antivirals such as Abacavir (anti HIV) and acyclovir (herpes treatment).[92] The structures of the last two are shown in Figure 1.16.

Building on this theme, kinases are an example of a drug target family that is host to a range of substructures which are known to be largely promiscuous within the target family.[100,109] The promiscuity for compounds within kinases with broad selectivity over other targets can be reasoned based on the high degree of similarity of kinase targets coupled with key structural knowledge of the key recognition interactions. The majority of work towards the design of kinase inhibitors has concentrated on inhibiting the ATP binding site. As the family of over 500 distinct proteins have all evolved to share ATP as a common natural ligand, it is unsurprising that there is broad conservation of size, shape

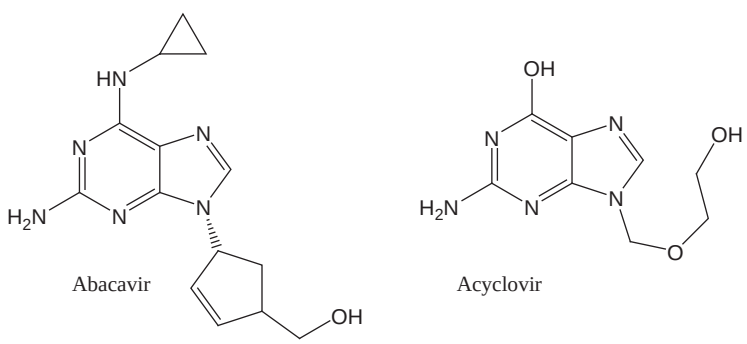


Figure 1.16 Representative structures of published purine-based antiviral compounds: Abacavir (left) and Acyclovir (right).

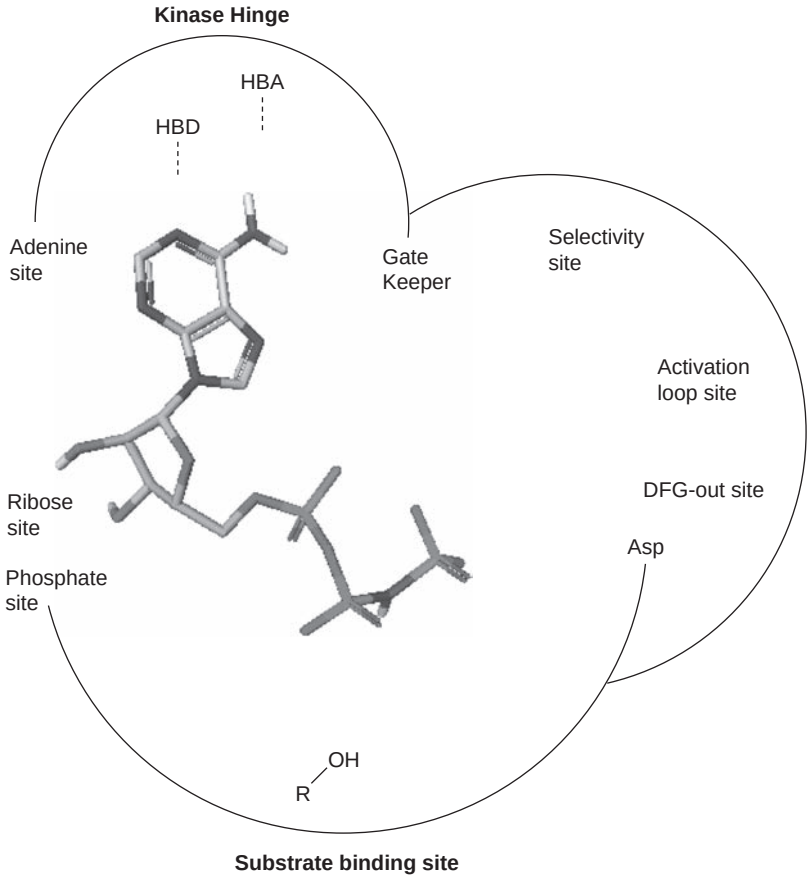


Figure 1.17 ATP purine recognition by kinases.

and residue selection within this site across kinases. Furthermore, the ATP site is well adapted to recognise the ATP purine ring head group via a series of hydrogen bonding interactions.[140] A schematic of ATP recognition by kinases is shown in Figure 1.17.

Aranov *et al*. from Vertex discussed in detail the concept of kinase privileged hinge-binding fragments and the notion of kinase targeted libraries whereby libraries of known promiscuous kinase inhibitor scaffolds were made to specifically target SAR in kinase drug space.[109] They investigated the idea of kinase likeness in the context of kinase privileged fragments. Initially they carried out an analogous method to that described by Murcko[97,102] to define the structures of kinase inhibitors in the context of their framework and side chain atoms. The analysis was performed on 119 published kinase inhibitors and revealed that the structural diversity at the level of rings and linkers was relatively low. A combination of four rings and eight linkers was found to describe 90% of the dataset.[109] In particular, amino-substituted

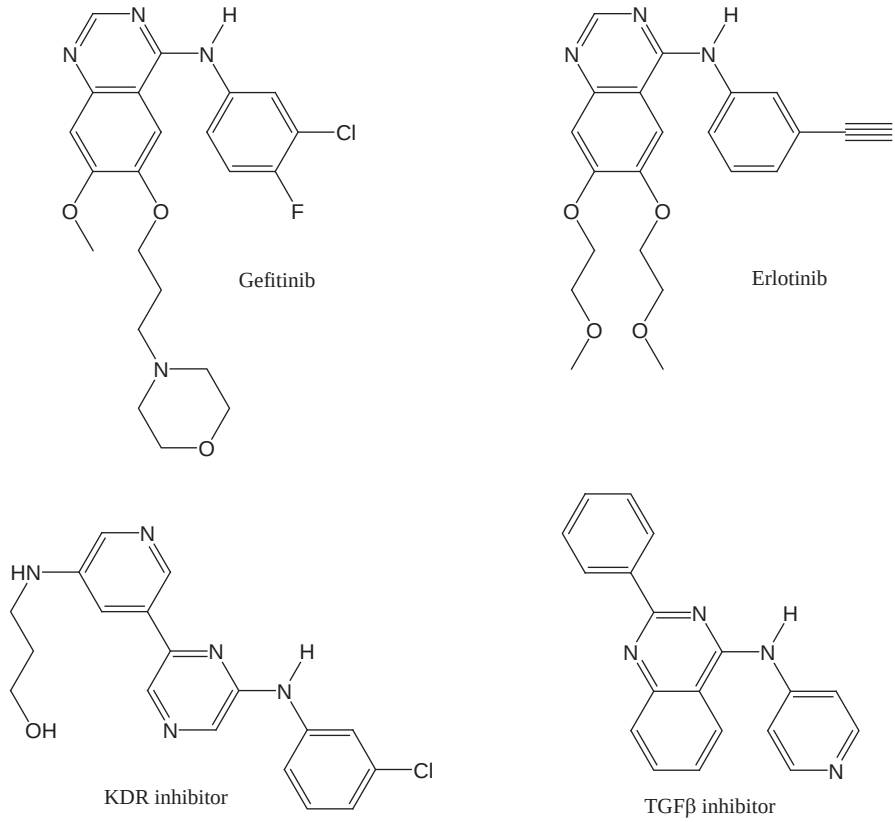


Figure 1.18 Representative structures of kinase inhibitor drugs.

heteroarylanilines have presented the majority of the kinase inhibitors undergoing clinical trials and most of the launched kinase drugs. Although the key hydrogen bond elements that are crucial for recognition of the ATP purine group are conserved for these bisarylamine groups, an analysis of X-ray structures has revealed that the closely related groups do in fact bind in different orientations and locations of the ATP site. Representative structures of kinase inhibitor drugs are shown in Figure 1.18.

Further to these observations, Aronov *et al*. proposed and internally validated a kinase-likeness rule termed the '2-0' rule. This rule stated that a compound is likely to have kinase activity if:

i) it contains two or more heteroaromatic nitrogens;
ii) it contains one or more heteroaromatic NH groups;
iii) it contains one or more anilines; and
iv) it contains one or more nitriles.

When tested against the Vertex file, this rule was observed to accurately describe between 80% and 100% of known kinase hinge-binding inhibitors. When used prospectively, the authors suggested that a five-fold enrichment in the discovery of new kinase inhibitors had been observed and that this was likely due to the fact that the rule of thumb ensured that the key hydrogen bonding recognition elements for hinge binding were present.[109]

1.4.7 HMG-CoA Reductase Inhibitors

Statins are a class of hypolipidemic drugs used to control hypercholesterolemia (elevated cholesterol levels) and to prevent cardiovascular disease. They act through inhibition of HMG-CoA. The world's best selling drug, Atorvastatin (Lipitor™), with worldwide sales of around $13.8 billion in 2008 (see Table 1.2), belongs to this drug class.

Two distinct classes of HMG-CoA inhibitors appear to have been marketed so far. One class, containing for example Atorvastatin, is made up of synthetic statins. The other class, containing for example Pravastatin, which was initially known as CS-514 and was originally identified in the bacterium *Nocardia autotrophica*[141], is made of natural products; another example is Mevastatin, which was obtained from *Penicillium citrinum*.[142,143] Pravastatin is also an active metabolite of mevastatin.[144] Both classes of compounds (illustrated in Figure 1.19) can be regarded as containing privileged substructures, such as for example the acid side chain in Cerivastatin, Rosuvastatin as well as Atorvastatin, and the lactone side chain as well as the bicyclic core in the other class of statins. However, this is debatable since the second class in particular is very similar in terms of molecular structure (differing effectively by no more than two atoms) and is based on natural products rather than having been synthesised on the basis of receptor SAR and observed preference for a particular substructure.

In addition, the two classes of statins are quite dissimilar in molecular structure. Therefore, it appears that there is no particular single privileged substructure responsible for activity against HMG-CoA in marketed statins, but that there are at least two distinct chemical structures that may express a very similar pharmacophore, which then accounts for the common activity against the target.

1.5 Privileged Substructures and Chemical Space

A growing number of substructures have been classed as privileged over the past two decades from a variety of analyses across industry and academia. It is evident that rigid aromatic and polyaromatic rings are common features of privileged structures. This makes sense when one considers the nature of the majority of targeted binding pockets. The majority of these have been

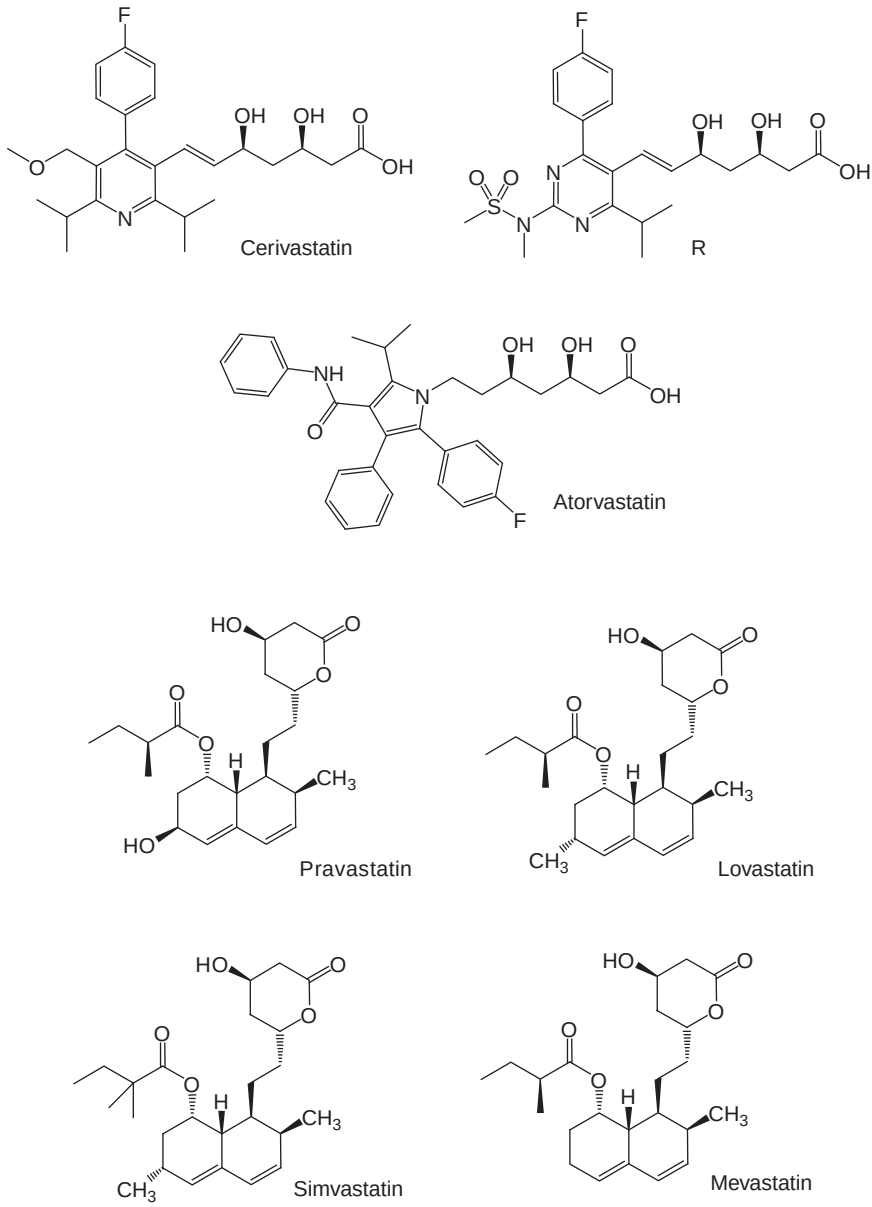


Figure 1.19 Cerivastatin (Baycol™), Rosuvastatin (Crestor™), Atorvastatin (Lipitor™), Pravastatin (Pravacol™), Lovastatin (Mevacor™) and Simvastatin (Zocor™) and Mevastatin.

hydrophobic pockets with π-stacking to phenylalanines and tyrosines being commonly observed; hydrophobic and aromatic interactions between ligands and protein targets play significant roles in the overall binding energy. Additionally the rigidity of many privileged groups enables presentation of the peripheral functionality in an ordered fashion, incurring minimal entropic penalty.[100,145]

It is worth considering why such groups have been discovered as privileged; are they truly privileged? In particular, it is interesting to note that many leads have been discovered from HTS strategies whereby a company's compound file is tested against a new target. The nature of the chemical matter in these collections is composed of a combination of substrate from previous drug programmes and file enrichment library enabled compounds. As a consequence, hit identification for a new programme potentially provides established groups a disproportionate opportunity to be rediscovered as hits over defining opportunities for discovery of series that describe entirely new chemical space. When this is considered in conjunction with the fact that templates that have a proven safety track record are often viewed as appealing leads, it seems hardly surprising that the same structural elements have been optimised for multiple drug programmes.[27]

A recent analysis of 1386 marketed drugs revealed that 15% are contained within other drugs and that 30% contain other drugs as substructure fragments.[145] A variety of recent papers have discussed these concepts of chemical and biological space in the context of drug discovery. In particular discussions consider future opportunities and methods to inspire new directions for drug discover chemistry to find additional chemical matter to enable targets that have been either unsuccessful or classed as undruggable due to the fact that current chemical matter is unsuitable. Opportunities such as using natural products as bio-active templates for further elaboration, broader fragment screening strategies and further library manipulation of currently existing templates via the development of more inventive library designs have all been considered.[58,145,146]

Although privileged substructures are a useful concept, there are aspects of this principle that are still ambiguous. It is not clear whether privileged substructures are a result of nature's preference for particular molecular entities, or whether it is the chemist's preference for particular synthetic routes, biased by synthetic feasibility and precedence. Either way, we suggest that it is a useful concept in the design of drugs which highlights the link between active compounds and their SAR. Also, chemists generally think in terms of two-dimensional Lewis structures, as used in this chapter, despite the fact that receptors don't recognise Lewis structures but rather electron densities around ligand atoms. However, it is much more practical and efficient to view molecules in the usual Lewis depiction rather than as for humans difficult to interpret surfaces. Therefore, the existence of the concept of privileged substructures could be more a result of how chemists are trained to recognise molecules rather than the actual recognition processes that happen when small molecules bind to a receptor.

1.6 Reasons for Compound Attrition

As pointed out earlier, only about 11% of compounds entering clinical development ever reach the market; more than one third are withdrawn for reasons like toxicology (24%) and clinical safety (12%), making toxicity-related factors one of the major contributors to compound attrition.[20] Smith and Schmid reviewed drug withdrawals over recent decades and found that, in the cases having the greatest impact, the reason for withdrawal was the interaction of a drug with a single receptor, ion channel or enzyme.[147] Once the mechanism has been identified, screens can be established; however, when the mechanism is more complex such as for example in organ toxicity, it is far more difficult to establish those screens.[147]

One of the most significant developments in compound attrition in the last ten years was the effect of QT prolongation on drug approvals. By 1998, QT prolongation emerged as a major safety issue affecting many classes of drugs. This was precipitated by the withdrawal of Serenading and Cisalpine from the US market because of sudden deaths associated with QT interval prolongation. The subsequent focus on QT led to the re-evaluation of many drugs on the market and in development, and is likely to have contributed to the lower NCE approval rates from 1998 onwards.[16] QT prolongation can be related to inhibition of the *human ether-a-go-go* related gene (hERG) potassium channel. The function of this channel is to conduct the rapidly activating delayed rectifier potassium current (IKr), which has a key role in the control of cardiac rhythm.[148] Examples of compounds removed from the market due to concerns with this issue include the antihistamine Terfenadine (withdrawn February 1998) and $5HT_4$ partial agonist Cisapride (withdrawn July 2000) (Figure 1.20). Both of these compounds are high affinity ligands for the hERG ion channel with IC_{50} of 56 nM and 6 nM, respectively.

Both compounds have structural similarities, for example the piperidine ring with a flexible hydrophobic substituent (phenylbutyl or phenyloxypropyl). However, the substructure is not specific to hERG activity, since a number of compounds with different substructures are also active. It appears to be more a case of having the relevant pharmacophoric elements, which have been

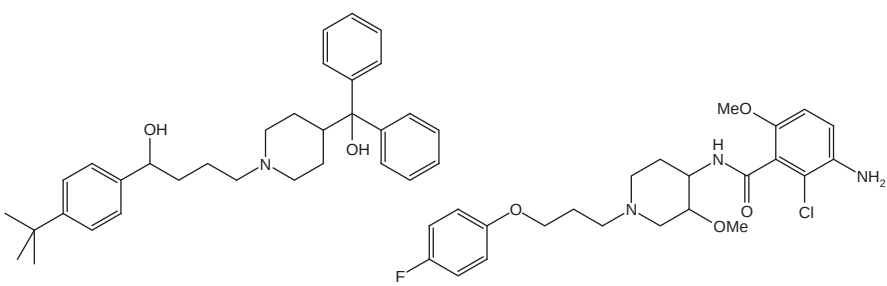


Figure 1.20 Terfenadine (left) and cisapride.

identified as an amine as a basis centre and at least two lipophilic groups at certain distances on either side of the amine.[149,150]

It is widely accepted that the withdrawal of compounds like Terfenadine and Cisapride has had a considerable impact on the pharmaceutical industry, particularly in terms of designing compounds that are less likely to have affinity for the hERG channel. There are successful examples of avoiding hERG assisted by molecular modelling, such as for example in the discovery of the anti-HIV drug Maraviroc.[133,149] More recently, evidence has been presented for a strong link of molecular properties like molecular weight and logP with the probability of polypharmacology or toxicity of compounds.[81,151] Therefore, it is not only the structure of compounds but also their physicochemical properties which can influence their toxicological profile, significantly adding to the complexity of compound attrition.

Attrition rates have also been analysed in terms of different disease areas and target class, finding that for example that the attrition rate of kinase inhibitors in oncology is only 53% compared with the overall attrition rate of anti-cancer drug candidates of 82%. This appears to indicate the benefits of developing molecularly targeted therapeutics for cancer.[152]

Seemingly minor differences can be responsible for the 'launched' or 'withdrawn' status for drugs, as in the example of Cerivastatin (Baycol™) and Rosuvastatin (Crestor™). Launched in 1997, Cerivastatin was voluntarily withdrawn from all markets worldwide by Bayer in 2001 following reports of side-effects of potentially fatal myopathy and rhabdomyolysis, in particular when the drug was co-administered with gemfibrozil. The structures of Cerivastatin and Rosuvastatin are shown in Figure 1.21.

At its peak, Baycol's global sales in 2000 exceeded $586 million. Launched by AstraZeneca in 2003, Rosuvastatin is now marketed in over 50 countries, with global sales over $2 billion in 2006. Both compounds are HMG-CoA reductase inhibitors and have been observed to have an antihypercholesterolemic and antihyperlipidemic effect by depleting cells of mevalonic acid, a cholesterol precursor. The event faced by Bayer in 2001 is similar to the global withdrawal

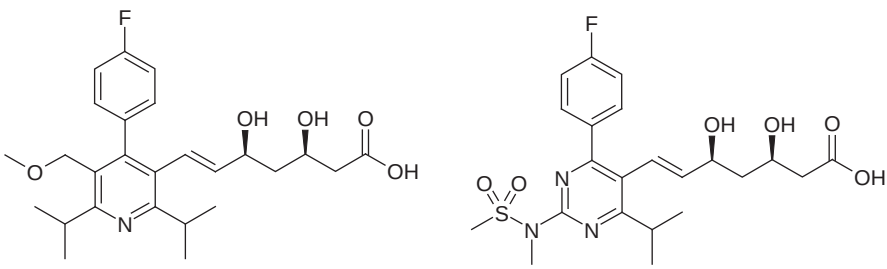


Figure 1.21 HMG-CoA reductase inhibitors on the market, withdrawn or discontinued in development: Cerivastatin (Baycol™, left) and Rosuvastatin (Crestor™, right).

of Vioxx™ (Rofecoxib) by Merck and Co. in 2004 (global sales over $2.5 billion in 2003).

Events like these have been categorised as a 'Black Swan'.[163] Coined by Nassim Nicholas Taleb,[153] the 'Black Swan' is an unpredictable event, which has a massive impact from a business perspective. The term 'Black Swan' is a metaphor for the first sighting of the black swan, which (a) invalidated the assumption that all swans are white—based on millions of previous observations; (b) changed our perception of those birds; and (c) was retrospectively 'assimilated' as a highly predictable event. Thus, the accumulation of past data cannot be used to predict the 'unknown unknown'. Considered by philosophers and military alike, three cognitive categories can be discussed: (a) the 'known knowns', for which sets and models are available, they have been validated externally and can be considered quite reliable; (b) the 'known unknowns', for which sets and models can be documented and proved, but otherwise lack external validation (*i.e.* true predictivity is of limited reliability); and finally, (c) the 'unknown unknowns', for which we do not have sets and models, thus lack any validation and predictivity.[163]

On the surface, the concept of the 'unknown unknown' does not appear to be applicable for, for example, Cerivastatin which has good affinity for a known target (HMG-CoA reductase), well-categorised pharmacokinetic profile (*e.g.* 60% orally bioavailable, 80% bound to albumin), and documented clinical effect (it lowers cholesterolemia). An 'unknown unknown' element was introduced by clinical practitioners who prescribed this lipid lowering drug in combination with an older antilipidemic, gemfibrozil (marketed since 1982 as Lipur™/Lopid™). Besides its serum lipid regulating effect, gemfibrozil also blocks CYP 2C8, the cytochrome P450 isozyme primarily responsible for metabolising Cerivastatin, leading to an unanticipated drug–drug interaction.[163]

A further example of the complexity of the reasons for attrition is the anti-inflammatory drug ibuprofen. After being launched in 1969, it became widely prescribed throughout the world in the wake of increasing concern about the hazard of gastric bleeding caused by aspirin. Such was its relative safety that in 1983 it became available in the UK as a non-prescription analgesic on account of its having the lowest overall rate of reporting of suspected adverse reactions among NSAIDs, some 20 million prescriptions having been issued over the preceding 15 years. However, ibuprofen was only selected to become a drug after its close analogue ibufenac, which only differs by a methyl group (Figure 1.22), had to be withdrawn soon after being launched

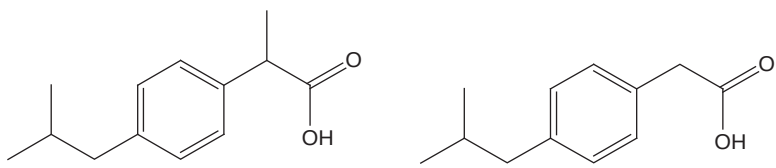


Figure 1.22 Ibuprofen (left) and its close analogue ibufenac.

in 1966.[31] A change as small as one methyl group can, therefore, make the difference between a safe drug and one which leads to unacceptable adverse reactions.

Interestingly the ibuprofen analogue, flurbiprofen, which is also marketed as an anti-inflammatory drug, also carries the motif of the alpha-substituted carboxylic acid, as do several other marketed analogues such as naproxen, ketoprofen and fenoprofen. However, a further analogue, benoxaprofen, which also has the alpha-methylcarboxylic acid motif, had to be withdrawn from the market after it was discovered that it had serious side effects in patients relating to renal or hepatic failure. However, the adverse reactions were attributed to a rather long half-life of the compound, particularly in elderly patients.[31] This highlights the complexity of the relationship between compound structure and adverse reactions, which make it very difficult to predict toxicity and side effects, even when the pharmacological or toxicological profiles of close analogues are known.[154] This may be in contrast to attempts to predicting adverse reactions from *in vitro* pharmacological profiles of structurally very similar compounds or close analogues containing identical substructures.[82,84,85]

Balancing early market access to new drugs with the need for the assessment of benefit and risk is an ongoing dilemma for the drug regulatory agencies. This dilemma is not new, but has been made more prominent by recent high-profile drug withdrawals and conflicting demands, including the need to improve the efficiency of drug development on one hand and the need to avoid exposing patients to unnecessary risks and possibly ineffective treatments on the other.[18]

Drug target and candidate selection are two of the key decision points within the drug discovery process for which all companies use certain selection criteria to make decisions on which targets to accept into their discovery pipelines and which compounds will pass into development.[154] These steps do not only help define the overall productivity of every company, but they are also decisions taken without full predictive knowledge of the risks that lie ahead or how best to manage them. In particular, the process of selecting new targets does not normally involve full evaluation of the risks in the mechanism under investigation, which may result in an inability to fully connect *in vitro* and animal model results to the disease setting. The resulting poor progression statistics of many compounds in the clinic is at least partially the result of a lack of understanding of disease pathophysiology. Notably, the lack of efficacy is still a major reason for failure in the clinic. Creating a more holistic understanding of disease pathophysiology and an early confidence in the mechanism under investigation could help facilitate the selection of not only the most appropriate targets but also the best mechanisms for disease intervention and how to select and optimise the best compounds.[154]

However, despite the continuing improvements in our understanding of the origins of diseases and how to treat them, the hurdles to get drugs to market may appear higher every year, perhaps accounting for the increasing rate in attrition and the drop in productivity. Bringing a drug to the market today may

well be far more difficult than for example 50 or 100 years ago. As Walter Sneader pointed out in his book *Drug Discovery, A History*:[125] '*It is fair to say that if an attempt were to be made today to introduce either paracetamol or aspirin into medicine, they might be denied a license'*.

The knowledge about the pharmacology of drug candidates (*e.g.* through broad-spectrum screening for example against a panel of common targets) may also identify relationships with compound attrition and aid selection of clinical candidates.[82,84,85,155,156] Perhaps new knowledge-based approaches (*e.g.* systems toxicology) will be able to identify attrition risks more effectively and reduce attrition rates by utilising toxicogenomics knowledge that combines molecular expression data sets from transcriptomics, proteomics, metabonomics and conventional toxicology with metabolic, toxicological pathway and gene regulatory network information relevant to human disease.[157,158]

1.7 Summary and Outlook

We hope that we have shown that drug discovery is an extremely difficult endeavour, which is becoming ever increasingly complex due to the changes in the regulatory requirements as well as the pressure on cost and productivity.

Despite these pressures, the pharmaceutical industry keeps discovering new drugs, albeit at what appears to be a slower rate. Whether the apparent reduction in productivity is a temporary phase or will be a continuing trend is not possible to determine at this time. However, it is almost inconceivable that without a significant change in drug discovery paradigm and increase in productivity (particularly drug output), the industry in its current state will continue to be as attractive to investors it appeared to be in the past. These changes in paradigm will need to come from the business end of the industry, which is of course research. The trend has been to suggest that the downward trajectory in productivity and diminishing returns on investment are purely a 'science problem'.[159] In addition, pharmaceutical research has been considered a knowledge-based and skill-based approach with research largely operating in 'chemogenomics knowledge space'.[160] Although scientific innovation is clearly part of the solution, a recent analysis suggested that stronger management attention to well-known value-creation levers such as for example cost, speed and decision making, could increase productivity and return on investment very significantly.[159]

Recently, an analysis of pharmaceutical industry performance metrics and current portfolios has been used to project the future productivity of the industry over the years 2007–2012 for the top 14 pharmaceutical companies.[161] The results indicate, for example, that the collection of branded drugs each of which is projected to achieve at least $500 million in annual sales, will only rise slightly (1.1%) over the five years. This is considered to be largely a consequence of loss of exclusivity for major products such as Pfizer's Lipitor (Atorvastatin), Wyeth's Effexor (Venlafaxine), Johnson & Johnson's

Risperdal (Risperidone) and Eli Lilly's Zyprexa (Olanzapine). However, biologics sales are projected to grow significantly in the region of 13% over the same time period. The companies with the largest percentage of biologics in 2007 were Amgen (96.7%, $13.6 billion), Roche (70.8%, $16.9 billion) and Wyeth (39.6%, $5.3 billion). The forecasted growth is driven by products such as Roche's Averting (Bevacizumab) and Abbott's Humira (Adalimumab), new products anticipated to launch during the forecast period such as Amgen's Denosumab and Johnson & Johnson's Golimumab as well as vaccines, in particular GlaxoSmithKline's Cervarix, Merck's Gardasil and Wyeth's Prevnar. In addition, there is a projected overall 4.4% reduction in internally developed products, with products from in-licensing and acquisition rising correspondingly. Companies with the highest proportion of internally developed product-derived revenue in 2007 include Novartis (93.4%), Eli Lilly (84.2%) and GlaxoSmithKline (80.3%), whereas those with the lowest proportion include Roche (15.1%), Schering-Plough (11.8%) and Sanofi-Aventis (8.4%).

Although research in itself does not create revenue, it is here where future value is created. Therefore, new thinking is perhaps required in research and a better understanding of fundamental principles such as the influence of the molecular properties of drugs on activity, ADME and toxicity. These principles also include those of the rule of five as well as the concept of druggability, ligand efficiency and privileged substructures.

We hope that we have highlighted the relevance and importance of privileged substructures in drug design and discovery, using a number of examples spanning a large area of pharmacological and target as well as chemical space. New approaches to discovery such as indications discovery could also contribute to maximising the value out of the chemical matter already available that has already passed essential compound safety and attrition hurdles.[162] However, as outlined above, future target space may well look very different from current target space due to the exploration of new members of the proteome discovered through sequencing of the human genome. The future of drug discovery and the expansion of target as well as compound space are the subject of the last chapter.

1.8 Abbreviations

ADMET	absorption, distribution, metabolism, excretion, toxicity
ADP	adenosine diphosphate
ATP	adenosine triphosphate
DMPK	drug metabolism and pharmacokinetics
CCK	cholecystokinin
CMC	Comprehensive Medicinal Chemistry
CNS	central nervous system
COX	cyclooxygenase inhibitor
EGFR	epidermal growth factor receptor

FDA	Food and Drug Administration
GABA	gamma-aminobutyric acid
GPCR	G-protein coupled receptor
GTP	guanosine triphosphate
HERG	*human ether-a-go-go* related gene
HIV	human immunodeficiency virus
HMG-CoA	3-hydroxy-3-methyl-glutaryl-coenzyme A
HTS	high throughput screening
MW	molecular weight
NCE	new chemical entity
NHR	nuclear hormonal receptor
NME	new molecular entity
NMR	nuclear magnetic resonance
NSAID	non-steroidal anti-inflammatory drug
PAF	platelet activation factor
PDB	Protein Data Bank
PDE	phosphodiesterase
Ro5	rule of five
R&D	research and development
SAR	structure–activity relationship
TPSA	topological polar surface area

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CHAPTER 2

ADMET for the Medicinal Chemist

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2.1 Introduction

In order to become an effective therapy against a disease, a compound must exhibit potency *versus* a particular therapeutic target, combined with a degree of selectivity over other targets; this drives to an appropriate safety margin. In addition, the vast majority of drugs are delivered at sites which are remote from the site of action. Over millions of years of human evolution, the body has developed a host of defence mechanisms designed to protect against exogenous substances that may cause harm. These mechanisms now form barriers to the passage of therapeutic drugs from their site of administration to their site of action. The extent to which a drug can avoid these barriers will to a great extent determine the therapeutic potential of that particular compound. Successful drugs need to strike a balance between three major determinants of therapeutic potential:

1) Potency against a pharmacological target to drive efficacy
2) Selectivity over the large number of potential pharmacological/toxicological targets to drive safety

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3) Absorption, distribution, metabolism and excretion (ADME) to drive to an acceptable dose and frequency of administration.

Consequently, the modulation of human ADME of a candidate drug series has become one of the major goals for a medicinal chemist. In order to achieve this goal, medicinal chemists need to understand the impact of structure/physicochemistry on ADME properties and how the human body deals with chemicals. Furthering this understanding within the medicinal chemistry community is the aim of this chapter.

2.1.1 Physicochemical Principles for ADME

The ultimate ownership of the physicochemical properties of candidate drugs lies with the medicinal chemist. Since many of the physicochemical properties that drive ADME properties are calculable using *in silico* methods, the medicinal chemist should understand the properties of the molecules they design before they are synthesised. However, this simplistic view conveniently sidesteps the issue that the physicochemical requirements for potency *versus* a pharmacological target are often at odds with those required for optimal pharmacokinetics and a balance must be struck. To understand the trade-offs required to achieve this balance, it is first appropriate to consider the physicochemical requirements for pharmacological potency.

In crude terms, the pharmacological potency of a compound is determined by the free energy generated on binding of the molecule at the active site. The relationship between free energy of binding and Kd *versus* the target is represented by eqn (2.1):

$$\Delta G = -RT \ln Kd \tag{2.1}$$

In general, pharmacological potency is achieved by specific interactions (these tend to be polar in nature) and non-specific lipophilic interactions of the compound within the active site. Lipophilic groups add binding energy at rate of $0.7\,kcal^{-1}\,mol^{-1}$ for every methyl equivalent. Effectively, this suggests that the levels of desired pharmacological potency can be achieved by adding carbon to an active scaffold. If this approach is widely adopted, compounds become large and lipophilic as well as highly potent. Thus, compound series optimised solely for potency will tend to high molecular weight and lipophilicity, a situation that led to significant compound attrition due to poor pharmacokinetics across the industry prior to the introduction of drug metabolism into drug discovery.

As previously stated, the human body comprises a series of barriers to drug movement between the sites of administration and action. These barriers have been described previously[1] and consist of aqueous and lipophilic environments, as well as a large number of active processes (*e.g.* enzymic metabolism and transporter-mediated excretion). To perform well in the body, a compound must be able to exist in both aqueous and lipophilic environments. The

physicochemistry of a compound will, to a large extent, define how successful it will be in overcoming these biological barriers.

The most important physicochemical properties from an ADME perspective are lipophilicity, molecular weight and polar surface area. Lipophilicity is measured as the partitioning between an aqueous and an organic (lipophilic) environment. LogD is the partitioning between octanol and buffer at a particular pH to take into account ionisation—hence log $D_{7.4}$. Lipophilicity mimics the passage from aqueous solution through a membrane. When the lipophilicity of a compound is too low, membrane permeation is less likely. When lipophilicity is too high, aqueous solubility is compromised and, for example, oral absorption is unlikely. Molecular weight is important as larger molecules are likely to have greater difficulty permeating membranes due to steric interactions with triglycerides making up a lipid bilayer. Finally, polar surface area (PSA), an indicator of hydrogen bonding capacity, also plays a role since it will define the number of associated water molecules in aqueous solution. For passage across a membrane, these water molecules need to be removed—a process that requires energy. Thus, the more hydrogen bonding potential a molecule has, the more energy will be required to remove associated water molecules and the less likely it will be to cross a membrane.

The importance of these parameters for oral absorption was recognised over a decade ago.[2] From an analysis of approximately 50 000 compounds, Lipinski *et al*. proposed that poor oral absorption was most likely for a compound when:

- log P was greater than five;
- molecular weight was greater than 500;
- there were more than five hydrogen bond donors; and
- the sum of nitrogen and oxygen atoms was greater than 10.

This is the 'rule of five'.

The important physicochemical parameters of lipophilicity, molecular weight and polar surface area are, in fact, highly interrelated. Indeed, lipophilicity can be viewed as a combination of the molecular weight (how many carbons in the molecule) with polar surface area (how many heteroatoms). The Lipinski rules are explainable by the fact that, as molecular weight approaches 500, the probability increases that either lipophilicity or the polar surface area of a molecule will be too high, driving to poor aqueous solubility or membrane permeation respectively.

Other ADME processes are also highly associated with these physicochemical properties. For example, the clearance of a molecule is defined by a combination of structure and physicochemistry. Passive renal clearance is only highly apparent when log $D_{7.4}$ is below 0, whereas many metabolising enzymes are membrane-bound and show a preference for lipophilic compounds. Plasma protein binding (particularly to human serum albumen) is to a large extent determined by lipophilicity. Brain penetration requires passage across a membranous barrier that is a more significant hurdle than the gut wall

membrane and is driven by the lipophilicity, molecular weight and polar surface area of a molecule.

We have established that the ADME trinity of lipophilicity, polar surface area and molecular weight are interrelated and define the ADME characteristics of molecules. Smith and coworkers[3,4] put forward the concept of ADME space defined by the three axes of lipophilicity, molecular weight and polar surface area. The challenge for the medicinal chemist is always to balance ADME with pharmacological potency, a balance that is largely determined by the physicochemical properties required to achieve binding to the active site of the target.

The aminergic G-protein coupled receptors (GPCRs) are exemplified by natural ligands (*e.g.* adrenaline and dopamine) that exhibit physicochemical properties well within ADME space.[3] Drugs emerging from these particular targets (*e.g.* beta blockers and beta agonists) have tended to share the physicochemical properties of the leads, driven by the size and shape of the active site to which the modulators (and ligands) need to bind. Thus, drugs against the aminergic GPCRs tend to fall well within ADME space, meaning that potency and ADME properties can be rationalised in single molecules with drug-like potential. In contrast, peptidic GPCR ligands bind to extracellular sites of their target and tend to require high molecular weight to achieve adequate target binding. Thus, ligands for these particular targets are not ideally placed to promote drug-like ADME properties.

Targets that lie outside of ADME space cannot be ignored on the basis that drugs with an appropriate ADME profile cannot be delivered. When working under these circumstances, a degree of compromise needs to be considered. This is exemplified by the HIV protease compounds.[5] This topic is be explored in Chapters 10 and 11.

Another potential method of making a compound series more physicochemically tractable from an ADME perspective is the 'prodrug' approach. This has been extensively reviewed.[4,6] A prodrug is used to mitigate a physicochemical liability of a candidate through the addition of a pro-moiety that incorporates the desired physicochemical profile to address the ADME issue but can be removed once inside the body.

The most successful examples of prodrugs tend to be in the addition of polar functionality to improve aqueous solubility (and thus oral absorption) and the addition of lipophilicity to improve membrane permeation (and hence oral absorption). These approaches are not without their difficulties. In general, they can only be used successfully when the active drug is close to defined ADME space. For example, the addition of a pro-moiety to improve membrane permeation will have only limited impact on the degree of hydrogen bonding[4] (and hence polar surface area) and will derive most benefit from the addition of lipophilicity to the active moiety. The improvement in oral absorption will then depend upon the balance of lipophilicity and aqueous solubility as well as the extent of release of active principle from the prodrug once absorbed. The further away from ADME space the active principle is, the more lipophilicity will need to be added and the more likely the prodrug

strategy will fail. Therefore, the prodrug strategy is not an easy win for the medicinal chemist confronted with an ADME space issue.

It is clear that the pharmaceutical industry cannot ignore the challenge of targets that require physicochemistry outside of defined ADME space. However, the medicinal chemistry challenge of approaches to mine drugs out of these targets is an appreciable one. To enable these targets to yield acceptable drugs, a degree of compromise on the quality of the pharmacokinetic profile will need to be accepted. When working in these areas, the risks of slipping into unacceptable ADME properties will be significant and predictability will be lower. This suggests that larger numbers of compounds will need to be taken to the clinic at risk to achieve the goal.

From a medicinal chemistry perspective, this does not signal a return to the days of optimising chemical matter solely on the basis of potency. In recent years the terms of 'ligand efficiency'[7] and 'lipid efficiency'[8] have entered the literature. These calculations allow medicinal chemists to pose questions about whether they are using molecular weight and lipophilicity to the greatest advantage to improve potency. If used appropriately, we can be sure that each atom and each degree of lipophilicity is being used optimally in compound design.

2.1.2 Physicochemistry Summary

The therapeutic potential of a drug is defined in large part by its potency, selectivity over other targets and ADME properties. Potency and ADME properties are strongly defined by structure and physicochemistry. The extent to which a compound overcomes the barriers to drug delivery in the body will be defined by lipophilicity, molecular weight and polar surface area. The series is considered chemically tractable when the physicochemistry required for potency is within the ADME space defined by lipophilicity, molecular weight and polar surface area. However, many new targets require physicochemistry that is outside of ADME space, requiring alternative approaches.

2.2 Delivery of Drugs and Bioavailability

Typically, drugs are delivered at sites remote from the site of action. The major drug delivery routes are exemplified in Table 2.1.

The movement of a drug away from the site of administration is termed the absorption of that drug and the extent to which a drug overcomes the barriers to its passage to its site of action is termed the bioavailability. The distinction between the two terms is important as a drug can be completely absorbed but exhibit no oral bioavailability due to post-absorption metabolism.

In our description of the pharmacokinetics of a drug, the site of action is assumed to be the blood, which is also the site of measurement. For the intravenous route, all the dose is delivered directly into the blood. Consequently, the

Table 2.1 Overview of the potential routes of administration of drugs and the barriers to those routes.

Route	*Barriers*	*Comment*
Intranasal	Mucocilliary clearance	Limited to low doses (solubility)
Buccal	Swallowing	Limited to low doses (solubility)
Inhalation	Deposition in mouth and throat	Limited to low doses, patient needs device training
Dermal	Skin poorly permeable	Limited to low doses, accidental removal of dose by washing *etc*.
Intravenous	None	Limited to in-clinic delivery
Oral	Dissolution, gut permeability, first pass metabolism in gut and liver	Can deliver range of doses conveniently
Subcutaneous and intramuscular	Blood flow to site and irritation of tissue	Low doses

bioavailability of an intravenous dose is always 100% and all other dose routes must be measured relative to an intravenous dose.

Intravenous administration gives complete bioavailability and the potential to rapidly achieve therapeutic concentrations. A good example of a drug that is given by intravenous infusion is lidocaine, which is used to treat severe neuropathic pain in the clinical setting.[9]

There are several important considerations for a drug to be administered by the intravenous route. First, it must be sufficiently soluble in the proposed formulation (mainly saline with limited potential for organic co-solvents) to deliver the whole dose in an acceptable volume. Secondly, the compound must have prolonged chemical stability in the formulation, especially as the dose must be taken through sterilisation procedures. Overall, the requirement for venous cannulation means that intravenous administration tends to be limited to severe and life-threatening indications, and usually under qualified medical supervision.

2.2.1 Oral Delivery

Most drugs are formulated for delivery by the oral route. For the patient this route is the most convenient method of drug delivery. However, from a pharmacokinetic point of view, it represents a significant challenge. There are a number of barriers via the oral route that can limit oral bioavailability of a drug (Figure 2.1).

Modulation of oral bioavailability is a key parameter for many drug discovery programmes. High oral bioavailability is often required to limit interpatient variability and maintain an acceptable dose size.

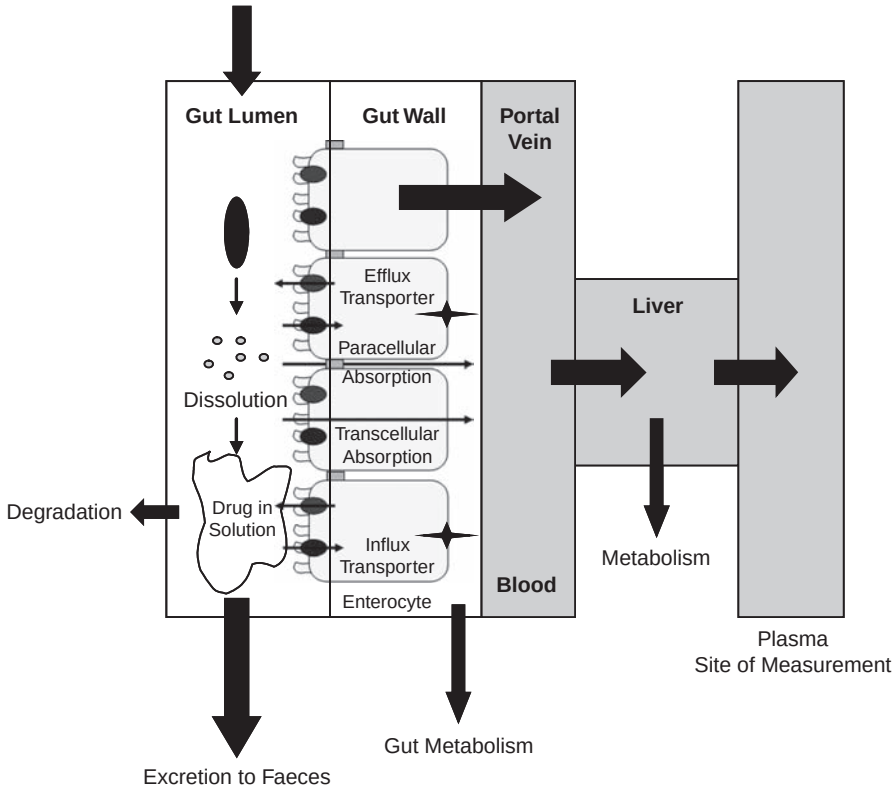

Figure 2.1 Summary of the barriers to drug delivery following oral administration.

The human gastrointestinal tract has evolved to be the organ of food digestion and nutrient absorption. In addition, it has in place a number of mechanisms for preventing the entry of unwanted molecules (*e.g.* toxins). These mechanisms often limit the oral bioavailability of drugs.

The physiology of the human gastrointestinal tract consists of three main parts: the stomach, small intestine and large intestine. These are considered in turn below.

The first organ that a drug will reach following oral administration is the stomach. The major function of the stomach is the preparation of food for further digestion. Gastric pH is acidic, ranging from 2 to 6, depending on the presence of food.

The small intestine is the major absorbing organ. It is divided into three parts: duodenum, jejunum and ileum. These regions display differences in their absorptive and secretory capabilities. The duodenum is responsible for neutralising the gastric acid to near physiological pH in preparation for absorption across the gut wall.

The major adaptation of the small intestine is the membrane surface area available for oral absorption. There is extensive inward folding on the luminal

surface of the small intestine. On the surface of these folds are finger-like projections called villi containing absorptive epithelial cells. These cells are characterised by small projections on their luminal surface called microvilli, which form the brush border membrane. The combination of luminal folds, villi and microvilli increases the membrane surface area for absorption by 600-fold over that of the internal surface of a cylindrical surface alone.

The large intestine comprises three sections: cecum, colon and rectum. The major role for the large intestine is reabsorption of water involved in digestion. It is not a major absorptive organ as fluid for dissolution is limited and the surface area for absorption is far less than for the small intestine.

To design compounds with acceptable oral bioavailability, it is important to understand a drug's journey from its site of administration into the systemic circulation. There are a number of steps in this process.

2.2.1.1 Dissolution

To be absorbed a drug must be in solution in the gastrointestinal tract lumen. The fluids in the lumen are predominantly aqueous in nature with a pH ranging from acidic to physiological. Consequently, an important parameter for the medicinal chemist to modulate within a chemical series is the aqueous solubility.

Oral drugs tend to be administered in tablet or capsule formulation. The formulation must first disintegrate before the active drug undergoes a dissolution process. These processes are governed in part by excipients in the formulation and in part by the physical form and aqueous solubility of the formulated drug.

Food may affect the absorption of drugs through the dissolution process. The delay in gastric emptying with food after a meal may result in a longer time to achieve maximal plasma concentrations, since the drug is held in the stomach for longer than in the fasted state. The release of bile salts on ingestion of food may result in enhanced dissolution of poorly soluble drugs. In addition, certain compounds may form complexes with food resulting in a reduction in their absorption.[10]

2.2.1.2 Permeation Across the Gastrointestinal Tract Epithelium

Once in aqueous solution, a drug must cross the gastrointestinal tract epithelium to be absorbed. The gut wall epithelial cell is called the enterocyte. The drug can cross the enterocyte layer by passive diffusion through the tight junctions between the enterocytes (paracellular absorption) or by crossing the enterocyte membrane (transcellular absorption). The route for passive absorption of a drug depends upon the physicochemistry of the drug.

Paracellular absorption involves the passage of the drug through the aqueous filled channels between the epithelial cells. Due to this size constraint, drugs that are predominantly absorbed by the paracellular route are generally of low

molecular weight (<300) and relatively polar (log $D_{7.4}$<0). An example of such a drug is the beta blocker, atenolol. Paracellular absorption is limited to the small intestine as, in the large intestine, aqueous pores are fewer in number and smaller. In addition, there is an important species difference in paracellular absorption with pore size being comparable in human and rodent, but significantly larger in the dog.[11] For this reason, the oral bioavailability of atenolol is limited to approximately 50% in rat and human, but is complete in dog.[12,13]

The majority of oral drugs cross the enterocyte by the transcellular route. As outlined previously, the physicochemistry of the drug will determine the extent of transcellular absorption. Lipophilicity, molecular size and hydrogen bonding potential will in large part determine the ability of a drug to cross the gut wall epithelial cell membrane (see above).

However, the vision of the enterocyte membrane as a simple lipid bilayer is overly simplistic. There are a number of proteins expressed within the membrane that have the potential to either facilitate (active absorption) or hinder (active efflux) the passage of drug molecules across the membrane.

2.2.1.3 Active Absorption of Drugs

Given that the major role of the gastrointestinal tract is the digestion of food and the absorption of nutrients, proteins have evolved to facilitate the active uptake of nutrients with poor membrane permeation characteristics (*e.g.* di- and tri-peptides). A small number of drugs take advantage of these uptake transporters to facilitate their absorption into the body.

For example, the human peptide transporter 1 (hPEPT1) is a low affinity, high capacity system expressed on the apical brush border membrane of enterocytes.[14,15] hPEPT1 has been shown to mediate the transport of di- and tri-peptides into the systemic circulation from the gut lumen utilising a H^+ gradient dependent transport system. This influx transporter has been implicated in the absorption of a diverse range of drugs including β-lactam antibiotics such as cefadroxil (Figure 2.2).[16]

Targeting intestinal transporters by means of prodrugs has been a successful strategy for improving oral absorption. Uptake via hPEPT1 is thought to be the primary mechanism for valacyclovir absorption (Figure 2.3).[17] Once absorbed, valacyclovir is hydrolysed to its active form acyclovir. Consequently,

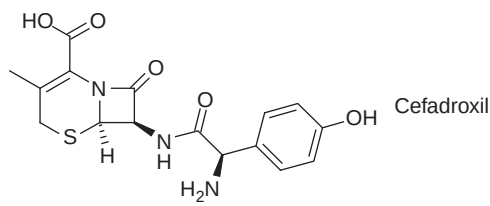

Figure 2.2 Structure of cefadroxil.

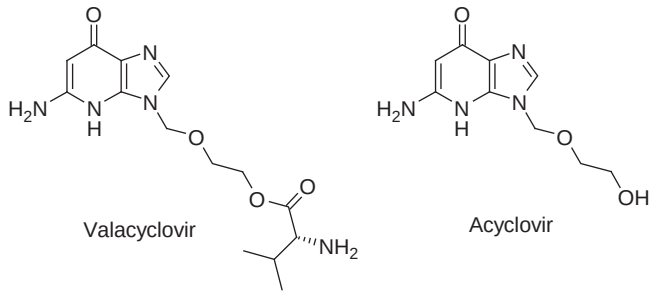

Figure 2.3 Structure of valacyclovir.

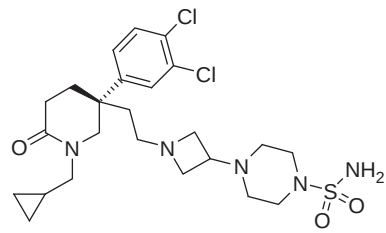

Figure 2.4 Structure of UK-224 671.

valacyclovir pharmacokinetics are characterised by non-linear absorption as a result of the saturation of this influx process at higher doses.[18]

2.2.1.4 Active Efflux of Drugs

In addition to uptake transporters, a number of efflux transporters are expressed at the gut lumen facing (apical) membrane of the enterocyte to limit the passage of molecules that could be potentially damaging to the body. They can act as a significant barrier to the absorption of drugs.

The most well-studied drug efflux transporter is P-glycoprotein (P-gp). P-gp is an ATP-binding cassette transporter highly expressed in many human tissues including the enterocyte.[19] It intercepts compounds as they pass through the enterocyte membrane and effluxes them back into the gut lumen.[20]

The functional activity of P-gp is saturable. P-gp substrates that are poorly membrane permeable and that are administered at non-saturating doses will demonstrate limited oral absorption or non-linear absorption.

There are a number of examples where P-gp has been shown to affect the oral absorption of its substrates. UK-224 671 is a potent selective NK_2 receptor antagonist (Figure 2.4) that has been shown to be a substrate for P-gp.[21,22] In P-gp knockout mice, the oral bioavailability of UK-224 671 was 22%, whereas in the wild-type mice expressing P-gp, the value was less than 5%. The low oral bioavailability translated to humans where UK-224 671 was shown to be

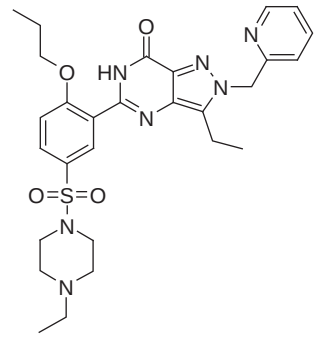

Figure 2.5 Structure of UK-343 664.

approximately 10% absorbed, presumably as a consequence of limited membrane permeation and P-gp mediated efflux.

UK-343 664 (Figure 2.5) is a potent and selective phosphodiesterase 5 (PDE5) inhibitor.[23] Over an 80-fold oral dose range (10–800 mg), it displayed a 1300-fold increase in Cmax and a 1900-fold increase in AUC_t for UK-343 664. This was accompanied by a reduction in the Tmax from 3.5 to 0.6 h. This non-linear profile was ascribed to saturation of P-gp in the gut as the doses were increased. At the lower doses, gut lumen concentrations were below the Km for P-gp and consequently it was possible to delay the rate and impact of the extent of absorption of UK-343 664. As the dose increased, the gut lumen concentrations increased significantly above the P-gp Km and this saturation led to an increase in the rate and extent of absorption of the compound.

It is possible for co-administered P-gp substrates to promote a P-gp drug–drug interaction by inhibition of this efflux transporter. For example, the oral bioavailability of digoxin can be enhanced by co-administration of talinolol as a result of P-gp inhibition.[24]

2.2.1.5 First-Pass Metabolism

Once a drug has crossed the enterocyte membrane, it has been absorbed into the body. However, it has not yet reached its site of action and two significant further metabolic barriers need to be overcome, both of which can contribute to reduction in the overall bioavailability of the drug.

(a) Gut Wall Metabolism. The small intestine contains enzymes that can metabolise drugs through both phase 1 (oxidative) and 2 (conjugation) reactions.[25,26] The involvement of the gut wall in the metabolism of drugs has been extensively reviewed.[27,28]

A range of P450 enzymes have been identified in the human small intestine including CYP3A4, 3A5, 2C9, 2C19, 2J2 and 2D6. CYP3A is the most highly expressed CYP accounting for around 80% of the total CYP content.[29] Even

though the total mass of CYP3A in the intestine is only 1% of that in the liver, clinical studies have demonstrated that intestinal metabolism can have a significant effect on the overall first-pass metabolism of some drugs.[30,31]

The oral bioavailability of midazolam in humans has been estimated at approximately 30%.[32] This is significantly lower than would be expected from the plasma clearance of midazolam (approximately 5 ml min^{-1} kg^{-1}) with respect to human hepatic blood flow, knowing that midazolam is completely absorbed. When midazolam was administered to patients undergoing hepatic transplantation,[33] the gut wall first-pass extraction following intraduodenal administration was calculated to be 0.43 (range 0.14 to 0.59). Thus, the oral bioavailability of midazolam is a consequence of complete oral absorption and loss of approximately 40% of the drug on first-pass through the gut wall, with the remainder lost by hepatic first-pass extraction.

The gut wall first-pass extraction of drug molecules is thought to be enhanced by the expression of CYP3A4 and P-gp together close to the apical membrane of the enterocyte. It is thought that these two proteins can act as a concerted barrier to the passage of their substrates across the enterocyte. The P-gp intercepts its substrates on passage through the enterocyte and effluxes them back into the gut lumen. Thus, the drug is cycled in and out of the enterocyte, allowing CYP3A4 a number of opportunities for metabolism. In this way, it is possible for a relatively low expression of enzyme to exert a significant gut wall first-pass extraction.

(b) Hepatic. The entire blood supply of the upper gastrointestinal tract passes into the hepatic portal vein which flows directly to the liver. Consequently, drug that has crossed the enterocyte and avoided gut wall metabolism is also subject to first-pass extraction by the liver. Extraction of drugs by the liver is discussed in detail later.

2.2.1.6 Oral Bioavailability

Oral bioavailability is the amount of drug that reaches the systemic circulation following oral administration relative to the same intravenous dose of the same compound. The oral bioavailability (F) of a drug is determined by the fraction of the dose absorbed (Fa), the fraction escaping gut wall first-pass extraction (Fg) and the fraction escaping hepatic first-pass extraction (Fh) as shown in eqn (2.2):

$$F = Fa \times Fg \times Fh \tag{2.2}$$

Given that the physicochemical factors determining absorption and first-pass extraction are often in opposition, the oral route provides significant challenges to the medicinal chemist in attaining the goal of high oral bioavailability.

When it proves too difficult to balance the physicochemistry for potency with that required for extensive oral bioavailability, it is possible to consider alternative delivery routes.

2.2.2 Intranasal Delivery

The nasal route has become a popular alternative to the oral route because it provides an easy method of administration. The nasal cavity presents the main barrier to drug absorption; low permeability of the nasal epithelium, rapid mucociliary clearance and enzymatic degradation in the mucus layer are limiting factors. The nasal cavity is easily accessible and is extensively vascularised.[34] Compounds that are administered by this route avoid hepatic first-pass metabolism and so absorption will determine bioavailability.

Several peptides and proteins are given as nasal sprays.[35] These tend to be inactive when given orally, as they are quickly destroyed in the gastrointestinal tract, but enough is absorbed from the nasal mucosa to provide a suitable therapeutic effect.

Nasal administration is primarily suitable for highly potent, low dose and aqueous soluble drugs since only a limited volume can be sprayed into the nasal cavity.

2.2.3 Inhaled Delivery

The inhaled route has traditionally been used for the delivery of small molecules to the lung (*e.g.* in the treatment of asthma). An example is the β2 agonist, salmeterol. This asthma drug is administered by inhalation device at doses as low as 50 μg for the treatment of the bronchoconstriction associated with an asthma attack.[36] Due to its rapid systemic clearance, plasma concentrations of salmeterol are low, which reduces the potential for systemic side effects. Thus, salmeterol can be considered lung focused.

More recently, the lung has been considered as a delivery route for large macromolecules[37] by virtue of its large surface area for absorption and the highly vascularised nature of the tissue.

2.2.4 Sublingual Delivery

Delivery of drugs via the oral cavity is a useful route of administration. In the oral cavity, the primary absorptive areas are the non-keratinised buccal and sublingual mucosae. The buccal mucosa is the lining of the cheek and the lips, while the sublingual mucosa is the membrane on the ventral surface of the tongue and floor of the mouth. The oral mucosa is relatively permeable and has an abundant blood supply, which drains from the mouth to the superior vena cava.

Drugs absorbed from the mouth pass straight into the systemic circulation without entering the hepatic portal system, thus escaping first-pass metabolism and degradation by gastric acids.

Glyceryl trinitrate (for the treatment of acute angina) is given sublingually[38] to avoid extensive first-pass metabolism following oral administration. Its lipophilicity ensures rapid and effective absorption via the sublingual route. Disadvantages of this route include potential irritation of the mucous

membrane, as well as excessive salivation which promotes swallowing and hence loss of dose.

2.2.5 Rectal Delivery

The rectal mucosa is highly vascularised with a rich blood and lymph supply. However, absorption through the rectal mucosa is often unreliable and incomplete. The route a drug takes depends on how it distributes within the rectum and this is somewhat unpredictable. If a drug is absorbed from the lower rectum via the inferior or middle haemorrhoidal veins, it can avoid first-pass metabolism because these veins empty into the vena cava and bypass the hepatic portal system. Substances that cross the upper rectal mucosa will be carried by the superior haemorrhoidal vein to the hepatic portal circulation.

The rectal route can be useful to patients who have difficulty in swallowing or have nausea or gastric pain. It may offer an advantage for drugs that are destroyed by gastric acid or by microflora or enzymes in the intestine. In addition, drugs may be given rectally when oral ingestion is prohibited because the patient is unconscious. An example of this is the rectal administration of diazepam employed in the acute treatment of epileptic seizures.[39]

2.2.6 Transdermal Delivery

Transdermal absorption of a drug through the skin to the systemic circulation can occur via a transfollicular or transepidermal pathway. To be effective in any transdermal delivery system, a drug must be absorbed in sufficient quantities and the extent of absorption depends upon molecular weight and the lipid and water solubility of the drug. Physiological factors including surface area of application, skin condition and location can affect drug penetration.

Transdermal application is of utility for systemic delivery of small lipophilic and potent molecules that require low input rates to achieve effective therapy. An example of a drug that is administered transdermally is fentanyl for the treatment of pain.[40,41]

2.2.7 Subcutaneous and Intramuscular Administration

Subcutaneous administration can be used in either acute or chronic therapies and may be self-administered. It is often the route of choice for large molecules such as insulin. Drug is either injected or delivered via a device placed in the interstitial tissue beneath the dermis—most commonly in the upper arm, the upper thigh, the lower part of the abdomen and the upper part of the back.

Intramuscular administration involves the injection of drug into the muscular layer below the subcutaneous tissue. The most common sites of administration are the shoulder, the buttocks and the upper thigh muscles.

Absorption of most drugs administered via these routes is dependent on blood flow to the site as the capillary wall between the injection site and the

blood offers little resistance to the passage of drugs. Absorption can be manipulated by heating, vasodilation through massaging the injection site or pharmacologically to increase the blood flow and thus absorption rate. Absorption can occur through the blood or lymph, which is mainly determined by the molecular weight of the drug. Lower molecular weight drugs are absorbed into blood vessels whilst higher molecular weight molecules of greater than 2000 daltons preferentially use the lymphatic system. Other properties that influence drug absorption via these routes include those affecting dissolution rate and lipophilicity.

An example of a drug administered either subcutaneously or intramuscularly is interferon α2a for the treatment of hepatitis C or certain cancers.[42]

2.3 Tissue Distribution of Drugs

The pharmacokinetic approach that considers the blood as the site of action for a drug is overly simplistic. Most drugs modulate targets that are in the tissues supplied by the blood. As stated earlier, all of an intravenous dose is administered into the blood. On administration, several distribution processes begin to take place. Within the blood itself, the drug can bind to plasma proteins and distribute into the cellular component of the blood (mainly erythrocytes).

Blood is rich in proteins which make up the plasma. The predominant plasma proteins are albumin, alpha 1 acid glycoprotein and lipoproteins. Drugs can bind to these proteins, with the extent of binding dependent on their physicochemistry. Highly lipophilic compounds tend to show high binding to albumin. In addition, acidic groups will also increase binding to albumin as these interact with the basic lysine residues of the protein. Thus, lipophilic acids tend to be most highly bound to plasma protein. Neutral compounds will bind to albumin with the extent being solely determined by lipophilicity. Basic compounds bind to alpha 1 acid glycoprotein by virtue of their positive charge and to albumin by virtue of their lipophilicity. Plasma protein binding is an important parameter to understand in drug disposition and further detailed information is available in the literature.[43–47]

In addition to binding to plasma proteins, compounds can distribute into the cellular component of blood. From a pharmacokinetic perspective, this is a much neglected compartment since most bioanalytical methods measure plasma concentrations and dispose of the cellular fraction.

Drug distribution into erythrocytes has been extensively reviewed.[48] Drugs can distribute into erythrocytes through non-specific interactions with membranes and proteins within the erythrocyte such as haemoglobin. In addition, certain drugs (*e.g.* acetazolomide) can bind specifically to carbonic anhydrase and as such exhibit extensive erythrocyte distribution. Drugs that distribute evenly between the plasma and cells of blood will exhibit a Kb/p of 1 and the plasma pharmacokinetics will reflect those of blood. However, extensive distribution ($Kb/p > 1$) will lead to a disconnect between the plasma pharmacokinetics and those determined in blood. Thus, it is important to understand the

blood distribution behaviour of a compound. The Kb/p for acetazolomide is 2.9.[48]

Arterial blood flows to the tissues and into the microvasculature within the tissues. The endothelial walls of the blood vessels within most tissues are relatively leaky, which allows certain constituents of the blood to perfuse the cells that make up the tissues. The aqueous portion of the blood readily escapes the blood vessels, whereas the plasma proteins tend to be slower to diffuse due to their molecular size. Erythrocytes do not generally escape the microvasculature. Consequently, drugs that are bound to plasma proteins or extensively distributed into erythrocytes tend to have more difficulty than unbound drug in distributing into the tissues.

Physicochemical factors generally determine the extent of distribution of drugs not bound to plasma proteins. Tissues are made up of cells, which in turn are predominantly made up of cell membranes. Cell membranes are made up of phospholipids, which are amphoteric in nature. The phospholipid membranes arrange into bilayers with the negatively charged phosphate head groups on the outside facing the extracellular water and the triglyceride tails on the inside, producing a highly lipophilic environment. Lipophilic bases tend to permeate these membranes well as the basic group interacts with the acid groups of phospholipids on the membranes and the lipophilicity facilitates passage through the hydrophobic lipid core. Lipophilic acids, as well as being highly bound to albumin, also tend to be repelled by the negatively charged phospholipid heads and consequently their tissue distribution is limited. The extent of distribution of neutral molecules tends to be determined by their lipophilicity, driven by interaction with the triglyceride tails of the cell membrane.[49]

Consequently, medicinal chemists can utilise physicochemical approaches to calculate expected distribution of drugs in the body either prior to synthesis (virtual molecules) or prior to *in vivo* administration.[46,50–52] Such knowledge of physicochemistry can also be used in compound design (*e.g.* to improve distribution to the required site or to generally improve the pharmacokinetics of a drug). For example, the introduction of basic centres has been used successfully to increase elimination half-life and thus the duration of effects of a number of drugs relative to their neutral analogues.[53–55]

In terms of pharmacokinetics, the extent of distribution of a drug is represented by the volume of distribution. This parameter relates the total amount of drug in the body at any given time to the plasma concentration at that time. It can only be calculated following intravenous administration. In its most simple form, it is obtained by extrapolating the terminal elimination phase back to determine the concentration at time zero. This is the volume the total dose would have to be instantaneously distributed into to achieve the time zero plasma concentration, C_0 (Figure 2.6).

Immediately following intravenous administration (A in Figure 2.6), all of the drug will be in the blood as no tissue distribution has taken place. Since the blood is the site of measurement, measured concentrations will be at their highest. Several minutes after the dose (B in Figure 2.6), blood will have flowed around the body many times, allowing distribution of the drug into the tissues

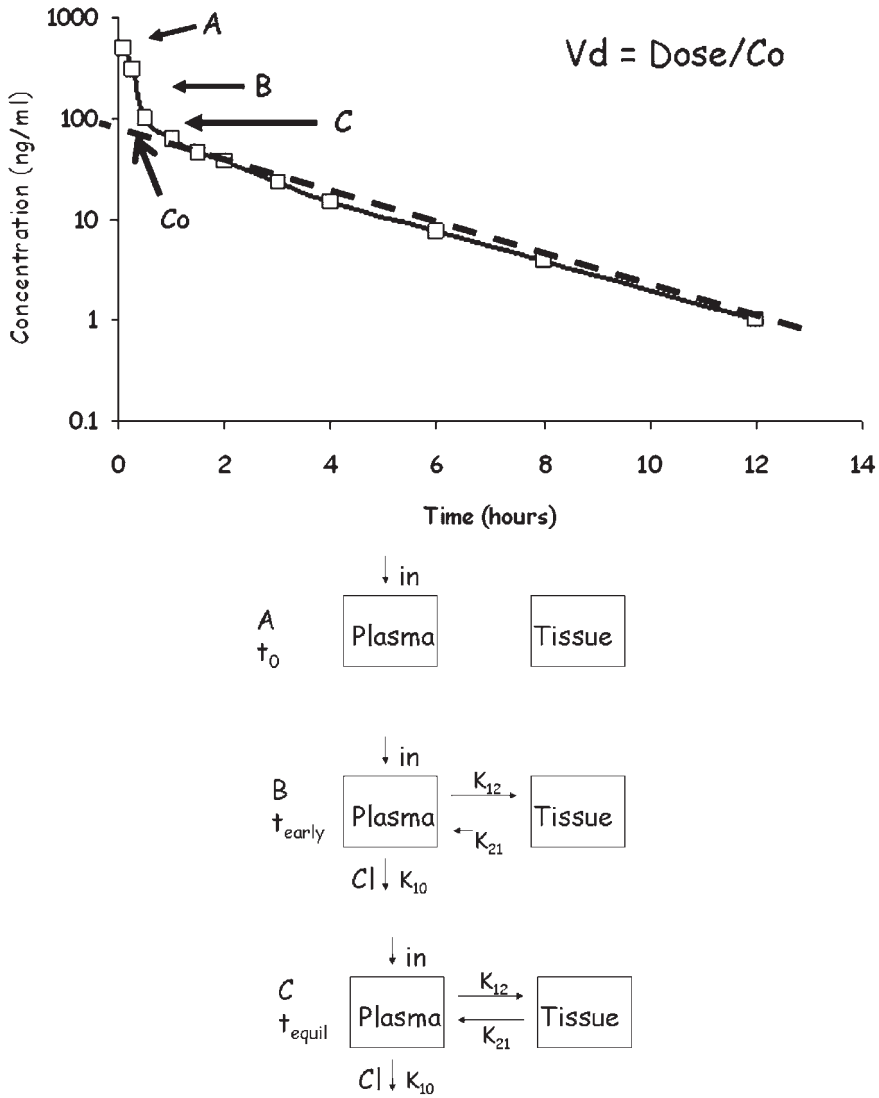

Figure 2.6 Plasma concentration versus time curve for an intravenous dose and corresponding schematic of drug distribution at early times following intravenous administration.

to take place. This will occur by the law of mass action down a concentration gradient. At this point, the rate into the tissues will be greater than the rate of return into the blood. When tissue distribution and blood clearance (see below) is taken into consideration, the concentrations in the blood will fall rapidly over this period.

At some point following intravenous administration (C in Figure 2.6), the unbound concentrations in the tissue will be in equilibrium with the unbound

concentrations in the blood. This is termed the steady state of distribution. After this point, tissue distribution is complete and plasma concentrations decline solely as a result of clearance from the blood. Extrapolation of this phase back to the concentration axis will give the concentration of the drug at time zero (Co) or the blood concentration if all the drug was distributed instantaneously following dosing. This is related to the distribution volume (Vd) and the dose by eqn (2.3):

$$\text{Co} = \text{Dose}/\text{Vd} \tag{2.3}$$

The volume of distribution of a drug is not related to an actual volume, but instead when compared with various physiological volumes, it provides an indication of how well a drug distributes into tissues. Compounds that are primarily restricted to the blood compartment will exhibit volumes of distribution that reflect blood volume (*i.e.* approximately $0.15\,\text{L}\,\text{kg}^{-1}$). Since blood volume is fixed and blood is the compartment that is measured, it is not possible for a compound to exhibit a volume of distribution value less than blood volume. Compounds that distribute into extracellular water, but which are not permeable enough to cross into the cell itself, will tend to exhibit volumes of distribution equivalent to extracellular water (*i.e.* around $0.4\,\text{L}\,\text{kg}^{-1}$). Compounds that equally distribute into cells can exhibit volumes of distribution equivalent to total body water (*i.e.* approximately $0.7\,\text{L}\,\text{kg}^{-1}$). Finally, compounds that enter tissues and bind extensively will exhibit volumes of distribution in excess of total body water (*i.e.* any value above $1\,\text{L}\,\text{kg}^{-1}$).

The relationship between volume of distribution and physicochemistry has long been established.[49] This is exemplified in Figure 2.7.

The determination of volume of distribution has traditionally always required intravenous administration. However, it is now possible to use physiologically based pharmacokinetic modelling (PBPK) to predict the distributional behaviour of compounds *in silico*. These models divide the body into a number of compartments and determine the partitioning into each tissue either by measurement or (more likely) by mechanistic equations that are based on tissue physiology and the phospholipid composition of tissues. These can be used to estimate distribution based on the physicochemistry of each compound.[56–58]

In order to exert a pharmacological response the drug must bind to its target. In the majority of cases, only unbound drug is available to interact with the target (Figure 2.8).

As stated earlier, drug is absorbed (or administered) into the blood, where it can bind to plasma proteins, distribute into red cells or begin to distribute into tissues. An equilibrium will eventually be reached between binding in blood and tissues. Assuming that the drug only distributes passively and that the laws of mass action apply, then at equilibrium unbound drug concentrations should be equivalent in all phases (*i.e.* the unbound concentration in the tissues should be the same as the unbound concentration in the blood). Determination of unbound concentrations in tissues is technically challenging. However,

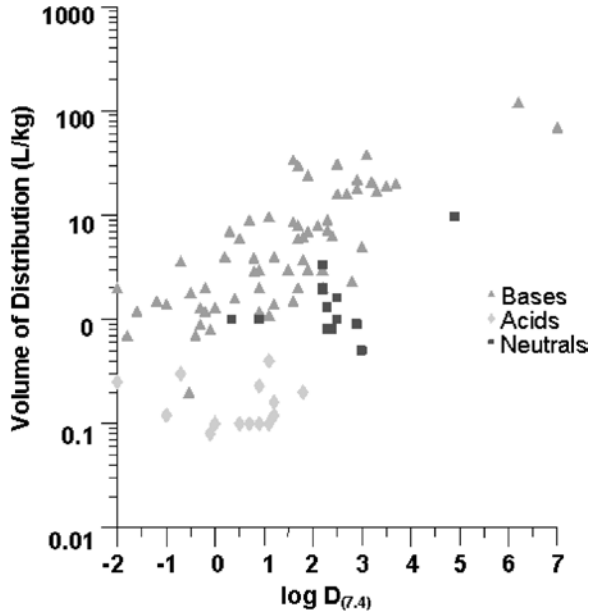

Figure 2.7 Relationship between $\log D_{(7.4)}$ and Vd for acids, bases and neutral compounds.

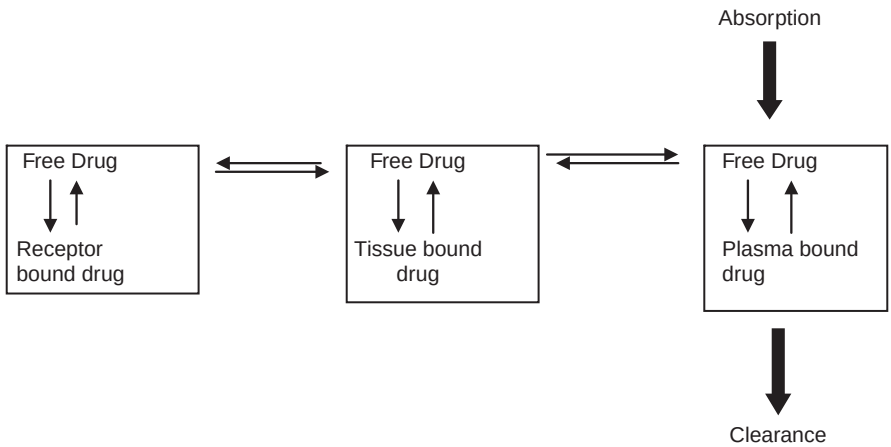

Figure 2.8 The role of free drug.

unbound drug in blood is relatively easy to determine and is used as a surrogate for target concentrations.

This concept is central to drug pharmacodynamics.[49,59,60] Supporting evidence for this hypothesis is shown in Figure 2.9. For a series of G-protein

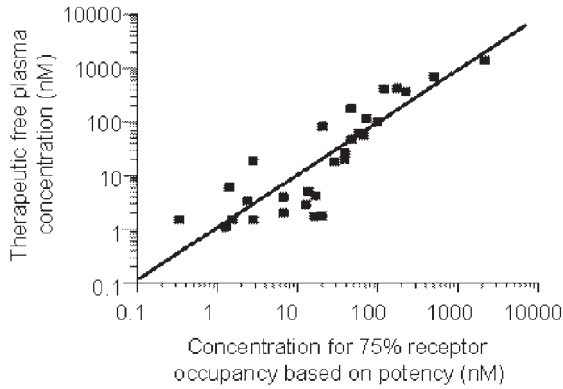

Figure 2.9 The relationship between unbound plasma concentration at efficacious dose and receptor occupancy for a series of GPCR antagonists (ref. 49).

coupled receptor (GPCR) antagonists, the unbound concentrations at therapeutic doses show a correlation with the concentration required for 75% receptor occupancy at the target.

Unbound concentrations in blood will only reflect those in the tissue if there is free movement of the drug between the blood and the tissue. In many tissues this is the case, but there are a number of tissues that are behind significant barriers to drug movement. The brain is the most important of these cases since many targets for drug modulation are in the central nervous system (CNS).

2.3.1 Distribution to the Central Nervous System

The endothelial cells of the blood vessels supplying the brain have evolved to form the blood–brain barrier, which can act to exclude the passage of drug molecules. The adaptations include:

- tight junctions that are extremely resistant to the passage of drugs;
- membrane phospholipid composition that differs from other endothelial cell membranes making them more rigid and resistant to passive permeation;
- expression of efflux transporters (*e.g.* P-gp)[61–63] and metabolizing enzymes.

These adaptations make the blood–brain barrier a significant hurdle for a drug to cross in order to reach the site of action. Consequently, the physicochemical constraints for a CNS targeted drug are much more severe than for a non-CNS drug (Figure 2.10).

As with all membrane permeation, lipophilicity, molecular size and hydrogen bonding capacity are important determinants of brain penetration. A drug is more likely to penetrate the CNS when polar surface area is below 90Å and molecular weight (MW) is below 450, in contrast to non-CNS drugs where the

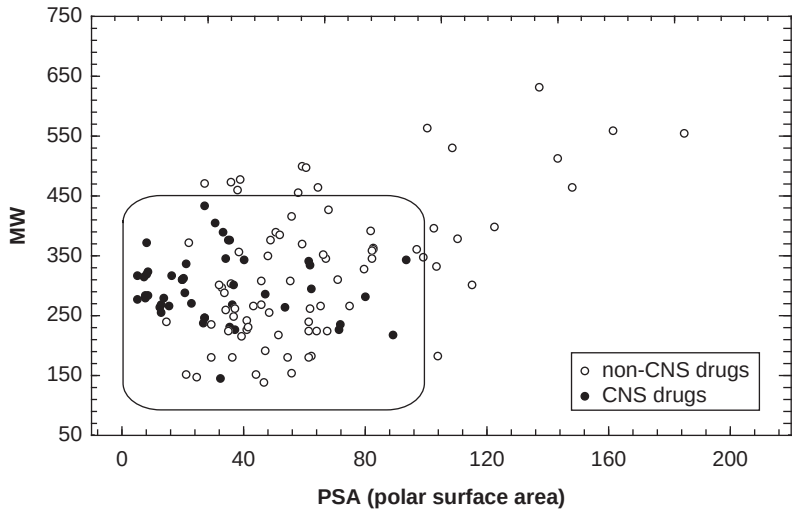

Figure 2.10 Physicochemical parameters for CNS and non-CNS drugs.

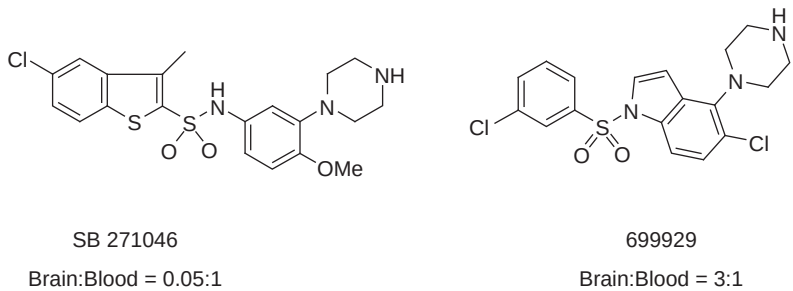

Figure 2.11 Conformational constraint and removal of hydrogen bonding capacity to promote CNS penetration.

physicochemical space is significantly larger. This reflects the relative difficulty of permeation across the blood-brain barrier *versus* the enterocyte membrane.

Physicochemical strategies to improve blood–brain barrier penetration have been suggested.[64] The potent 5HT6 antagonist SB271046 exhibited poor CNS penetration in rat (brain to blood ratio 0.05 to 1) by virtue of its poor membrane permeation and P-gp substrate potential. A medicinal chemistry strategy to remove the acidic NH, reduce hydrogen bonding potential and restrict conformation led to the identification of 699929. This compound exhibited greater CNS penetration in rat with a brain to plasma ratio of 3 to 1 (Figure 2.11)

Due to the presence of the blood–brain barrier, the unbound concentration in the plasma may not reflect that at a CNS target. As in other tissues it is the

unbound drug concentration in the brain that is important. Strategies that drive compound into the brain using excessive lipophilicity are likely to fail, since unbound concentrations are likely to be low.

A method has been developed in which brain binding in relation to plasma protein binding is compared and used to judge whether a compound will distribute well into the CNS. If this is the case, then unbound drug measured in plasma can be used as a surrogate for that in brain.[65] This topic of blood–brain penetration is also discussed in Section 11.8 when future targets are considered

2.4 Clearance, Extraction, Metabolism and Excretion

The aim of many drug discovery projects is to provide a drug candidate with a duration of action consistent with the required dose regimen. For many approaches, the desired regimen is once per day. In order to produce a once daily regimen, the drug must exhibit a significantly long elimination half-life relative to the dose interval (every 24 h). The elimination half-life is determined by the clearance and the volume of distribution. In addition, the importance of clearance in defining dose is exemplified by eqn (2.4):

$$\text{Dose} = \frac{C_{ss,avg} \times CL \times \tau}{F} \tag{2.4}$$

where:

$C_{ss,avg}$ is the average concentration at steady state (target concentration)
Cl is the plasma clearance of the molecule
τ is the dose interval
F is the fraction bioavailability by the particular route.

Thus, clearance is an important parameter for the medicinal chemist to understand as it will determine the dose necessary to achieve pharmacologically active concentrations. Indirectly, clearance will determine the frequency of dosing and drive peak-to-trough ratio as a component of elimination half-life.

2.4.1 Clearance

The clearance of a molecule is defined as the amount of blood (blood clearance) or plasma (plasma clearance) cleared of drug per unit time and body weight. The units of clearance are $\text{ml}\,\text{min}^{-1}\,\text{kg}^{-1}$. Clearance is dependent upon the ability of organs to metabolise or excrete the compound, the plasma protein binding of the molecule and the blood flow to the clearing organ. The importance of clearance as a parameter is that, for a given dose, it defines the exposure (or AUC) to a given compound as determined by

eqn (2.5):

$$\text{Clearance} = \frac{\text{Dose}}{\text{AUC}} \tag{2.5}$$

Thus, a compound with a low clearance will always exhibit a high exposure at a given dose, relative to a compound with a higher clearance.

The free drug hypothesis suggests that therapeutic efficacy is driven by the unbound concentrations of the drug. Thus, unbound clearance is the plasma clearance corrected for the fraction unbound in plasma.

In addition, the potential amount of blood cleared of drug in the absence of flow limitations and plasma protein binding considerations is defined as the unbound intrinsic clearance ($Clint_u$) of that particular molecule. For metabolised compounds, this is an important parameter as it is related to the Vmax and Km of the enzyme for metabolism of any particular compound. This is the parameter that medicinal chemists attempt to reduce when required to modify the plasma clearance of any particular molecule. $Clint_u$ is related to the clearance by the well stirred model as described by eqn (2.6):

$$\text{Cl} = \frac{\text{fu} \times \text{Clint}_u}{\text{fu}(\text{Clint}_u + \text{Q})} \tag{2.6}$$

where fu is the fraction unbound in plasma and Q is the hepatic blood flow.

2.4.1.1 Organ Extraction

The major drug metabolising and excreting organs are the liver, the kidney and the gut. The extent to which a compound can avoid metabolism and excretion by these organs will define the clearance and hence the dose and duration of action of that particular molecule. The fraction of a drug removed from the blood on single perfusion through any particular organ is termed the extraction of that compound.

For instance where extraction by an organ is 0.5 (50% of flow through it) the concentration of a particular compound is halved by passage through that organ. The clearance by that organ relates to the blood flow and the extraction (metabolism *etc*.). This process is continuous for all further passages through the extracting organ, such that plasma concentrations of the compound will fall in an exponential manner. Consequently, if we know the blood flow to a single extracting organ and the extraction ratio across that organ, we can calculate amount of blood freed per unit time (*i.e*. the clearance of that drug) from eqn (2.7):

$$\text{Clearance} = \text{Extraction ratio} \times \text{Organ blood flow} \tag{2.7}$$

However, since many drugs are cleared by more than one organ and single human organs cannot be studied in isolation, the clearance of a drug is always calculated from the plasma concentration *versus* time curve using eqn (2.5).

Clearance is most often calculated following intravenous administration because the total dose of the drug is known to be delivered into the blood. However, most drugs are delivered at sites remote from the blood, with the most popular route being oral administration. As stated earlier, following oral administration the drug must overcome the barriers of dissolution, absorption, gut wall metabolism and hepatic first-pass metabolism, dissolve in the gastrointestinal tract lumen contents and cross the gastrointestinal tract enterocyte membrane.

Thus, at a number of points between the site of administration and the blood, there is capacity for an oral drug to be lost. Consequently, the oral clearance will often be higher than the intravenous clearance. The unifying factor is the fraction bioavailable (F). Clearance is calculated using eqn (2.8).

$$\text{Clearance}_{(\text{oral})} = \frac{\text{Dose(oral)}}{\text{AUC}_{(\text{oral})} \times \text{F}} \tag{2.8}$$

Drugs can be cleared by a variety of organs in the human body. However, the major clearing organs are the liver and the kidney.

2.4.2 Clearance by the Liver

The liver is at the centre of drug clearance by virtue of its drug metabolising enzyme expression and its blood perfusion. The liver is perfused by blood from two sources, the hepatic vein and the hepatic portal vein. Compounds being presented to the liver through the hepatic vein are available for hepatic extraction, which contributes to the systemic clearance of that drug. The hepatic portal vein carries blood from the gut to the liver. Consequently, any drug that has been absorbed from the gastrointestinal tract is available for extraction by the liver, before it reaches the systemic circulation. The fraction of a drug removed from the portal vein by the liver prior to accessing the systemic blood is termed the hepatic first-pass extraction (see Section 2.2.1.5). This is a significant factor contributing to the oral bioavailability of a drug. When hepatic first-pass extraction is high, oral bioavailability will be low.

The hepatocyte is the liver cell responsible for the clearance of drugs (Figure 2.12). Hepatocytes contain an arsenal of drug metabolising enzymes and drug transport proteins that are capable of irreversibly removing compounds from the circulating blood.

2.4.3 Metabolism

2.4.3.1 Cytochrome P450

The family of enzymes that metabolise the majority of drugs are the cytochrome P450s (CYPs). These are a super-family of haem-containing enzymes that use NADPH to catalyse the single electron oxidation of substrates. The overall family consists of several hundred isoforms, with a variety of endogenous roles.

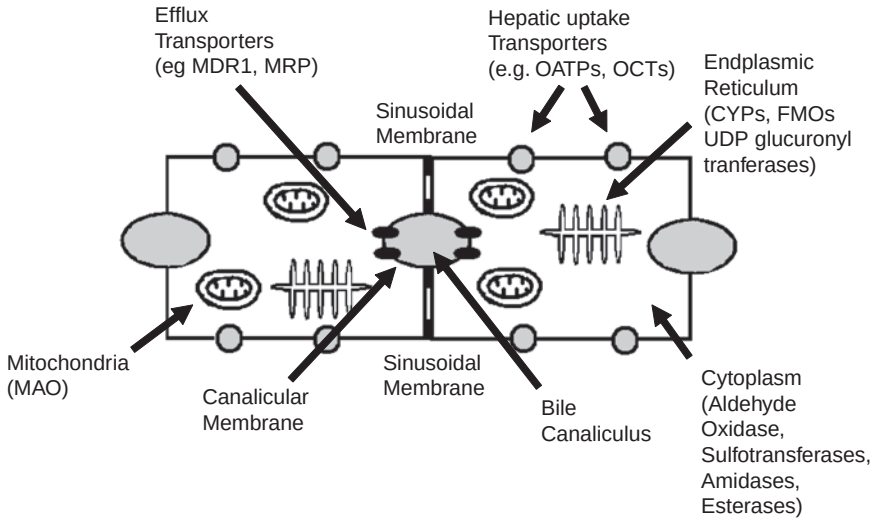

Figure 2.12 Representation of two hepatocytes showing the bile canaliculus.

However, there are only a small number of major human drug metabolising CYPs, with the most important being CYP3A4, CYP2C9 and CYP2D6.

The CYP enzymes are expressed in many tissues, but they are found in highest concentrations in the hepatocyte. They are membrane-bound enzymes found within the cell on the endoplasmic reticulum. Human liver microsomes are preparations of endoplasmic reticulum made by differential centrifugation of human liver homogenates. On preparation, these intracellular membranes form into spheres that express CYPs on their inner and outer surfaces.

The substrate structure metabolism relationships for these major drug metabolising CYPs have been extensively reviewed.[49,66] CYP2D6 will tend to metabolise compounds with a metabolically vulnerable site a certain distance away from a basic centre. Since CYP2D6 is polymorphically expressed across the human population (6–8% of the Caucasian population do not express CYP2D6), compounds that are predominantly metabolised by this isoform will exhibit significant variability in pharmacokinetic profile. For this reason, CYP2D6 substrates tend to be avoided in compound selection. CYP2C9 will tend to metabolise acids or compounds with a significant degree of hydrogen bonding. CYP3A4 tends to be more promiscuous, driven by a large active site, and will metabolise relatively large molecules with no major preference for ionisation.

These CYP isoforms share a common requirement for lipophilic compounds. In general, CYP metabolism is positively correlated with log D.[67]

Therefore, across a series of compounds, the most lipophilic analogue will tend to be more rapidly metabolised than its analogues with lower log D. As oxidative enzymes, CYPs will tend to oxidise lipophilic compounds at sites of significant electron density. This points the way to medicinal chemistry strategies to address CYP metabolism. Clearly, reducing log D is likely to lead to a reduction in

intrinsic clearance observed in human liver microsomes. However, as previously established, lipophilicity tends to be correlated positively with potency against the pharmacological target. Therefore, a balance needs to be struck. Perhaps a more informed strategy to reduce CYP metabolism would be to identify the sites of metabolic attack and reduce their susceptibility to metabolism by blocking with metabolically inert groups or by reducing electron density at that position. In this way, lipophilicity to drive potency can be maintained whilst reducing metabolic clearance due to CYP metabolism.

It is easily overlooked in compound optimisation (based on human liver microsomal intrinsic clearance) that the liver contains a vast armoury of other drug metabolising enzymes. Thus, it is not unusual to find that a successful campaign to lower the CYP mediated clearance within a series has not reduced the *in vivo* clearance as much as expected due to metabolism by other hepatic enzymes. This has been exemplified by Williams *et al.*[68] A review of the top 200 prescribed drugs suggests that metabolism is responsible for the clearance of approximately two-thirds. Of these, approximately two-thirds are predominantly CYP metabolised, suggesting that the emphasis on CYP-mediated metabolism is well placed. However, there is approximately a further third that are substrates for metabolic clearance mediated by enzymes other than CYPs. Of these, the most prevalent are UDP-glucuronosyltranferases and esterases, representing approximately 8% and 5% of the metabolised drugs, respectively. More minor contributions come from flavin monooxygenases (FMOs), monoamine oxidases (MAOs) and *N*-acetyltransferases.

2.4.3.2 Flavin Monooxygenase

Flavin monooxygenases (FMOs) are a family of membrane-bound endoplasmic reticulum enzymes consisting of six isoforms. FMO3 is the major human adult FMO expressed in the liver. Consequently, it is present in human liver microsomes and hepatocytes.

FMO3 utilises NADPH to produce the two electron oxidation of substrates at heteroatoms such as nitrogen and sulphur[69,70] to produce N- and S-oxides.

One of the endogenous substrates of human FMO3 is trimethylamine, which is cleared by N-oxidation. Humans lacking FMO3 cannot metabolise trimethylamine and exhibit the characteristic 'fish odour' syndrome, trimethylaminuria. Drugs that are in part cleared by FMO3 are nicotine (Figure 2.13), clozapine (N-oxidation), methimazole and cimetidine (S-oxidation).

Figure 2.13 Metabolism of nicotine by FMO3.

2.4.3.3 Monoamine Oxidases

Monoamine oxidases (MAOs) use FAD to catalyse the oxidative deamination of primary, secondary and tertiary amines. The reaction proceeds through oxidation of the amine to form an imine, which is then hydrolysed to form the aldehyde. The aldehyde is then further oxidised chemically or enzymatically to produce the carboxylic acid.[71–73]

Two forms of MAO (MAO A and B) are involved in the metabolism of endogenous catecholamine neurotransmitters such as dopamine and tryptamine. These enzymes are predominantly found in the outer mitochondrial membrane in a variety of tissues, including the liver. MAO A has been shown to be involved in the clearance of sumatriptan[74] producing the only human phase I metabolite, indole acetic acid (Figure 2.14).

2.4.3.4 Aldehyde Oxidase

Aldehyde oxidase is a molybdenum-containing liver cytosolic enzyme that can be involved in both oxidative and reductive reactions. It is responsible for the oxidation of a wide range of aldehydes and a number of nitrogen-containing heterocycles. For example, aldehyde oxidase is the major enzyme involved in the oxidation of the phthalazine containing compound, carbazeran (Figure 2.15).[75]

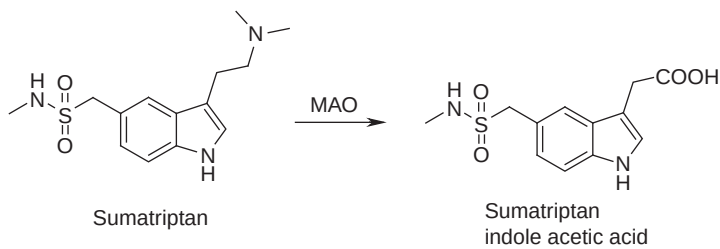

Figure 2.14 Metabolism of sumatriptan by MAO.

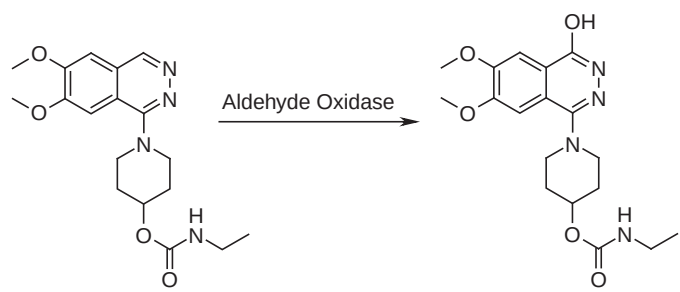

Figure 2.15 Metabolism of carbazeran by aldehyde oxidase.

Carbazeran exhibits a species difference in pharmacokinetics that is driven by differences in aldehyde oxidase activity.[76] In the dog, carbazeran exhibits greater than 60% oral bioavailability, whereas in humans oral bioavailability is less than 5%.[75] This species difference is driven by differences in hepatic first-pass extraction mediated by aldehyde oxidase. Rat and dog livers show negligible aldehyde oxidase activity, whereas human liver cytosol exhibits significant activity. Guinea pig liver cytosol is the most appropriate pre-clinical model for human aldehyde oxidase.

In addition, aldehyde oxidase is involved in the reductive metabolism of ziprasidone,[77] where it accounts for approximately two-thirds of the observed metabolism (Figure 2.16).

2.4.3.5 Hydrolases

Hydrolases encompass a wide range of enzymes that use water to breakdown their substrates. In general, they catalyse the reaction exemplified by Figure 2.17. Hydrolases comprise a huge family of enzymes (including esterases, amidases and peptidases) that have been thoroughly reviewed in terms of classification, mechanism of action and structure–activity relationship (SAR) by Testa and Krämer.[78]

Probably the most important group of hydrolases from a drug metabolism and prodrug point of view are the esterases including the cholinesterases

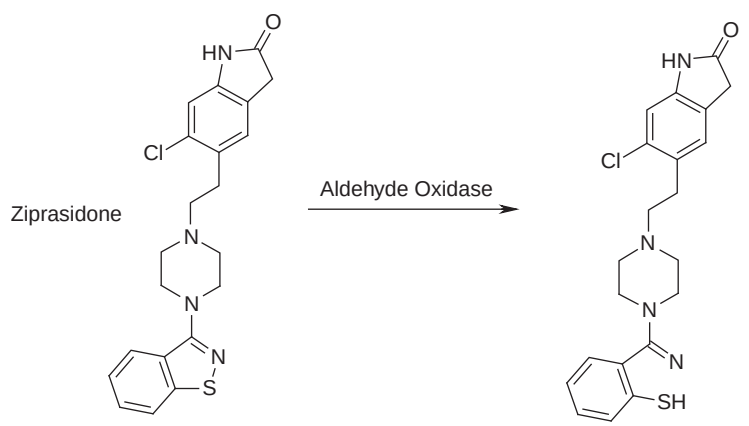

Figure 2.16 Metabolism of ziprasidone by aldehyde oxidase.

Figure 2.17 General reaction scheme for hydrolase enzymes.

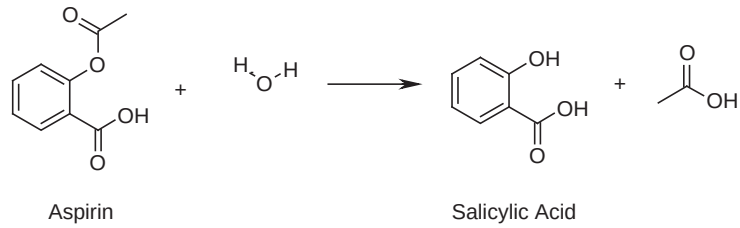

Figure 2.18 Metabolism of aspirin by carboxylesterase.

(predominantly in plasma), arylesterases (predominantly in blood cells and plasma) and carboxylesterases (predominantly in gut and liver).[79–81]

There are two major isoforms of human carboxylesterase. Human carboxylesterase 1 (hCE-1) is highly expressed in the liver, whereas hCE-2 is most highly expressed in the intestine. Both isoforms are not highly expressed in human blood, whereas in preclinical species there is significant expression in the blood. In addition, carboxylesterase-2 is not expressed in dog intestine. This points to some of the difficulties encountered in preclinical species by programs that target ester prodrugs.[6]

The carboxylesterases are serine hydrolases that catalyse the base-mediated hydrolysis of esters using a three amino acid triad of serine, histidine and glutamic acid. They are involved in the conversion of aspirin to acetyl salicylic acid (Figure 2.18).

2.4.3.6 UDP-glucuronosyltranferases (UGTs)

The glucuronidation of drugs is an important clearance pathway for a number of xenobiotics.[68] The conjugation of a drug molecule with glucuronic acid (most often at hydroxyl, carboxylic acid or nitrogen containing functions) reduces lipophilicity and increases water solubility, thus rendering the molecule more readily excretable in urine and bile.

The UGT enzymes catalysing glucuronidation are part of a larger family of UDP-glycosyltranferase enzymes that conjugate lipophilic compounds with glycosyl groups such as glucose, glucuronic acid and galactose.[82] The UGT 1 and UGT 2 sub-families use UDP-glucuronic acid as the activated sugar donor, whereas the UGT 8 sub-family uses UDP-galactose. Within the UGT 1 and 2 sub-families, there are a total of 18 enzymes, but only a small number catalyse the majority of xenobiotic glucuronidation.[68,83] For example, UGT2B7 metabolises 35% of the glucuronidation substrates within the top 200 drugs, with UGT1A4 responsible for 20% and UGT1A1 15%.

UDP-glucuronosyltransferase enzymes are expressed in many tissues within the body, with major expression sites in the liver, kidney and intestine. The UGT1 and UGT2 sub-families are expressed on the endoplasmic reticulum. It is possible to demonstrate glucuronidation in human liver microsomes,

provided UDG-glucuronic acid is added as a cofactor. Hepatocytes are also potential *in vitro* assays for glucuronidation.

2.4.3.7 Sulfotransferases (SULTs)

In terms of drug metabolism, sulfotransferases use 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to transfer a sulfate group onto phenol/hydroxyl and amine functions of their substrates.

The human sulfotransferase family of enzymes have a variety of roles from xenobiotic metabolism to regulation of endogenous processes. There are two main classes of SULTs: membrane-bound enzymes that in general are involved in endogenous processes and the cytosolic SULTs that are responsible for drug metabolism.[84] The cytosolic SULTs have been extensively reviewed.[85]

There are three sub-families of cytosolic SULTs representing approximately 13 enzymes. The SULT1 sub-family is responsible for the majority of drug sulfation, with SULT1A1 and SULT1A3 being the most active. SULT1A1 exhibits highest expression in human liver, whereas SULT1A3 is hardly expressed in liver and shows significant expression in the intestine. Thus, these isoforms are well placed to exert a first-pass sulfation on their substrates following oral administration. Examples of drugs that undergo significant sulfation are salbutamol, paracetamol and diflunisal.

2.4.3.8 N-Acetyltransferases (NATs)

N-Acetyltransferases are cytosolic enzymes that use acetyl-coenzyme A to acetylate their substrates at amine functions. Arylamine *N*-acetyltransferases have been extensively reviewed.[86]

There are two human NATs. NAT1 exhibits a wide range of tissue expression, including liver and lung. It tends to be mainly involved in endogenous function, although it does have some drug substrates. NAT2 is the major drug metabolising NAT, with expression in liver and intestine. NAT2 is polymorphically expressed with 50–55% of Caucasians and 10% of Asians classified as slow acetylators. This is important as the anti-tuberculosis drug isoniazid is metabolised by NAT2 and shows higher circulating concentrations in slow acetylators that makes them more prone to the peripheral neuropathy side effect.

Recently, Rawal *et al.*[87] documented their efforts to address NAT2 metabolism in a research project. They found that UK-469 413 was metabolised by NAT2, unusually at the piperazine nitrogen (Figure 2.19). As would be expected for a NAT2 substrate, this compound was not turned over in rat and human liver microsomes, but was cleared at greater than liver blood flow in the rat. Further investigations showed that it was N-acetylated in rat and human liver cytosol and hepatocytes. Incubations with singly expressed enzymes showed that UK-469 413 was a specific substrate for NAT2. Analogues with methyl groups adjacent to the piperazine nitrogen or bridging of the piperazine

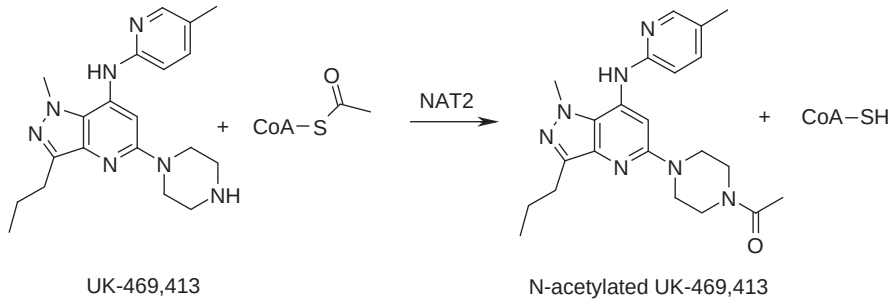

Figure 2.19 Metabolism of UK-469 413 by *N*-acetyltransferase.

ring tended to block N-acetylation, suggesting that steric bulk is an appropriate strategy to reduce NAT2 clearance.

2.4.4 Biliary Elimination

In addition to the metabolic capacity of the liver, hepatic clearance can occur via biliary elimination. This is a three-stage process that largely involves active transport of compounds from the blood into the bile. A review of the large variety of drug-transporting proteins is beyond the scope of this chapter but this been comprehensively reviewed elsewhere.[20,88]

The first stage in biliary clearance is the passage of the compound across the sinusoidal membrane of the hepatocyte. For the majority of drugs, this process occurs by passive diffusion, although these compounds tend to be substrates for metabolic clearance that occurs within the hepatocyte. The products of this metabolism can be excreted from the body in the bile. However, compounds with inappropriate physicochemistry (high molecular weight, significant charge and/or polarity) for rapid passage across the sinusoidal membrane can be recognised in the blood and transported into the hepatocyte by drug transport proteins (*e.g.* OATP1). By virtue of their physicochemistry, these types of molecules are less prone to metabolic clearance, and so the second step in hepatobiliary elimination is transfer of the molecule to the biliary canalicular membrane (by diffusion or intracellular transfer proteins). Once at the canalicular membrane, these compounds can be recognised by efflux proteins (*e.g.* P-gp and MRP2) and actively transported into the bile, to be excreted in the faeces.

The study of biliary clearance in humans is difficult because the bile duct empties into the gall bladder, which subsequently delivers bile into the faeces. In the absence of bile duct surgery, it is impossible to determine the amount of a compound excreted in the bile. Rather this information needs to be inferred from the observed biliary clearance in animals and the excretion of unchanged drug in the faeces. One such example is susalimod, which is completely eliminated unchanged in bile in animals and shows a high excretion of unchanged compound in the faeces of humans following intravenous administration.[89]

2.4.5 Clearance by the Kidney

The main role of the kidney is to regulate water loss from the body and to produce urine.

Blood flow to the Bowman's capsule is filtered through the glomerulus into the proximal convoluted tubule. Water and small molecules can pass freely through the glomerulus whereas cells and large molecules such as proteins are excluded. Thus, the fluid in the proximal convoluted tubule is simply a filtrate of plasma. The blood flow to the glomerulus is approximately 1–2 ml min^{-1} kg^{-1}, suggesting that around 30 litres of blood is filtered through the human kidneys per day. The purpose of the rest of the nephron (the proximal and distal convoluted tubules and the Loop of Henle) is to concentrate this fluid to produce urine, ensuring water homeostasis and balance of salts. Thus, these areas have a significant blood supply to enable water reabsorption by an osmotic process.

The kidney is predominantly an organ of excretion, rather than metabolism. Clearance of compounds by the kidney can be affected in three ways: glomerular filtration, renal reabsorption and renal secretion.[90]

2.4.5.1 Glomerular Filtration

In humans, blood flows to the glomerulus at a rate of 1 to 2 ml min^{-1} kg^{-1}. This is called the glomerular filtration rate (GFR). Any drug that is not bound to plasma proteins in the blood will be filtered with the aqueous component. Once in the filtrate, the fate of a compound will depend upon its ability to cross the membranes of cells lining the nephron. Compounds that are significantly hydrophilic will not be able to be reabsorbed passively across the cell membranes and therefore will remain to be excreted in the urine. For such compounds, the renal clearance (CLr) of the unbound drug will be the GFR as indicated by eqn (2.9):

$$\mathrm{Clr} = \mathrm{fu} \times \mathrm{GFR} \tag{2.9}$$

Since these compounds tend to be largely unbound in plasma and not metabolised to any great extent (because they do not cross membranes), they are likely to exhibit a plasma clearance at GFR (*i.e.* 1–2 ml min^{-1} kg^{-1}).

In common with many of the hydrophilic beta blockers, nadolol is 100% excreted unchanged in human urine with a plasma clearance of 1.6 ml min^{-1} kg^{-1} equivalent to GFR.[91]

2.4.5.2 Renal Reabsorption

The unbound fraction of lipophilic drugs will also be filtered through the glomerulus. However, the filtered component is likely to be reabsorbed across the membranes of the cells lining the nephron. For these compounds, depending on the lipophilicity, very little compound will remain in the urine and renal

clearance will be negligible (*i.e.* much less than GFR). However, these types of compounds are more likely to exhibit clearance by (hepatic) metabolism.

A good example of a compound undergoing significant renal reabsorption is fluconazole,[92,93] which is 80% excreted unchanged in urine. The clearance of fluconazole in humans is $0.23\,ml\,min^{-1}\,kg^{-1}$, which is approximately a fifth of GFR, suggesting that up to 80% is reabsorbed following glomerular filtration.

2.4.5.3 Renal Secretion

Some drugs are excreted in the urine at renal clearances that are greater than GFR. Such compounds are substrates for transporter proteins that are expressed in the cells lining the nephron (*e.g.* P-gp). The drugs can be actively removed from the blood and secreted into the filtrate, and subsequently excreted into the urine. For example, an intravenous dose of ranitidine is approximately 70% excreted unchanged in human urine. The renal clearance of ranitidine is approximately $7\,ml\,min^{-1}\,kg^{-1}$, which is significantly higher than GFR (*i.e.* $1.5\,ml\,min^{-1}\,kg^{-1}$). The difference between the renal clearance and GFR is the renal secretion clearance of the compound (*i.e.* $5.5\,ml\,min^{-1}\,kg^{-1}$).[94,95]

Overall, renal clearance of xenobiotics can be both passive by filtration and active *via* transport proteins. Renal clearance is inversely correlated with lipophilicity, positively correlated with polarity and related to ionisation state.[96] Generally, compounds with log D values <0 will tend to be passively renally excreted at GFR. However, as lipophilicity increases in a series, passive renal excretion is reduced to significantly lower than GFR due to passive reabsorption across the cells lining the nephron. In addition, drugs can be actively reabsorbed from or secreted into the urine if they are substrates for transporter proteins. In the case of active renal secretion, the renal clearance values can be significantly in excess of GFR.

2.4.6 Clearance Summary

Throughout compound optimisation strategies, medicinal chemists most often attempt to balance potency improvements with strategies to reduce clearance. This is not surprising since clearance is a key parameter in modulating oral bioavailability and elimination half-life and is a major determinant of daily dose.

All molecules within the body are subject to clearance, such that the human body renews itself on a regular basis. The same is true of drug molecules. Indeed, a compound that is not cleared would only require a single dose to be effective for a lifetime. Therefore, it is important that drug molecules exhibit a degree of clearance and it is only the rate of clearance that medicinal chemists seek to modulate.

The clearance of a potential drug molecule is dependant upon its physicochemistry and its structure. Drugs can be cleared in many ways from metabolism to excretion of unchanged drug in urine and faeces.

In general, hits emerging from high throughput screening (HTS) tend to exhibit a significant degree of lipophilicity, as specific interactions with the target are not

optimised and potency is driven by lipophilic interactions. Consequently, it is not unusual for these types of molecules to be high affinity substrates for metabolising enzymes such as CYPs. Medicinal chemistry strategies have emerged that allow reduction in CYP-mediated metabolic clearance, whilst maintaining and improving potency against the target. On many occasions these strategies have led to clearance by other enzymes, such as FMO and UDP-glucuronyl transferases, albeit at lower rates. In addition, metabolically inert molecules can also be substrates for clearance by drug transporters in the liver and kidney.

The challenge of maintaining/improving potency whilst lowering clearance will continue to be a major one for medicinal chemists. Thus, it is important that chemists responsible for compound optimisation strategies continue to understand the range of clearance routes arrayed against potential drug molecules.

2.5 Toxicology related to ADME

Metabolism is a key part of the detoxifying strategy employed by the body to deal with xenobiotics such as drug molecules. However, there is growing evidence that, in some cases, these strategies may be counterproductive and that metabolism is capable of acting as a toxification process.

Some molecules undergo metabolism to yield chemically reactive products or intermediates. Such molecules are at an increased risk of covalent binding to proteins within the body[97] and are also at an increased risk of rare but potentially serious idiosyncratic adverse drug reactions (IADRs).[98–100] These drug reactions are frequently not picked up in pre-clinical toxicology and may not be revealed until after launch when a drug is used by large numbers of people. In addition, many of the structural fragments known to be activated by metabolism to chemically reactive groups also cause signals in genetic toxicity testing and adducts with DNA itself have been reported for some of these fragments. The 'reactive metabolite' hypothesis states that the covalent binding of metabolic products to biomolecules such as DNA and proteins is a necessary step for these molecules to show genetic toxicity[101] or other toxicological outcome.[102] Furthermore, the drug–protein adducts may act as a 'hapten' triggering the immune reaction which is a feature of many, but not all IADRs.[103]

In Section 2.1 we saw the key role that physicochemical properties play in the metabolic fate of a drug. In the case of toxicology liabilities related to ADME, there is a closer link with functional groups and structural motifs with a specific chemical reactivity than with physicochemistry *per se*. However, the physicochemical properties of a drug will govern the rates of the biochemical reactions which result in toxic products and the distribution of these metabolites.

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CHAPTER 3

Carboxylic Acids and their Bioisosteres

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3.1 Introduction

Exposing the importance of the carboxylic acid functional group is best achieved by examining the number of endogenous processes and individual molecules which rely on the intrinsic chemical nature (*e.g.* pKa and hydrogen bonding characteristics) of this functional group. From amino acid conjugation (peptide synthesis → proteins) and post-translational protein acylation, to triglycerides, bile acids, prostanoids, messenger molecules and hormone catabolites, it is evident that the carboxylic acid represents a key functional group contributing to the biochemistry critical to mammalian physiology. Not surprisingly then, there exists an extensive number of drugs possessing the carboxylic acid functional group. The compounds represent a heterogeneous group comprising, among others, non-steroidal anti-inflammatory drugs (NSAIDs), β-lactam antibiotics, statins, fibrates, and even food additives such as preservatives and flavouring agents; these compounds range from hydrophilic to lipophilic organic compounds.

Over 450 drugs containing a free carboxylic acid group are marketed in various countries worldwide (see Table 3.1 for select examples). In addition to the

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Table 3.1 Examples of marketed drugs containing carboxylic acid functionality.

Amoxicillin	Ubenimex	Diflunisal	Ofloxacin	Ramatroban	Repaglinide
Cefadroxil	Ketorolac	Alprostadil	Thymopentin	Tiagabine	Ganirelix
Cetraxate	Acitretin	Methotrexate	Captopril	Cefpodoxime	Tirofiban
Sofalcone	Argipidine	Baclofen	Imipemide	Raltitrexed	Imidapril
Chenodeoxycholic acid	Mebrofenin	Methyldopa	Cilastatino	Acitazanolast	Cefprozil
Pentagastrin	Quinapril	Bumetanide	Indobufen	Marbofloxacin	Hirulog-1
Sincalide	Lisinopril	Metirosine	Lomefloxacin	Ecabet	Valsartan
Cefoxitin	Zaltoprofen	Aminocaproic acid	Desglugastrin	Olopatadine	Olmesartan
Flucloxacillin	Melphalan	Difenoxin	Enrofloxacin	Pidotimod	Pazufloxacin
Sulphasalazine	Azelaic acid	Dinoprostone	Norfloxacin	Tosufloxacin	Finofibric acid
Amphotericin B	Proamipide	Lysine	Aceclofenac	γ-Linolenic acid	Eptifibatide
Ciprofloxacin	Tranilast	Leucovorin	Rufloxacin	Sparfloxacin	(S)-(+)-Ketoprofen
Aspoxicillin		Cefdinir	Iloprost	Adapalene	δ-Aminolevulinic acid
Eflornithine	Butoctamide	Perindopril	Seratrodast	Prulifloxacin	Zanamivir
Enoxacin	Aztreonam	Flunoxaprofen	Felbinac	Ulifloxacin	Bepotastine
Pefloxacin	Acemetacin	Cilazapril	Erdosteine		Orbifloxacin
Etodolac	Oxaprozin	Fosinoprilat	Spirapril	Eicosapentaenoic acid	Pregabalin
Diacerhein	Pirprofen	Nadifloxacin	Ursodiol	Dexibuprofen	Telmisartan
Moexipril	Alminoprofen	Cefaclor	Droxidopa	Sarpogrelate	Ziconotide
Lonidamine	Piretanide	Cefprozil	Tamibarotene	Fosfosal	Triproamylin
Fleroxacin	Norfloxacin	Docosahexaenoic acid	Actarit	Proxigermanium	Prezatide
Bucillamine	Cefotiam	Somatostatin-14	Mofezolac	Betamipron	Nesiritide
Amlexanox	13-*cis*-Retinoic acid	Levocabastine	Ademetionine	Telmesteine	Recombinant human parathyroid hormone (1-84)

Delapril	Gemfibrozil	Proglumide	Nateglinide	Pazufloxacin	Glycopin
Vigabatrin	Clinofibrate	Romurtide	Ceftibuten	Zofenoprilat	Alvimopan
Cefminox	Carprofen	Sermorelin	Limaprost	Salmeterol	Glycyrrhizinic acid
Gabapentin	Cefmenoxime	Cefalexin	Gadopentetate	Eprosartan	Alatrofloxacin
Epalrestat	Triflusal	Pranoprofen	Unoprostone	Febuxostat	Alitretinoin
Cefixime	Levodopa	Frusemide	Tranexamic acid	Angiotensin II	Bexarotene
Alacepril	Carbidopa	Panipenem	Temocapril	Fudosteine	Etacrynic acid
Enalaprilat	Acetylcysteine	Daptomycin	Ibuprofen	alpha-Lipoic acid	Thymalfasin
Acrivastine	Artesunic acid	Tosufloxacin	Flurbiprofen	Trovafloxacin	Enfuvirtide
Sarafloxacin	Sulindac	Trandolapril	Levofloxacin	Folinic acid	Vedaprofen
Tropesin	Tolmetin	Acipimox	Cetirizine	Cefoselis	Bendamustine
Ramipril	Aspirin	Ciprofibrate	Gadoterate	Sitafloxacin	Gemifloxacin
Cefodizime	Indomethacin	Pseudomonic acid	Loracarbef	all-*trans*-Retinoic acid	4-Aminosalicylic acid
Benazepril	Mefenamic acid	Leuprorelin	Gatifloxacin	Gadobenate	Fexofenadine
Moxifloxacin	Cefamandole	Probenecid	Rosoxacin	Ampicillin	Simvastatin
Teriparatide	Amineptine	Valproic acid	Lidofenin	Atorvastatin	Ertapenem
Garenoxacin	5-Aminosalicylic acid	Deferasirox	Naproxen	Mitiglinide	Cefmetazole
Niacin	Chlorambucil	Fenbufen	Diclofenac	Pitavastatin	
Ceftobiprole	Lumiracoxib	Salicylic acid	Meclofenamate	Rosuvastatin	
Levocetirizine	Lodoxamide	Ambrisentan	Sulbactam	Doripenem	
Exenatide	Penicillin V	Bezafibrate	Vancomycin	Montelukast	
Tolfenamic acid	Ceftazidime	Folic acid	Fluvastatin	Ertapenem	
Cefuroxime	Pyrantel	Cinoxacin	Fenoprofen	Penicillin G	

biochemical processes that underscore the importance of this functional group, it is equally important to point to the physiochemical properties of the carboxylate ion which make it an attractive moiety for installation into drug candidates. For example, ionisation of a carboxylic acid, at physiological pH (7.4), improves its ability to hydrogen bond with neighbouring water molecules, and thus may improve its overall water solubility. Likewise, a carboxylic acid group can also serve to promote intermolecular hydrogen bonding at a particular pH, thus resulting in an alteration in a physiochemical property (Figure 3.1). Continuing with the theme of the importance of carboxylic acids to mammalian physiology, it is the intramolecular hydrogen bonding type which results in the three-dimensional structure of proteins.[1]

There may be multiple factors which vary the relative acidity (*i.e.* pKa) of a carboxylic acid group, including neighbouring group and long-range inductive effects. The simple straight- and branched-chain carboxylic acids shown in Table 3.2 depict the attenuation of pKa observed with the introduction of electron-withdrawing neighbouring groups and long-range substitutions;[2] it is noteworthy that the relative acidities of carboxylic acids are also related to their ability to stabilise the developing charge upon ionisation. The hydrophilic nature of a particular drug may govern absorption, distribution and elimination, and thus may bear on its overall *in vivo* disposition in a mammal.

While the ionisation state of the carboxylic acid functional group is an important determinant to a drug's physiochemical properties, it is not necessarily the governing characteristic contributing to the *overall ionisation state* of a

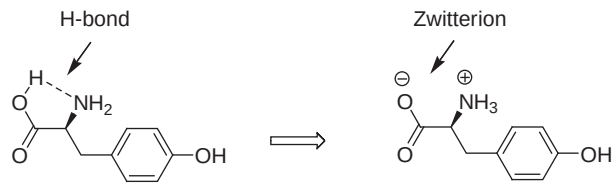


Figure 3.1 Example of intramolecular hydrogen bonding in the amino acid tyrosine yielding a zwitterion intermediate.

Table 3.2 Inductive and neighbouring group effects on the pKa of the carboxylic acid group.

Acid	*pKa*
HCOOH	3.77
(CH$_3$)$_3$CCOOH	5.05
ClCH$_2$COOH	2.86
HOCH$_2$COOH	3.83

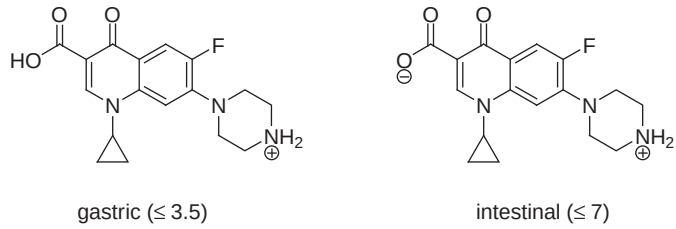


Figure 3.2 Ionisation state of ciprofloxacin within the gastrointestinal tract.

particular drug in a particular *in vivo* compartment. For example, the fluoroquinoline antibiotic ciprofloxacin contains several ionisable groups, including a carboxylic acid group and multiple amine functionalities. Because of the pH gradient unique to the gastrointestinal tract, it is the piperazine moiety (*i.e.* secondary amine) which governs the charge state within the upper gastrointestinal tract (gastric region). A subsequent elevation in pH in the proximal intestine results in a zwitterionic state for ciprofloxacin; importantly it is in the upper gastrointestinal tract where efficient ciprofloxacin absorption occurs (Figure 3.2).[3]

Mammalian systems are quite capable of processing and metabolising a range of endogenous carboxylic acid containing compounds (*e.g.* triglycerides, bile acids, prostanoids and hormone catabolites). These systems include but are not limited to glucuronidation, amino acid and thioester (acyl CoA) conjugation as well as active uptake and efflux transport. It is not surprising then that the physiological mechanisms predisposed to process endogenous substrates are quite suited to metabolise drugs bearing this functional group.

3.2 Carboxylic Acid Containing Non-steroidal Anti-inflammatory Drugs (NSAIDs)

NSAIDs are compounds with analgesic, antipyretic and, in higher doses, anti-inflammatory effects. The term 'non-steroidal' is used to distinguish these compounds from steroids (*e.g.* dexamethasone), which have a similar mode of anti-inflammatory action. There are >20 different NSAIDs currently in worldwide use. All classical NSAIDs introduced in the 1960s and 1970s contain either a free carboxylic acid group or the acidic enol–carboxamide moiety (*e.g.* piroxicam). As shown in Figure 3.3, carboxylic acid based NSAIDs can be subcategorised into: (a) salicylic acid, (b) anthranilic acid, (c) acetic acid, and (d) propionic acid derivatives.

3.2.1 Discovery of Aspirin

The history of NSAIDs dates back many thousands of years to the early uses of plant preparations that contained salicylate.[4] A feasible commercial synthesis of salicylic acid from phenol and carbon dioxide was formulated by Kolbe in

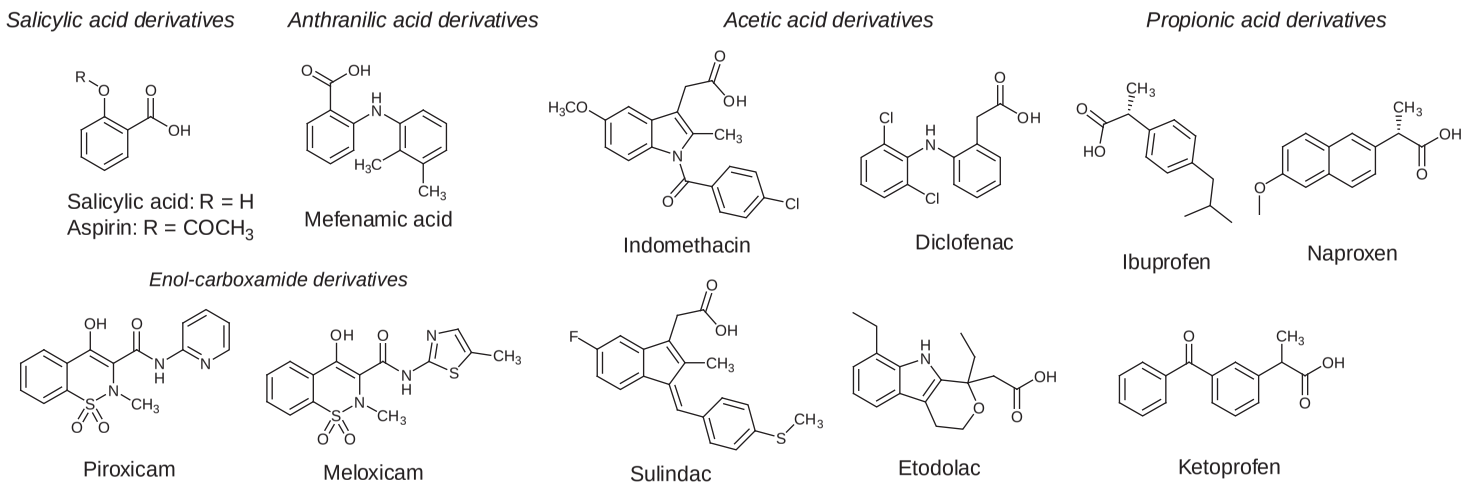
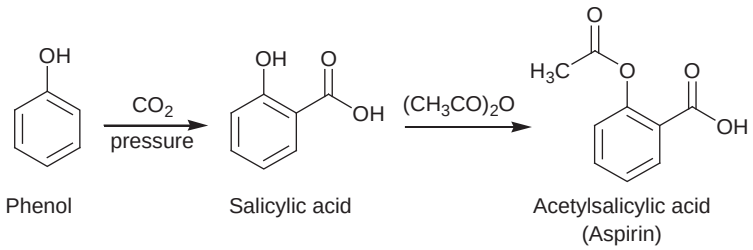


Figure 3.3 Structures of classical NSAIDs.



Scheme 3.1 Synthesis of aspirin, the first carboxylic acid containing NSAID.

1874 (Scheme 3.1), which led to the introduction of sodium salicylate in the treatment for chronic rheumatoid arthritis and gout.

The search for a superior salicylic acid derivative was initiated at Bayer in 1895. Chemist Felix Hoffman, to whom the task was presented, also had a personal reason for this endeavour; his father had been taking salicylic acid for many years to treat arthritis and encountered emesis as a major side effect. Hoffman found a way of acetylating the phenol group of salicylic acid to form acetylsalicylic acid (aspirin) (see Scheme 3.1).[4] After initial laboratory tests, Hoffman's father was given the drug; it was pronounced effective and later confirmed as such in 'impartial' clinical trials. The drug was introduced in 1899 with a report suggesting that aspirin was a convenient way of delivering salicylic acid to the body.[5] That aspirin is a mere prodrug for salicylic acid has been debated since its discovery, but as discussed below, aspirin clearly has potent actions of its own that are not shared by salicylic acid.

It was not until 1971 that Vane and co-workers proposed that the anti-inflammatory and analgesic properties of NSAIDs are due to inhibition of prostaglandin biosynthesis, which is catalysed by the enzyme prostaglandin endoperoxide synthase or cyclooxygenase (COX).[6–8] COX catalyses the formation of prostaglandins and thromboxane from the fatty acid substrate arachidonic acid (itself derived from the cellular phospholipid bilayer by the action of phospholipase A_2).[9]

COX activity originates from two distinct and independently regulated enzymes, termed COX-1 and COX-2.[10,11] COX-1 is the constitutive isoform and is mainly responsible for the synthesis of cytoprotective prostaglandins in the gastrointestinal tract. COX-2 is inducible and short-lived; its expression is stimulated in response to pro-inflammatory mediators and the isozyme plays a major role in prostaglandin biosynthesis in inflammatory cells (monocytes/macrophages).[12] Classical NSAIDs act as non-selective inhibitors of COX-1 and COX-2 isozymes.[13] Inhibition of COX-1 is thought to be responsible for the gastrointestinal liabilities associated with most NSAIDs, while inhibition of the inducible COX-2 isozyme is thought to be responsible for the anti-inflammatory effects.[14] The hypothesis led to substantial research efforts towards the discovery of selective COX-2 inhibitors and has resulted in the introduction of celecoxib, valdecoxib and rofecoxib into the market as the next generation of NSAIDs with reduced gastrointestinal liabilities.[15]

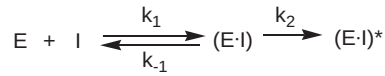


Figure 3.4 Kinetics of COX inhibition by NSAIDs.

3.2.2 Mode of Inhibition of COX Activity by NSAIDs

The interaction of NSAIDs with COX follows a two-step kinetic sequence (Figure 3.4) as originally proposed by Rome and Lands in the 1970s.[16] The first step involves the formation of a rapidly reversible (E · I) complex between COX and NSAIDs, leading to competitive inhibition. The second step is the time-dependent conversion of the initial (E · I) complex to one, [E · I]*, in which the inhibitor is bound more tightly. Formation of the [E · I]* complex occurs in seconds to minutes and is thought to reflect the induction of a subtle protein conformational change.

Based on their mode of inhibition, NSAIDs can be sub-categorised into: (a) competitive inhibitors, (b) slow, tight-binding inhibitors and (c) covalent modifiers.[16] Competitive inhibitors comprises of purely reversible COX inhibitors. Most compounds in this category compete reversibly with the fatty acid substrate, arachidonic acid, for binding at the COX active site. Examples of NSAIDs that fall into this category include ibuprofen, piroxicam, naproxen and mefenamic acid.

Slow, tight-binding inhibitors exhibit more complex kinetics than simple, competitive inhibitors. Like competitive inhibitors, they too form the initial, reversible (E I) complex, but this complex is converted to a more stable (E I)* complex in a time-dependent fashion at an enzyme–inhibitor ratio of 1 : 1.[16,17] *In vitro*, dissociation of the inhibitor from the (E I)* complex occurs very slowly. Examples of NSAIDs in this category include indomethacin, meclofenamic acid and diclofenac. Aspirin is the only NSAID that covalently modifies COX-1 and COX-2.

The mechanism of COX inactivation involves initial, reversible binding at the active site, followed by irreversible acetylation of an active site Ser^{530} residue in the two isozymes (Figure 3.5).[18–20] Ser^{530} is not important for COX activity; mutagenesis of this residue to alanine does not affect catalysis or arachidonate binding, suggesting that covalent modification of this residue by aspirin inhibits COX activity by simply blocking arachidonic acid binding to the COX active site.

3.2.3 Molecular and Structural Basis for COX Inhibition by NSAIDs

Crystal structures of complexes of sheep COX-1, mouse COX-2 and human COX-2 with NSAIDs have been solved.[21–24] Despite their structural diversity and differences in modes of inhibition, all NSAIDs bind in the substrate

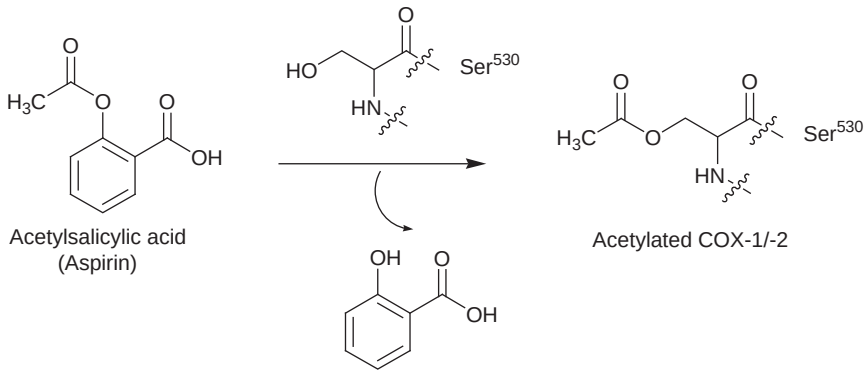


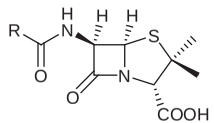
Figure 3.5 Covalent modification of COX-1 and COX-2 by aspirin.

access channel with their carboxylic acid moiety ion paired to an active site Arg^{120} residue. The Arg^{120} residue also ion pairs with the carboxylate of arachidonic acid. Site-directed mutagenesis of the arginine residue in COX-1 to glutamine or glutamate renders the protein resistant to inhibition by carboxylic acid containing NSAIDs.[25,26] Crystallisation of COX-1 acetylated by bromoacetylsalicyclic acid not only confirms Ser^{530} acetylation but also reveals a salicylate ion-paired to Arg120.[22] Arg^{120} is part of a hydrogen bonding network with Glu^{524} and Tyr^{355} which stabilises substrate–inhibitor interactions and closes off the upper part of the COX active site from the spacious opening at the base of the channel. Disruption of this hydrogen bonding network opens the constriction and enables substrate–inhibitor binding and release to occur.

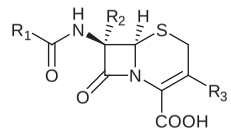
It is important to note that selective COX-2 inhibitors are actually competitive inhibitors of both COX-1 and COX-2, but exhibit selectivity for COX-2 in the time-dependent step by binding tightly at the active site and causing a conformational change in the isozyme structure (see Chapter 5 for a detailed description of sulfonamide-based selective COX-2 inhibitors).

3.3 Carboxylic Acid Containing β-Lactam Antibiotics

β-Lactam antibiotics are a broad class of antibacterial agents that include penicillin derivatives, cephalosporins and cephamycins.[27–29] Common structural features in these compounds is the presence of the β-lactam nucleus and a carboxylic acid moiety (Figure 3.6). The differences in these various derivatives (other than chemical structure) are related to absorption properties, resistance to penicillinases and specificity for organisms for which they are most effective.[27–29] Penicillin antibiotics are historically significant because they were the first drugs that were effective against many previously serious diseases such as tuberculosis, syphilis, and staphylococcus infections.

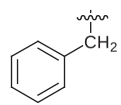


General scaffold of penicillins

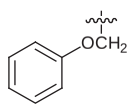


General scaffold of cephalosporins/cephamycins

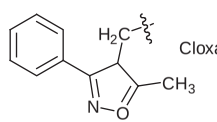
Penicillin G (Bicillin): R =



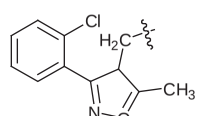
Penicillin V: R =



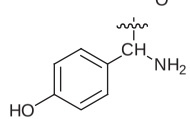
Oxacillin (Bactocill): R =



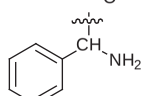
Cloxacillin (Cloxapen): R =



Amoxicillin: R =

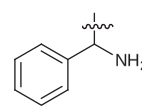


Ampicillin: R =



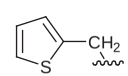
Cephalosporins: R_2 = H; Cephamycins: R_2 = OCH_3

Cefaclor: R_1 =



R_2 = H R_3 = Cl

Cefoxotin: R_1 =



R_2 = OCH_3 R_3 =

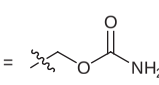


Figure 3.6 β-Lactam antibiotics.

3.3.1 Discovery of Penicillins

The discovery of penicillin in 1928 is attributed to Scottish scientist and Nobel laureate, Sir Alexander Fleming, who noticed a green mould growing in a culture of *Staphylococcus aureus;* where the two had converged, the bacteria were lysed.[30–32] This led to the discovery of penicillin, which was produced by the mould. This serendipitous observation began the modern era of antibiotic discovery. In the early 1940s, the chemical structure of penicillin was determined by Dorothy Crowfoot Hodgkin, while a team of Oxford scientists led by Sir Howard Florey and Ernst Boris Chain demonstrated the *in vivo* bacterial action of penicillin and also discovered a method of producing the drug in adequate quantities to treat humans.[30–32] Florey and Chain shared the 1945 Nobel Prize in Medicine with Fleming for their work. Strides in the fermentation technology arena and the discovery of moulds containing the highest quality of penicillin allowed the mass production of the drug; approximately 2.3 million doses were prepared in time for the Allied invasion of Normandy in the spring of 1944.

3.3.2 Mechanism of Action of β-Lactam Antibiotics

Unlike sulfonamide antibacterial agents that exhibit bacteriostatic activity, penicillins and cephalosporins/cephamycins are bacteriocidal, *i.e.* they destroy existing bacteria.[33,34] The biosynthesis of bacterial cell wall peptidoglycan is catalysed and controlled in its final stages by a class of transpeptidase enzymes, which act on the D-alanyl-D-alanine peptide appendages of glucosyl polysaccharides. The enzymes, after removing the COOH-terminal D-alanine, use the new carbonyl to form a peptide bond with an amino acceptor group on a neighbouring polysaccharide peptide. This transpeptidation produces a cross-linked cell wall network. Wall biosynthesis is inhibited by penicillins and cephalosporins.[34] The β-lactam is able, because of a structural resemblance to the D-alanyl-D-alanine segment, to compete with the catalytic process and form a transient penicilloyl–enzyme complex (Figure 3.7); the biochemical phenomenon has been proven *via* solution of the crystal structures of cephalosporin bound to a bifunctional serine-type D-alanyl-D-alanine carboxypeptidase/transpeptidase.[35–37] Penicillins function as affinity labels of the peptidoglycan transpeptidase by irreversibly acylating a catalytically active serine residue (see Figure 3.7); covalent binding at the active site prevents the substrate from binding.[38–42] The beauty of the penicillins (and cephalosporins) is that the β-lactam ring is not exceedingly reactive; consequently, few non-specific acylation reactions occur with these molecules in mammals.

Although penicillins are 'wonder drugs' in their activity against a variety of bacteria, and are still used widely today, many strains of bacteria have become resistant to their effects. This has been attributed to the excretion of the enzyme β-lactamase in resistant bacteria, which catalyses the hydrolysis of the β-lactams covalently attached to the target transpeptidase enzyme.[43] In the 1970s, several naturally occurring β-lactams, which lacked the general penicillin or

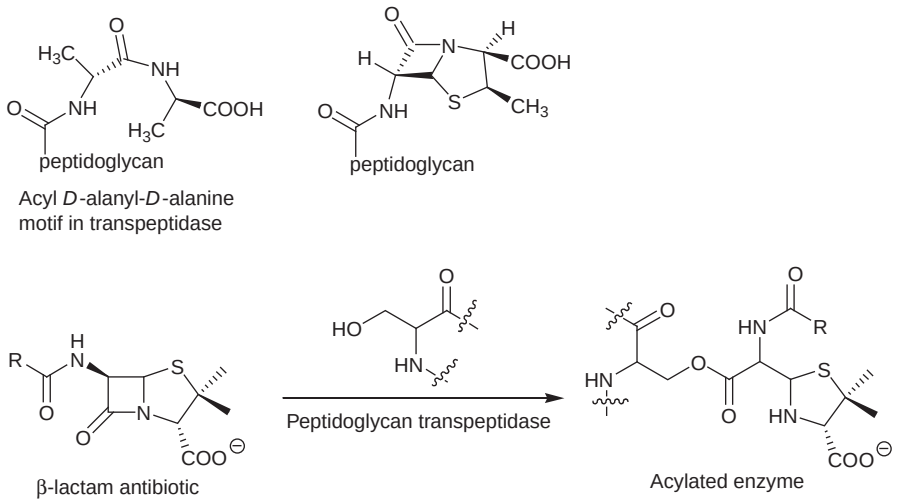


Figure 3.7 Mechanism of pharmacological action of β-lactam antibiotics.

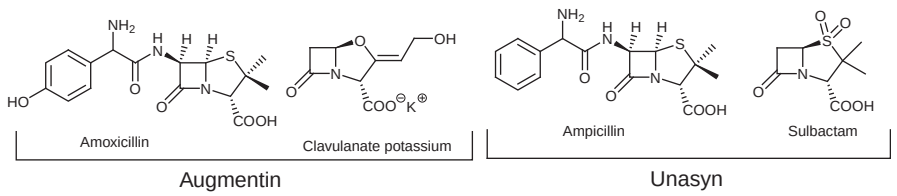


Figure 3.8 Penicillin–β-lactamase inhibitor combination to combat bacterial resistance.

cephalosporin structure were isolated from various organisms and were found to be potent mechanism-based inactivators of β-lactamases (Figure 3.8).[44,45] These compounds are used in combination with penicillins to destroy penicillin-resistant strains of bacteria. For example, the combination of amoxicillin and clavulanate (a β-lactamase inactivator) is sold as Augmentin, and ampicillin plus sulbactam is sold as Unasyn (see Figure 3.8).[46–48] The β-lactamase inhibitors have no antibiotic activity, but they protect the penicillin from destruction so that it can interfere with cell wall biosynthesis.

3.4 Carboxylic Acid Containing Statins

The discovery of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors—called statins—was a breakthrough in the prevention of hypercholesterolemia (high cholesterol) and related diseases. Hypercholesterolemia is considered to be one of the major risk factors for atherosclerosis,

which often leads to cardiovascular, cerebrovascular and peripheral vascular diseases.[49,50] HMG-CoA reductase is the rate-limiting enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol and other isoprenoids.[51,52] Inhibition of this enzyme in the liver by statins results in decreased cholesterol synthesis and an increased synthesis of low-density lipoprotein (LDL) receptors, which leads to the increased LDL clearance from the bloodstream, ultimately reducing the risk of atherosclerosis and diseases caused by it.[51–54]

3.4.1 Discovery of the Statins

Over hundred years ago, a German pathologist named Rudolph Virchow discovered the presence of cholesterol in the arterial walls of humans that died from occlusive vascular diseases such as myocardial infarction.[54] In the 1950s, the Framingham heart study led by Thomas Royle Dawber revealed the correlation between high blood cholesterol levels and coronary heart diseases;[55] this led scientists to explore novel ways of lowering cholesterol levels without significant changes in diet and lifestyle. Because the primary goal was to inhibit cholesterol biosynthesis in the body, HMG-CoA reductase became a natural target. In the 1970s, Akira Endo and Masao Kuroda initiated research into inhibitors of HMG-CoA reductase.[56] The Japanese team reasoned that certain microorganisms may produce inhibitors of this enzyme as a defence mechanism against other organisms, as mevalonate is a precursor of many substances required by organisms for cell wall maintenance. During the course of these studies, the team isolated mevastatin (Figure 3.9), a potent HMG-CoA inhibitor from a fermentation broth of *Penicillium citrinum*.[56,57] Likewise, in 1978, Alfred Alberts and co-workers at Merck discovered a new natural product-based HMG-CoA reductase inhibitor in a fermentation broth of *Aspergillus terreus*, which later became known as lovastatin (Figure 3.9)—the first commercially marketed statin.[58] Commercially available statins are categorised into two groups: fermentation-derived and synthetic. Fermentation-derived statins include lovastatin, simvastatin, and pravastatin, whereas, synthetic statins include atorvastatin, fluvastatin, cerivastatin, pitavastatin and rosuvastatin (see Figure 3.9).

In addition, statins have sometimes been grouped according to their structure into type 1 and type 2 statins.[59] Type 1 statins (*e.g.* lovastatin, pravastatin and simvastatin) possess a substituted decaline-ring structure which resembles mevastatin. Type 2 statins (*e.g.* atorvastatin, cerivastatin, fluvastatin and rosuvastatin) have larger hydrophobic groups linked to the carboxylic acid side chain.

One of the main differences between the type 1 and type 2 statins is the replacement of the butyryl group in type 1 statins with a fluorophenyl group in type 2 statins (see Figure 3.9). The fluorophenyl group is responsible for additional polar interactions that causes tighter binding of the statin to the HMG-CoA reductase enzyme.[59] As discussed later in great detail, the type 1 statins simvastatin and lovastatin are commercially available in the inactive lactone forms; they undergo metabolism to their active hydroxy acid forms

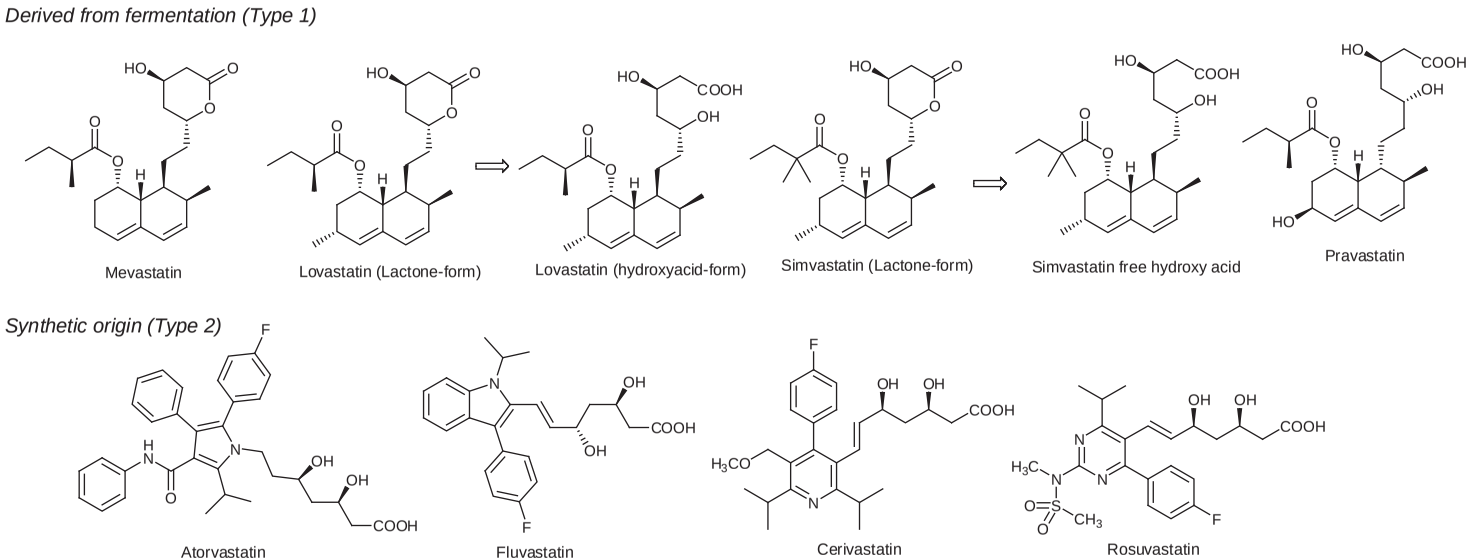


Figure 3.9 Categorisation of statins based on origin and chemical structure.

in vivo. In contrast, type 2 statins are commercially available in their active hydroxy acid forms.

3.4.2 Molecular and Structural Basis for Inhibition of HMG-CoA Reductase by Statins

Biochemical studies have shown that statins bind reversibly to the HMG-CoA reductase enzyme with nanomolar range affinity. The affinity of the natural substrate, 3-hydroxy-3-methylglutaryl-CoA towards conversion to malonyl-CoA (Figure 3.10) is in the micromolar range.[60,61]

The essential structural components of all statins are a dihydroxyheptanoic acid unit and hydrophobic ring system architecture. The statin pharmacophore is a modified hydroxyglutaric acid component, which is structurally analogous to the endogenous substrate and product transition state intermediate (see Figure 3.10). Because HMG-CoA reductase reveals a stereoselective bias in statin binding, all statins require a 3*R*,5*R* stereochemistry in the dihydroxyheptanoic acid unit for inhibition. Co-crystallisation of HMG-CoA reductase with statins reveals that the carboxylic acid inhibitors exploit a shallow hydrophobic groove to accommodate their hydrophobic domains.[59,62–64]

The specificity and the tight binding of statins is due to orientation and bonding interactions that form between the statin and the HMG-CoA reductase. Polar interactions are formed between the HMG-like moiety in the statin and residues that are located in the *cis* loop of the enzyme. The terminal carboxylic acid group in statins forms a salt bridge with a positively charged lysine (Lys^{735}) residue in the active site. In addition, Lys^{691} participates in a hydrogen bonding network with Glu^{559}, Asp^{767} and the hydroxyl group of the hydroxyglutartic acid component in statins.[59]

3.5 Carboxylic Acid Containing Fibrates

Fibrates are a class of lipophilic carboxylic acid derivatives which are used in accessory therapy in many forms of hypercholesterolemia, usually in combination with statins.[65] Although less effective in lowering LDL than statins,

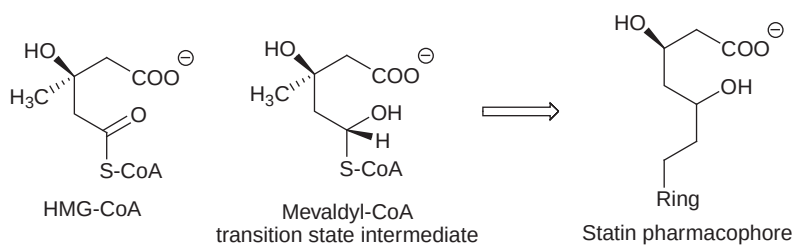


Figure 3.10 Molecular/structural basis for inhibition of HMG-CoA reductase by statins—the statin pharmacophore.

fibrates improve high-density lipoprotein (HDL) by ~20–30% and triglyceride levels by ~40%.[65,66]

Although used clinically since the 1930s, the mechanism of action of fibrates remained unelucidated until it was discovered in the 1990s that fibrates activate PPAR (peroxisome proliferator-activated receptors), especially PPARα in muscle, liver and other tissues.[67,68] The PPARs comprise a family of ligand-activated transcription factors that play a key role in lipid homeostasis *via* modulation of carbohydrate, fat metabolism and adipose tissue differentiation. There are three members of the family: PPARα, PPARδ(or β) and PPARγ.[69] In humans, PPARα activation results in increased clearance of triglyceride-rich very low-density lipoprotein and upregulation of ApoA1, the principal lipoprotein component of HDL.[70] As a consequence, the fibrates lower triglyceride and raise HDL levels. Fibrates prescribed commonly include fenofibrate, gemfibrozil, clofibrate, ciprofibrate and bezafibrate (Figure 3.11). Fenofibrate and clofibrate are sold as the corresponding ester derivatives; the active metabolites, responsible for the pharmacological activity, are fenofibric acid and clofibric acid, respectively.

Though effective for dyslipidemia, fibrates are weak PPARα agonists (EC_{50} ~30–50 μM) and their subtype selectivity is poor. Much research effort has been invested within the pharmaceutical industry to improve upon the potency of human PPARα agonism and several groups have reported the discovery of potent PPARα agonists (EC_{50}'s in the low nanomolar range) and their effects in animal models of dyslipidemia.[71–74] The archetypal small molecule PPAR agonists are structurally divided into three parts, for example, (1) a carboxylic acid head piece, (2) a linker part, and (3) a hydrophobic tail part. Examples of some novel, selective PPARα agonists are shown in Figure 3.12 Increase in the

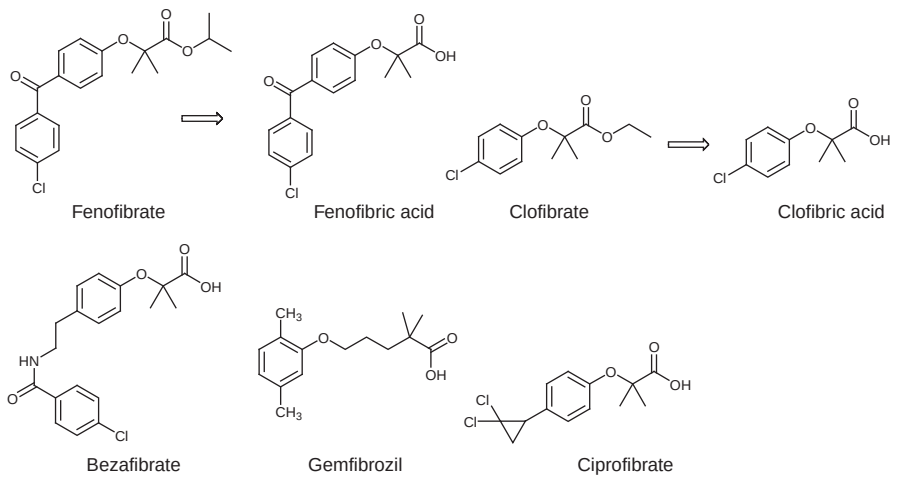


Figure 3.11 Fibrates in the treatment of dyslipidemia.

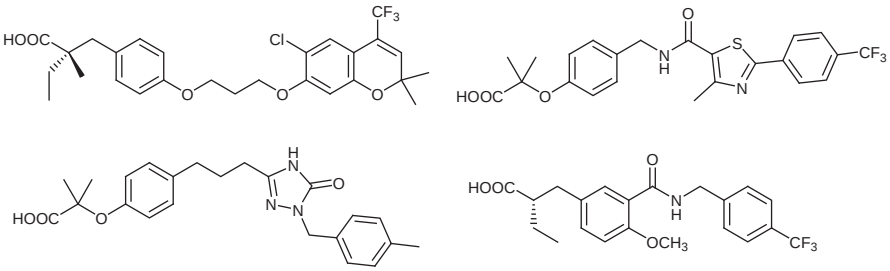


Figure 3.12 Novel PPARα agonists for the potential treatment of dyslipidemia.

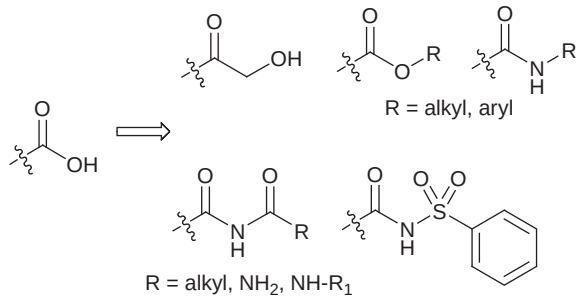
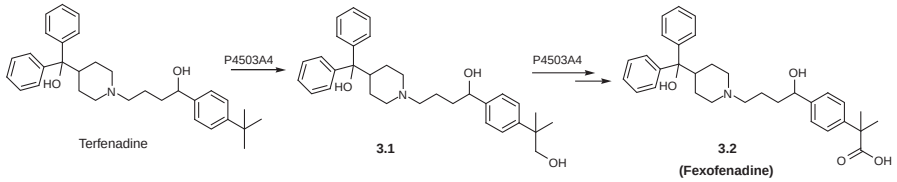


Figure 3.13 Non-classical bioisosteres of the hydroxyl group in a carboxylic acid moiety.

amphipathic (lipophilic) character generally results in improved PPAR agonism against the respective subtypes.

3.6 From Terfenadine to Fexfofenadine–an Interesting Case Study on the Utility of the Carboxylic Acid Moiety in Drug Discovery

The selective H_1 antihistamine terfenadine (Seldane®) (Figure 3.13) was the first non-sedating antihistamine to be introduced for the treatment of allergic rhinitis. It exhibited little or no incidence of central nervous system (CNS) sedative effects associated with the first-generation antihistamines.[75,76] Since its introduction in the 1980s, terfenadine ranked as one of the most widely prescribed drugs in the United States. However, a published report of ventricular arrhythmia associated with terfenadine use first appeared in 1990, wherein a patient developed *Torsades de Pointes* while on the recommended daily doses of terfenadine concomitantly with cefaclor, ketoconazole and progesterone.[77] The risk of cardiac arrhythmia caused by QT interval prolongation was traced back to excessively high serum concentrations of terfenadine, which occurred due to



Scheme 3.2 Discovery of non-cardiotoxic and non-sedating H_1 antagonist fexofenadine.

ketoconazole-mediated inhibition of its principal metabolic elimination pathway.[78–80]

Terfenadine is extensively metabolised by cytochrome P450 (P450) 3A4 *via* initial hydroxylation on its *t*-butyl group to the primary alcohol metabolite **3.1** followed by its oxidation to the corresponding carboxylic acid metabolite **3.2**[81] (Scheme 3.2). Under normal recommended dosages, terfenadine is not associated with cardiotoxicity because very low, free systemic concentrations of terfenadine are achieved *in vivo*, a consequence of its extensive and rapid first pass metabolism.[82] However, toxic effects on the heart's rhythm and electrical conduction such as ventricular tachycardia and *Torsades de Pointes* are discerned upon concomitant administration of terfenadine with P4503A4 inhibitors.

In early 1997, given the increased number of cases of pharmacokinetic interactions between terfenadine and P450 3A4 inhibitors,[83,84] the US Food and Drug Administration (FDA) recommended that terfenadine be removed from the market and that physicians consider alternative medications for their patients. Terfenadine was formally removed from the US market in late 1997.

Of much interest against this backdrop were the findings that the carboxylic acid metabolite **3.2** retained all the primary pharmacology (non-sedating H_1 antagonism) associated with terfenadine and that *in vivo* metabolite **3.2** appears to exert most, if not all, of the pharmacological actions associated with the administration of the parent compound.[82] Importantly, **3.2** was devoid of the cardiotoxic potential associated with the parent compound in the clinic; subsequently, **3.2** (later named as fexofenadine) replaced terfenadine on the market.[85]

3.7 Bioisosteres of the Carboxylic Acid Moiety

Non-classical bioisosteres for the carboxylic acid moiety consists of replacements which involve (a) only the hydroxyl portion or (b) both the hydroxyl and carbonyl fragments of the functional group. The determination of suitable replacements for the carboxylic acid group is often based on the ability of the bioisostere to possess similar acidity and to exhibit similar physiochemical properties.

3.7.1 Non-classical Bioisosteres of the Hydroxyl Portion of the Carboxylic Acid Group

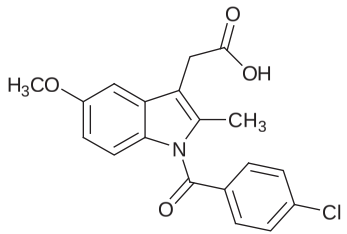
The types of non-classical bioisosteres typically used as hydroxyl replacements are similar to the non-classical bioisosteres of the phenolic hydroxyl group.[86] Of the prototypic fragments shown in Figure 3.13, replacement of the hydroxyl group in the COOH motif with a phenylsulfonamide results in the formation of a acylsulfonamide, which possesses a pKa comparable to the carboxylic acid moiety (see Chapter 5 for a detailed description of this bioisostere).

Because the molecular size of these individual fragments is larger than that of the hydroxyl group,[86] these non-classical bioisosteres are unlikely to be suitable in cases where pharmacological activity is adversely effected by an increased molecular size in the vicinity of the carboxylic acid group. These non-classical bioisosteres tend to be most effective in those instances where the role of the hydroxyl group in the carboxylic acid group is to act as either a hydrogen bond acceptor or donor. An interesting example where non-classical bioisosteres of the hydroxyl group significantly impacted primary pharmacology is evident with recent studies on the conversion of certain non-selective NSAIDs into non-ulcerogenic, selective COX-2 inhibitors.[87–89]

3.7.1.1 Neutral Derivatives of Non-selective Carboxylic Acid Containing NSAIDs as Selective COX-2 Inhibitors

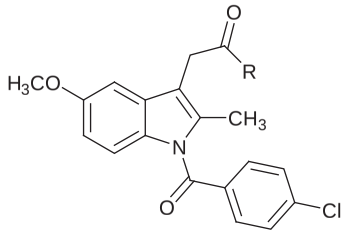
Derivatisation of the carboxylic acid moiety in the selective COX-1 inhibitors from the arylacetic and anthranilic acid class of compounds—exemplified by indomethacin and meclofenamic acid, respectively—affords potent and selective COX-2 inhibitors (Figure 3.14).[87–89] Within the indomethacin series, esters (compounds **3.3** and **3.4**) and primary and secondary amides (compounds **3.5** and **3.6**) are superior to tertiary amides (compound **3.7**) as selective inhibitors. Furthermore, increase in the lipophilicity of the amide and/or ester substituent also increased COX-2 inhibitory potency and selectivity (see Figure 3.14—compounds **3.3** and **3.5** and compounds **3.4** and **3.6**). Elaboration of structure–activity relationship (SAR) efforts also led to the discovery of the corresponding reverse esters (*e.g.* compound **3.8**) and reverse amides (*e.g.* compound **3.9**) of indomethacin as selective COX-2 inhibitors.[90]

Inhibition kinetics reveal that the neutral indomethacin derivatives behave as slow, tight-binding inhibitors of COX-2 and that selectivity is a function of the time-dependent step, as is the case with the diarylheterocycle-based COX-2 inhibitors celecoxib, valdecoxib and rofecoxib. Studies with site-directed COX-2 mutants, however, indicated that the molecular basis for COX-2 selectivity of indomethacin derivatives differs from the parent NSAIDs and from diarylheterocycles. For example, the Arg^{120} residue, which is a critical determinant of fatty acid substrate arachidonate as well as inhibitor binding, is not important for inhibition by indomethacin amides and esters. COX-2 selectivity was shown to arise from novel interactions at the opening and at the apex of the arachidonic acid-binding site.[87,89,90] In *in vivo* animal models of acute inflammation,



Indomethacin

IC_{50} (COX-2) ~ 0.01 μM
IC_{50} (COX-1) ~ 0.006 μM

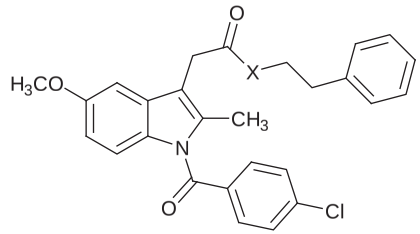


3.3: R = OCH_3

IC_{50} (COX-2) ~ 0.25 μM
IC_{50} (COX-1) ~ 33 μM

3.4: R = NH_2

IC_{50} (COX-2) ~ 0.70 μM
IC_{50} (COX-1) ~ 25 μM

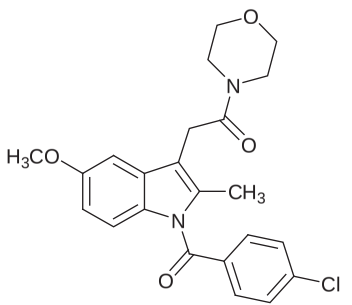


3.5: R = O

IC_{50} (COX-2) ~ 0.05 μM
IC_{50} (COX-1) > 66 μM

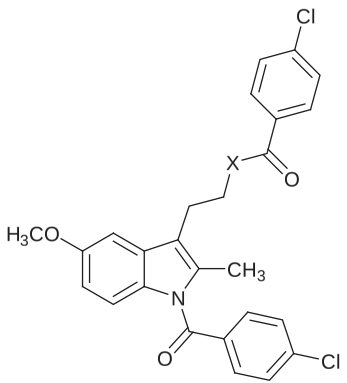
3.6: R = NH

IC_{50} (COX-2) ~ 0.06 μM
IC_{50} (COX-1) > 66 μM



3.7

IC_{50} (COX-2) > 33 μM
IC_{50} (COX-1) > 66 μM

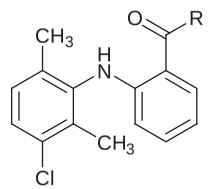


3.8: R = O

IC_{50} (COX-2) ~ 0.05 μM
IC_{50} (COX-1) > 66 μM

3.9: R = NH

IC_{50} (COX-2) ~ 0.05 μM
IC_{50} (COX-1) > 66 μM



Meclofenamic acid: R = OH

IC_{50} (COX-2) ~ 0.05 μM
IC_{50} (COX-1) ~ 0.04 μM

3.10: R = $NH(CH_2)_2OC_6H_5$

IC_{50} (COX-2) ~ 0.15 μM
IC_{50} (COX-1) ~ 66 μM

Figure 3.14 Neutralisation of the carboxylic acid group in non-selective COX inhibitors indomethacin and meclofenamic acid yields selective COX-2 inhibitors.

candidate compounds were also shown to possess oral anti-inflammatory activity without the ulcerogenic effects associated with the parent NSAIDs.[87] In a manner similar to indomethacin, some secondary amide derivatives of the fenamic acid NSAID, meclofenamic acid, also demonstrated potent and selective COX-2 inhibition.[89] The 2-phenoxyethylamide derivative **3.10** was the most selective inhibitor, with a COX-2 selectivity ratio of ~440 (Figure 3.14). Unlike indomethacin SAR, only the amide derivatives of meclofenamic acid demonstrated COX-2 selectivity. The esters were either inactive or non-selective COX inhibitors. The reason(s) for this discrepancy is unclear. Finally, it is interesting to note that simple derivatisation involving amidation and/or esterification is not a universal strategy to convert all traditional carboxylic acid NSAIDs into selective COX-2 inhibitors. For instance, amidation or esterification of naproxen, sulfindac and/or ibuprofen yields inactive compounds (A. S. Kalgutkar, unpublished observations).

3.7.2 Non-classical Bioisosteres of the Entire COOH Moiety

Non-classical bioisosteres as replacement of the entire carboxylic acid group are also widely known; in particular, sulfonamides, tetrazolyl and thiazolidinedione derivatives as non-classical bioisosteric replacements have yielded many commercially successful medicines. Of late, the boronic acid group has also emerged as a carboxylic acid surrogate and has resulted in the discovery of the peptidyl proteasome inhibitor, bortezomib. Discussion on the sulfonamide group as a non-classical bioisostere of the carboxylic acid moiety is provided in Chapter 5.

3.7.2.1 5-Substituted-1H-tetrazoles as Carboxylic Acid Bioisosteres

5-Substituted-1*H*-tetrazoles are excellent non-classical bioisosteres of carboxylic acid derivatives.[91–94] Because of the presence of a free N–H bond, the tetrazole moiety can exist in a ~1:1 ratio of the 1*H*- and 2*H*-tautomeric forms (Figure 3.15). The free N–H bond in tetrazole analogs also imparts acidity due to its ability to stabilise negative charge *via* electron delocalisation. Both aliphatic and aromatic tetrazoles possess pKa values (~4.5–4.9) which are comparable to carboxylic acids.[95–97] Like their carboxylic acid counterparts,

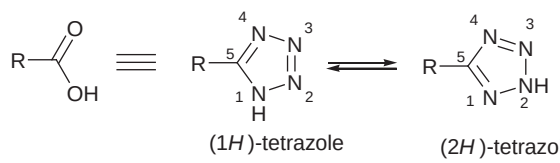


Figure 3.15 5-Substituted-1*H*-tetrazoles as non-classical bioisosteres of carboxylic acids.

tetrazoles are ionised at physiological pH; however, it is important to note that tetrazoles in the anionic form are ~10-fold more lipophilic than the corresponding carboxylate derivatives.[98]

3.7.2.2 *Tetrazole-based Angiotensin II Receptor Antagonists: the Discovery of Losartan and Related Analogs*

The renin–angiotensin system (RAS) plays a key role in blood pressure regulation and sodium balance.[99] RAS can be interrupted at various levels; it can be blocked either by inhibition of rennin or angiotensin-converting enzyme, or *via* direct antagonism of the G protein-coupled angiotensin II receptors.

The angiotensin II type I (AT_1) receptor is the best elucidated in terms of its biochemistry and signalling functions. The receptor is activated by the vasoconstricting octapeptide angiotensin II, which in turn results in an increase in cytosolic Ca^{2+} concentrations (through activation of phospholipase C). Amongst other actions, blockade of the AT_1 receptor directly causes vasodilation, reduces secretion of vasopressin, reduces production and secretion of aldosterone; the combined effect of which is reduction of blood pressure.

In the 1990s, numerous pharmaceutical companies were engaged in research directed at discovering novel AT_1 receptor antagonists for the treatment of hypertension. Although potent peptide-based AT_1 receptor antagonists (*e.g.* saralasin) have been used as pharmacological tools for many years, the therapeutic utility has been limited primarily due to poor oral bioavailability and significant agonist activity at the receptor.[100,101]

Figure 3.16 depicts key medicinal chemistry milestones that led to increasing more potent and, eventually, orally bioavailable *non-peptide* AT_1 receptor antagonists. Benzimidazole derivatives **3.11** and **3.12** were initially disclosed by Takeda as non-peptide hits that demonstrated weak but selective AT_1 receptor antagonist properties.[102,103] Another attractive feature was the lack of functional agonist activity at the receptor.[102,103] Because the C-terminus carboxylic acid group in angiotensin II is essential for binding to the receptor,[104] researchers at Du Pont decided to incorporate a second carboxylic acid moiety on the *N*-benzyl group in **3.11** and **3.12**, which led to **3.13**, a vastly improved AT_1 receptor antagonist.[105] Further improvements in potency were gained by 'enlarging' the molecular size and lipophilicity of **3.13**; although analogs such as **3.14** and **3.15**, which incorporated an additional phenyl ring *via* amide, ether, amine and/or thiol linkage exhibited significant gains in antagonist potency, the compounds were devoid of oral bioavailability in preclinical species.[105,106]

A breakthrough was achieved when the biphenyl carboxylic acid derivative EXP7711 (compound **3.16**) was synthesised and shown to be an orally active AT_1 receptor antagonist.[106] While orally active, **3.16** was slightly less potent than **3.14** in inhibiting angiotensin II binding.[107] In the hope of further improving oral activity and potency of the biphenyls, a number of carboxylic acid bioisosteres were evaluated.[108] The tetrazole bioisostere proved to be the

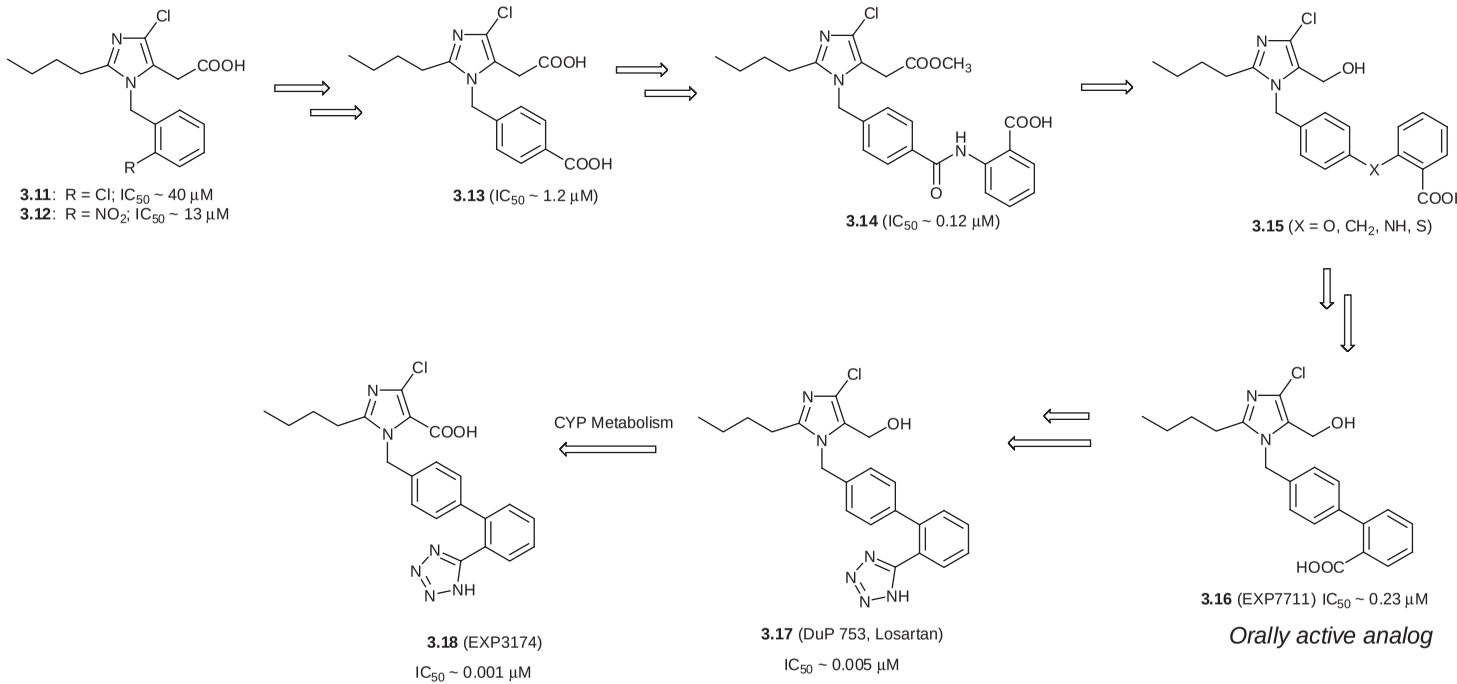


Figure 3.16 Historical overview of the discovery of the tetrazole-based angiotensin II receptor antagonist, losartan.

key in the discovery of the first non-peptide AT_1 receptor antagonist losartan (DuP 753) (**3.17**) with good oral bioavailability, vastly improved antagonist potency ($IC_{50} \sim 5.5$ nM) and a long duration of action.[109–111] It is noteworthy to point out that the carboxylic acid metabolite of losartan, *i.e.* EXP3174 (compound **3.18**), is an active metabolite with greater AT_1 receptor antagonism than the parent compound.[112,113]

The value of the tetrazole motif in the discovery of novel, selective and orally-active AT_1 receptor antagonists is clearly evident from the fact that five out of the six drugs in this class that are currently marketed for the treatment of hypertension contain the tetrazole group. The list includes losartan, irbesartan, olmesartan medoxomil (active metabolite: olmesartan), valsartan and candesartan cilexetil (active metabolite: candesartan); telmosartan contains a carboxylic acid group instead of the tetrazole motif (Figure 3.17). It is important to note that site-directed mutagenesis studies have provided evidence that the tetrazole moiety in the non-peptide antagonists interacts with a protonated lysine and histidine at the recognition site of the AT_1 receptor in a manner similar to the interaction of the carboxy terminus of the natural ligand angiotensin with the receptor.[114,115]

3.8 Absorption, Distribution, Metabolism and Excretion (ADME) Profile of Carboxylic Acids

Structural diversity within the carboxylic acid based drugs results in subtle differences in physiochemical properties [*e.g.* molecular weight (MW), clogP, log D] which then influences aqueous and lipid solubility, and subsequently the pharmacokinetic disposition profile.

3.8.1 Oral Absorption

With the exception of some β-lactam-based antibiotics, most small molecule carboxylic acid based drugs are administered by the oral route and hence absorption into the target tissue (*e.g.* liver in the case of statins and systemic circulation in the case of NSAIDs) is essential for their pharmacological action. As such, several marketed carboxylic acid drugs obey Lipinski's 'rule of five' (see Section 2.1.1) for good oral absorption; Table 3.3 depicts the aqueous solubility, Caco-2 cell permeability and human oral bioavailability characteristics of a series of low MW NSAIDs.[116]

The presence of the free carboxylic acid group in drugs also provides a convenient handle for preparation of salts to improve aqueous solubility (and therefore oral absorption and bioavailability). In other cases, the carboxylic acid moiety can be neutralised to corresponding ester prodrug derivatives to improve oral absorption profile by virtue of improved membrane permeability especially in the case of polar carboxylate analogs (*e.g.* certain β-lactam antibiotics).

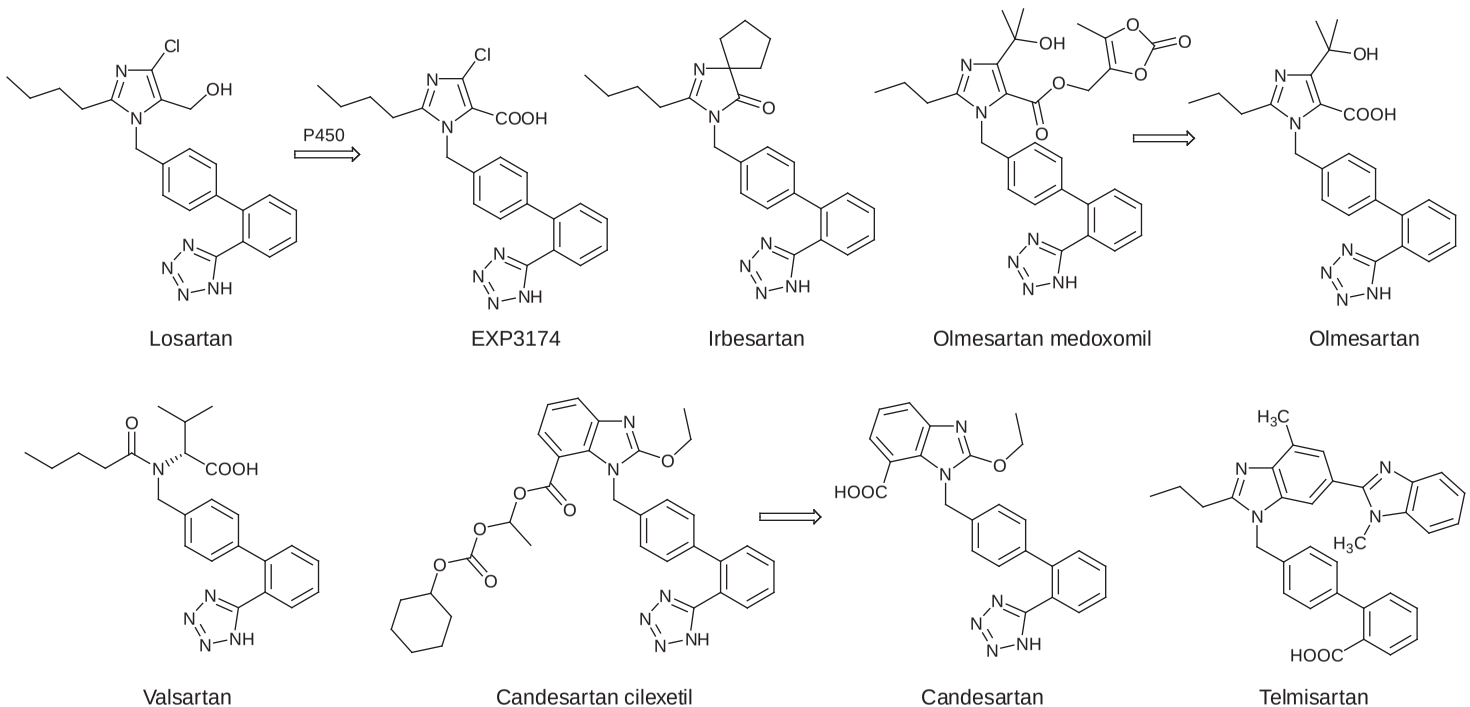
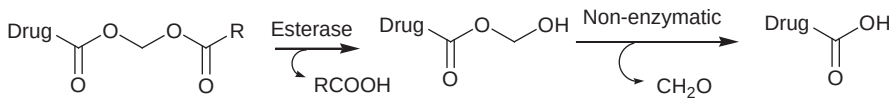


Figure 3.17 Structures of the marketed angiotensin II receptor antagonists for treatment of hypertension.

Table 3.3 Structure, dose, Caco-2 permeability and solubility characteristics of NSAIDs.

Compound	MW	High dose (mg)	Oral F (%)	P_{app} (Caco-2)[a]		Equilibrium solubility[b]	
				AB	BA	pH 1.2	pH 7.4
Diclofenac	295	50	54	20.2	21.3	1.0	15 900
Indomethacin	357	50	98	10.4	24.5	1.0	1300
Ibuprofen	206	800	>80	9.60	19.2	60	2300
Sulindac	356	200	88	6.30	12.2	7.0	1300
Aspirin	180	975	68	25.5	19.1	6200	6400
Ketorolac	255	20	100	4.30	18.6	110	1300
Naproxen	230	500	99	12.3	20.0	5.0	2500
Diflunisal	250	500	90	12.5	17.0	3.0	2500
Salicylic acid	138	750	100	17.6	20.5	180	2400

[a] $P_{app} \times 10^6$ cm sec^{-1}.
[b] μg mL^{-1}.



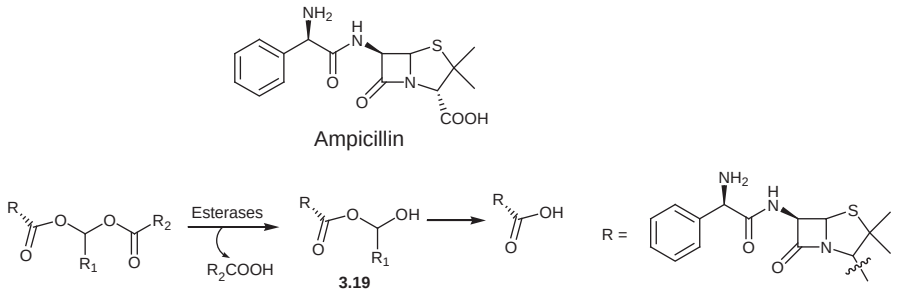
Scheme 3.3 Tripartate prodrug concept.

3.8.1.1 *Carboxylic Acid Prodrugs as a Tactic to Improve Oral Absorption*

The carboxylic acid group can be coupled with various alcohol derivatives to afford neutral ester analogs. Because, esterases are ubiquitous in mammals and can hydrolyse structurally diverse substrates, metabolic regeneration of the parent carboxylic acid drug is often a facile process. It is possible to prepare ester derivatives with virtually any degree of hydrophilicity or lipophilicity; furthermore, electronic and/or steric factors (on the carboxylic acid or alcohol moieties) can be manipulated to control the rates of enzymatic hydrolysis and therefore ester stability.

In some cases, prodrugs can be ineffective because the ester bond is too labile or too stable. A remedy towards this situation is the design of tripartate (self-immolative) prodrugs where the carrier is linked to the drug *via* a linker group (Scheme 3.3).[117,118] This feature allows for different kinds of functionalities to be incorporated for varying stabilities; it also displaces the drug farther from the hydrolysis site, which decreases steric interference by the carrier. The drug–linker connection, however, must be designed so that it cleaves spontaneously (*i.e.* is self-immolative) after the carrier has detached. A practical approach to accomplish this is *via* the double prodrug as shown in Scheme 3.3.

The tripartate prodrug strategy has been employed to improve the oral absorption of ampicillin (Scheme 3.4). The absorption of ampicillin in healthy



Bacampicillin: $R_1 = CH_3$; $R_2 = OC_2H_5$
Pivampicillin: $R_1 = H$; $R_2 = t$-Bu

Scheme 3.4 Double ester prodrugs of ampicillin: discovery of bacampicillin and pivampicillin.

humans was shown to be dose-dependent with decreased absorption at higher doses. In healthy humans, the absorption decreased from 72% to 45% when the oral dose increased from 500 to 3000 mg.[119] The dose-dependent intestinal absorption is in accordance with the carrier-mediated absorption process of the β-lactam derivative observed in animal studies, although the decrease in the absorption at very high doses (>3000 mg) could also be partially a result of the incomplete dissolution of ampicillin in the intestinal tract.[119–122] Although simple alkyl and/or aryl esters of ampicillin are rapidly hydrolysed in rodents, they are resistant to esterase-mediated hydrolysis in humans presumably due to steric hindrance of the ester carbonyl by the fused β-lactam ring system.

A solution to the dilemma was the construction of a double ester, an acyloxymethyl ester such as bacampicillin or pivampicillin (see Scheme 3.4) which extends the terminal ester carbonyl away from the fused β-lactam ring system and eliminates the inherent steric hindrance with the human esterases.[118,123,124] Hydrolysis of the terminal ester (or carbonate in the case of bacampicillin) affords the unstable hydroxymethyl ester **3.19** which spontaneously decomposes to ampicillin. Unlike ampicillin, bacampicillin is almost completely absorbed, and ampicillin is liberated into the systemic circulation in <15 minutes.[125,126] An additional example of the effectiveness of the tripartate prodrug strategy is evident with the acyloxy prodrug of the angiotensin II receptor antagonist candesartan (see Figure 3.17).

3.8.2 Distribution and Clearance

Because of extensive binding to albumin in plasma, many carboxylic acid drugs (especially NSAIDs) possess low tissue affinity, resulting in a small volume of distribution at steady state (Vd_{ss}), approaching plasma or blood volume (0.1 to 0.2 $L\,kg^{-1}$). The pharmacokinetic parameters [plasma clearance (CL_p), Vd_{ss}, half-life ($T_{1/2}$)], plasma free fraction (f_u) and physiochemical parameters of

Table 3.4 Physiochemical and pharmacokinetic attributes of carboxylic acid-based drugs.

Drug	*MW*	*cLogP*	*Log $D_{7.4}$*	f_u	*CL_p (mL/min/kg)*	*Vd_{ss} (L/kg)*	*$T_{1/2}$ (hr)*
Diclofenac	296	4.73	0.95	0.005	3.5	0.22	1.4
Ibuprofen	206	3.68	0.80	0.006	0.82	0.15	1.6
Ketoprofen	254	2.76	−0.25	0.008	1.6	0.13	2.1
Ketorolac	255	1.62	−0.95	0.0068	0.35	0.11	5.1
Cerivastatin	459	3.68	1.5–1.7	0.01	2.9	0.33	1.8
Fluvastatin	411	4.04	1.0–1.2	0.0079	16	0.42	0.70
Pravastatin	424	2.04	−0.75	0.5	14	0.46	0.78
Atorvastatin	558	4.46	1.11	0.01	8.9	5.4	7.8
Rosuvastatin	481	1.89	−0.25	0.12	14	1.20	20
Penicillin G	334	1.75	−2.06	0.40	6.9	0.24	0.70
Sulbactam	233	0.31	−5.11	0.62	5.1	0.32	1.1

structurally diverse carboxylic acid containing drugs in humans are shown in Table 3.4.[127]

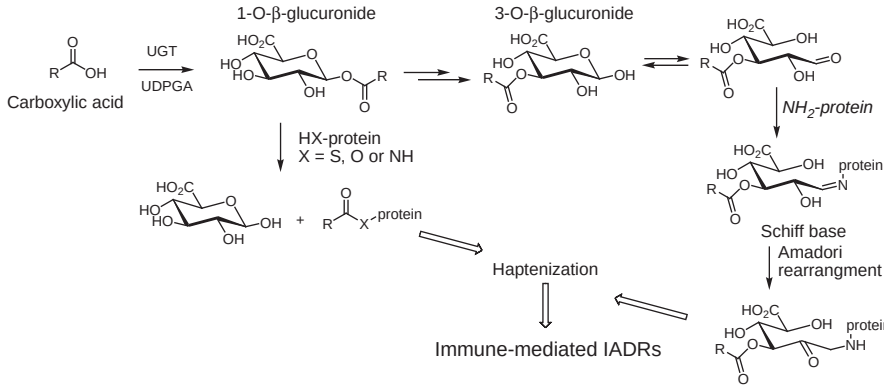
Within this context, it is noteworthy to comment on statin lipophilicity in relationship to their localised pharmacological action in the liver. The more lipophilic statins tend to achieve higher levels of exposure in non-hepatic tissues, while the hydrophilic statins tend to be more hepatoselective. The difference in selectivity is because lipophilic statins passively and non-selectively diffuse into both hepatocyte and non-hepatocyte, while the hydrophilic statins rely largely on active transport into hepatocyte to exert their effects. High hepatoselectivity is thought to translate into reduced risks of adverse effects including myopathy and rhabdomyolysis. Of the marketed statins, cerivastatin was the most lipophilic (log $D_{7.4}$) and also had the largest percentage of serious adverse effects due to its ability to inhibit vascular smooth muscle proliferation; as a result, it was voluntarily removed from the market by the manufacturer.

3.8.3 Metabolism of the Carboxylic Acid Moiety

Not surprisingly, the metabolism of carboxylate containing drugs mirrors that of many endogenous processes. The opening discussion is a characterisation of the principal routes of carboxylate biotransformation, and importantly, the core set of pathways responsible for eliciting the pharmacokinetics, pharmacology and toxicology commonly associated with carboxylic acid containing drugs.

3.8.3.1 Glucuronidation

From a quantitative perspective, glucuronidation is the most important route of carboylic acid biotransformation yielding the corresponding β-1-*O*-acyl glucuronides (also referred to as acyl glucuronides) (Scheme 3.5); these are



Scheme 3.5 Mechanism of covalent adduction of acyl glucuronide metabolites to proteins: plausible contributor to idiosyncratic drug toxicity of some carboxylic acid drugs.

more polar than the parent acids due to the hydrophilic nature of the linked glucuronic acid moiety. The biotransformation is catalysed by the family of uridine 5′-diphospho-glucuronosyl transferases (UDP-glucuronosyl transferases, UGT, EC 2.4.1.17) that require uridine-diphosphate glucuronic acid (UDPGA) as a co-factor. Depending on the structural features, molecular weight and recognition pattern for active uptake and/or efflux, acyl glucuronides can be eliminated *via* renal or biliary excretion. Following biliary excretion, acyl glucuronides may also be hydrolysed to regenerate the parent carboxylic acid (aglycone) that can be reabsorbed from the gut into the portal circulation *via* a process referred to as enterohepatic recirculation.[128,129] Acyl glucuronide hydrolysis is usually catalysed by β-glucuronidase enzymes, although non-specific esterases can also participate in this process.[130] Particularly noteworthy are the examples in which rearranged isomers of some acyl glucuronides (*vide infra*) display resistance to glucuronidase-mediated hydrolysis (*e.g.* diflunisal–acyl glucuronide) and thus present variations in enterohepatic recirculation.[131] Provided the acyl glucuronide is released into the systemic circulation, hydrolysis can also occur in plasma. Numerous carboxylic acid containing drugs including members from the NSAID, statin and fibrate classes of compounds are subject to some degree of acyl glucuronidation as a component of their elimination mechanism.[132]

3.8.3.2 Role of Acyl Glucuronide Metabolites in Drug Toxicity

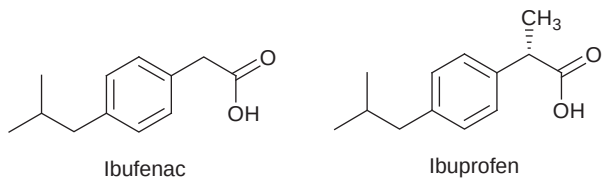
Because acyl glucuronides are ester derivatives, they are intrinsically electrophilic. The notion that acyl glucuronides could react with biological nucleophiles on proteins originated from observations that glucuronides of bilirubin and a number of other carboxylic acid containing drugs (NSAIDs and fibrates)

were able to form covalent adducts with human serum albumin *in vitro*.[133–139] The *in vitro* observations have also been extended to the *in vivo* situation with NSAIDs such as zomepirac and tolmetin.[136,138,139] For instance, an *in vivo* study in human volunteers revealed a linear correlation between the area under the curve of zomepirac acyl glucuronide (but not the parent zomepirac itself) in plasma and the amount of zomepirac covalently bound to plasma proteins.[135] The mechanism of covalent adduction of acyl glucuronides to proteins can proceed *via* two different pathways (Scheme 3.5).[140–144] The first is a transacylation mechanism where a nucleophilic amino acid on a protein macromolecule attacks the carbonyl group of the primary acyl glucuronide, leading to the formation of an acylated protein and free glucuronic acid. The second mechanism involves condensation between the aldehyde group of a rearranged acyl glucuronide and a lysine residue or an amine group of the N-terminus, leading to the formation of a glycated protein. The formation of the iminium species is reversible but may be followed by an Amadori rearrangement of the imino sugar to the more stable 1-amino-2-keto product.

A structural relationship between acyl glucuronide degradation to the Schiff base and covalent binding has been established utilising carboxylic acid derivatives with varying degrees of substitution on the carbon α to the carbonyl group in the parent compounds (*i.e.* acetic, propionic and benzoic acid derivatives).[140–144] The results of these studies suggest that a higher degree of alkyl substitution at the α-carbon leads to lower reactivity with biological nucleophiles giving rise to a general rank order of reactivity (acetic acid > propionic acid > benzoic acid). These observations imply that inherent electronic and steric properties must modulate the rate of acyl glucuronide rearrangement.

Of much interest within this context are the findings that acyl glucuronides of acetic acid-based NSAIDs including ibufenac, tolmetin and zomepirac—all of which have been withdrawn due to cases of idiosyncratic hepato- and/or renal toxicity—exhibit the highest level of glucuronide rearrangement and covalent binding, whereas mono-α-substituted acetic acids (2-substituted propionic acids) such as ibuprofen and naproxen exhibit intermediate level of acyl glucuronide rearrangement and covalent binding.

The pair of NSAIDs, ibufenac and ibuprofen (Figure 3.18), provides one of the most dramatic examples of structure–toxicity relationships in drug discovery. While ibuprofen is one of the safest over-the-counter anti-inflammatory agent on the market, its close-in analogue ibufenac was withdrawn due to



Ibufenac Ibuprofen

Figure 3.18 Structure–toxicity relationships for acyl glucuronidation—the *magic methyl*: ibufenac (hepatotoxin) *vs* ibuprofen (non-hepatotoxin).

severe hepatotoxicity. The daily doses of both NSAIDs are comparable (400–800 mg) and the only structural difference between the two drugs is the presence of the α-methyl substituent in ibuprofen. Both NSAIDs are subject to extensive acyl glucuronidation in animals and humans;[145,146] in the case of ibuprofen, it has been shown that the presence of the extra α-methyl substituent slows acyl glucuronide rearrangement to the electrophilic carbonyl intermediate capable of covalently modifying critical proteins potentially leading to toxicity.[146]

3.8.3.3 Inhibition of UGT and Transport Proteins by Acyl Glucuronides

A less common consequence, but noteworthy nonetheless, is the potential for inhibition of active transport proteins by acyl glucuronide metabolites. This occurrence was most recently reported in an account of an apparent renal transporter-mediated drug–drug interaction (DDI) induced by the fibrate, gemcabene.[147,148] What was originally described as a synergstic lowering of blood pressure during the concomitant administration of the angiotensin-converting enzyme inhibitor, quinapril, and the fibrate gemcabene, was later discovered to be the gemcabene-induced increase in the serum concentrations of the active metabolite, quinaprilat, *via* inhibition of its renal excretion by gemcabene and its acylglucuronide metabolite (Figure 3.19). Employing a rat *in vivo* model and human transporters *in vitro*, it was demonstrated that the acylglucuronide of gemcabene was a moderate inhibitor of human OAT3 ($IC_{50} = 197\,\mu M$; rOat3 $IC_{50} = 133\,\mu M$), the transporter responsible for the renal uptake of quinaprilat.

Similarly, a hepatotoxicity characterised by transient hyperbilirubinemia was observed in rats receiving oral administration of the drug candidate, MLN8054[149] (Figure 3.20). Particularly noteworthy was the efficiency at which the acyl glucuronide metabolite disrupted bilirubin homeostasis *via* the inhibition of OATP ($IC_{50} \sim 0.5\,\mu M$) mediated uptake of bilirubin as well as MRP2 ($IC_{50} \sim 3\,\mu M$) and MRP3 ($IC_{50} \sim 7\,\mu M$) mediated excretion of conjugated

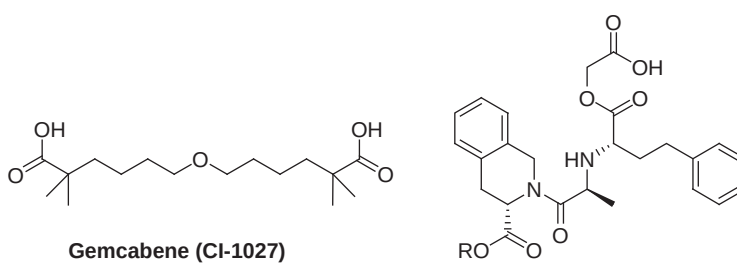


Figure 3.19 Clinical DDIs mediated by acyl glucuronide metabolites.

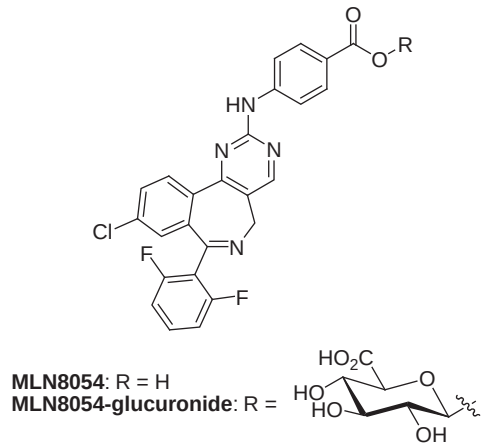
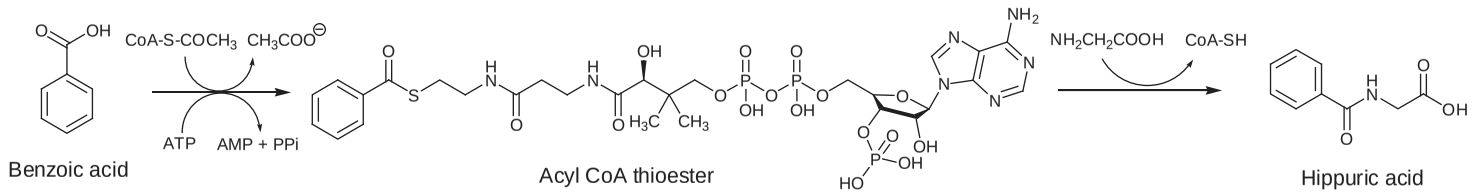


Figure 3.20 Inhibition of hepatobiliary transport of bilirubin by the acyl glucuronide of MLN8054.

bilirubin.[149] In addition to active transport inhibition, subsequent UGT inhibition data (IC_{50} ~200 μM) would also implicate the disruption of UGT1A1-mediated bilirubin conjugation by the acyl glucuronide of MLN8054, intensifying the disruption in bilirubin homeostasis. Importantly, the efficient production of the acylglucuronide and hepatobiliary concentrations was only observed in rat. Thus, the clinical manifestation of hyperbilirubinemia is not expected.

3.8.3.4 Amino Acid Conjugation

Amino acid conjugation of carboxylic acids is an alternative to acyl glucuronidation and is considered to be a detoxication reaction leading to the formation of amide metabolites. The conjugation of benzoic acid with glycine to form hippuric acid (Scheme 3.6) was discovered in 1842, making it the first biotransformation reaction described in the literature.[150] The specific amino acid involved in conjugation usually depends on the bioavailability of that amino acid from endogenous and dietary sources. Glycine conjugates are commonly observed as metabolites of carboxylic acids in mammals; glycine conjugation in mammals follows the order herbivores > omnivores > carnivores. Conjugation with L-glutamine is most common in primate drug metabolism. It does not occur to any significant extent in non-primates. In mammals, taurine is an alternate amino acid acceptor to glycine, although arginine, asparagine, histidine, lysine, glutamate, aspartate, alanine and serine conjugates also have been detected as carboxylic acid metabolites to some degree or other in mammals.[151] In addition, several dipeptides including glycylglycine, glycyltaurine and glycyclvaline are known to participate in this conjugation pathway.[151]



Scheme 3.6 Mechanism of amino acid conjugation of carboxylic acids: conversion of benzoic acid to hippuric acid, the first biotransformation reaction described in the scientific literature.

As is usually the case with enzyme-catalysed reactions, the ability of carboxylic acids to undergo amino acid conjugation depends on steric hindrance around the carboxylic acid group and upon substituents on the aromatic ring or aliphatic side chain. For instance, in rats, ferrets and monkeys, the major pathway of phenylacetic acid biotransformation is amino acid conjugation.[152–155] However, due to steric hindrance, diphenylacetic acid cannot be conjugated with an amino acid, so the major pathway of diphenylacetic acid biotransformation in the same three species is acyl glucuronidation.[152,154,156]

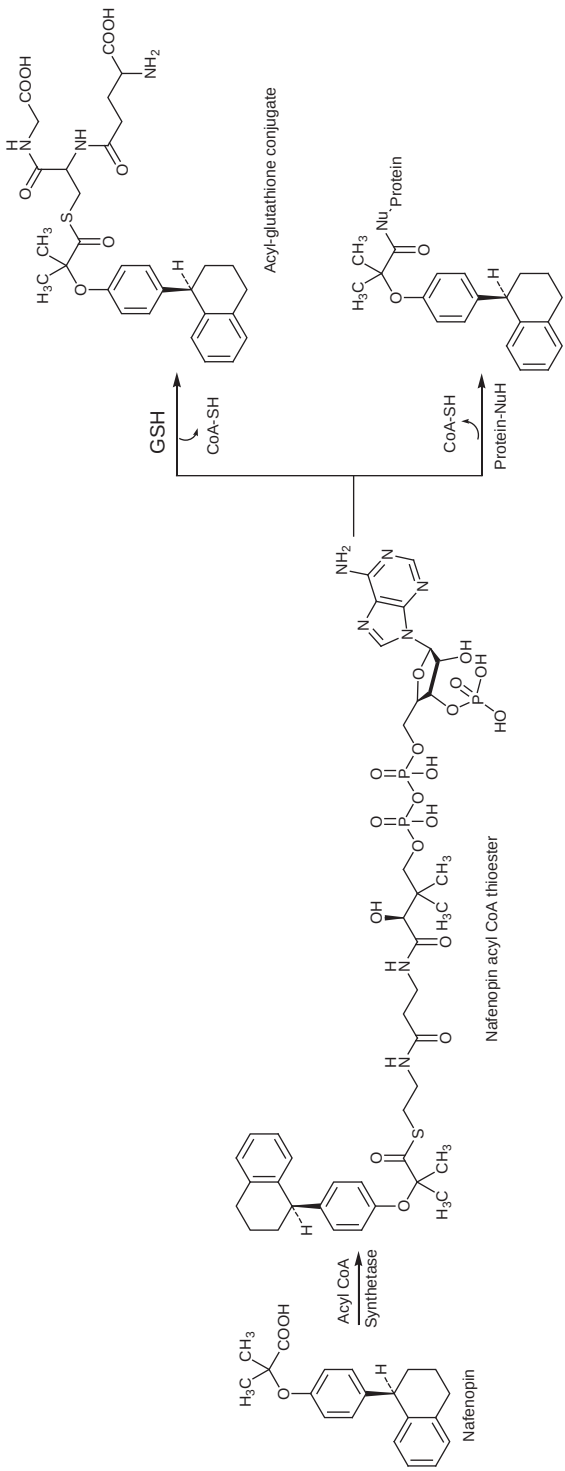
3.8.3.5 Mechanism of Amino Acid Conjugation

The mechanism of amino acid conjugation, as illustrated in the conversion of benzoic acid to hippuric acid, is shown in Scheme 3.6. The carboxylic acid moiety in xenobiotics and/or drugs is converted to the corresponding coenzyme A thioester derivative by mitochondrial acyl CoA synthetases (long-chain fatty acid-CoA ligases) and requires ATP. Conversion to the CoA thioester produces a more hydrolytically stable product that can be transported in the cell readily but is still quite reactive toward the appropriate amine nucleophiles. The appropriate cytosolic and/or mitochondrial amino acid *N*-acyltransferase then catalyses the condensation of the amino acid and the coenzyme A thioester to give the amino acid conjugate. This step is analogous to amide formation during the acetylation of aromatic amines by *N*-acetyltransferase. Two different types of *N*-acyltransferases have been purified from mammalian hepatic mitochondria. One prefers benzoyl-CoA as substrate, whereas the other prefers arylacetyl-CoA.

3.8.3.6 Role of Acyl CoA Metabolites in Covalent Modification of Proteins

The intermediate acyl CoA metabolites of carboxylic acids are thioester derivatives, which possess sufficient electrophilicity towards nucleophilic reactions with amino acid residue(s) on proteins as well as with the endogenous antioxidant glutathione (GSH).[157–160] For instance, the hypolipidemic drug nafenopin (see Scheme 3.7) was able to transacylate liver proteins following *in vitro* incubations with liver homogenates, resulting in amide and thioester linkages with protein lysine and cysteine amino acid residues, respectively, and that the AUC of nafenopin protein acylation correlated linearly with the AUC of nafenopin-CoA formation.[161] Conjugation of CoA thioesters with GSH has also been discerned.[161]

As has been previously demonstrated for acyl glucuronides, the substitution pattern around the acyl CoA metabolites greatly influences chemical reactivity; increasing substitution at the α-carbon generally correlates with a decrease in reactivity with nucleophiles.[162] Alongside acyl glucuronides, covalent adduction of acyl CoA metabolites of NSAIDs such as zomepirac



Scheme 3.7 Acyl CoA thioester metabolites of carboxylic acid derivatives as electrophiles.

and tolmetin with proteins and GSH has been proposed as a mechanism for the idiosyncratic immune-mediated toxicity associated with these drugs.[157–160]

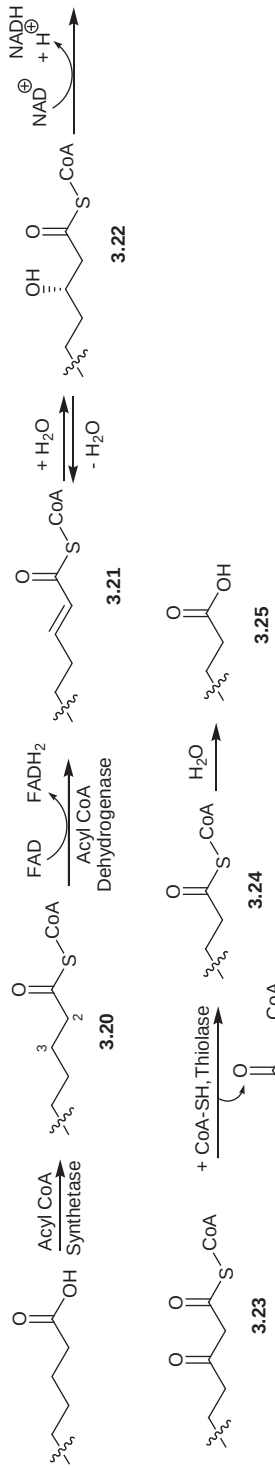
A remarkable feature in the metabolism of NSAIDs such as ibuprofen (see Figure 3.3) is the unidirectional chiral inversion from the pharmacologically inactive (*R*)- to the active (*S*)-enantiomer. Such inversion has been documented in several *in vivo* studies with 2-arylpropionic acid-based drugs and xenobiotics.[163] The mechanism of enantioselective inversion is believed to involve the initial enantioselective formation of the acyl CoA thioester followed by epimerisation by 2-arylpropionyl-CoA epimerase (this involves the intermediacy of a symmetrical conjugated enolate anion), followed by hydrolysis to regenerate the free acids. For each 2-arylpropionic acid drug studied, almost no acyl CoA formation is observed for the *S*-enantiomers, while the respective acyl CoA thioester derivatives are readily detected for most *R*-enantiomers. The enantioselective covalent binding of the acyl CoA thioester of *R*-2-phenylpropionic acid to hepatic tissue has been also demonstrated.[164]

3.8.3.7 β-Oxidation of Carboxylic Acids

β-Oxidation is the process by which fatty acids are broken down in mitochondria and/or in peroxisomes by stepwise oxidation of the carbon chain (two carbons for each cycle) to generate acetyl-CoA, the entry molecule for the Krebs cycle.[165] Mechanistically, β-oxidation comprises of an initial CoASH-dependent activation of the carboxylate moiety to afford the CoA thioester intermediate **3.20** (Scheme 3.8). Acyl-CoA-dehydrogenase mediated dehydrogenation (at the C2 and C3 carbons in **3.20**) yields olefin **3.31**, which undergoes a stereospecific hydration at the double bond to afford the corresponding *L*-β-hydroxyacyl CoA intermediate **3.22**. Oxidation of the alcohol group in **3.22** by NAD^+ generates the β-ketoacyl CoA derivative **3.23**, which undergoes cleavage at the α,β-bond by the thiol group of another molecule of CoA in a reaction catalysed by β-ketothiolase. This biochemical reaction results in the formation of one molecule of acetyl-CoA and one molecule of the acyl CoA derivative **3.24**. Hydrolysis of thioester bond in **3.24** yields the carboxylic acid metabolite **3.25**, which is two carbons shorter.[165]

3.8.3.8 β-Oxidation of Statins

The dihydroxyheptanoic or heptanoic acid side chain in statins is particularly prone to β-oxidation. Pentanoic acid derivatives of simvastatin and lovastatin, corresponding to the loss of a two-carbon unit from the dihydroxy hepatanoic acid side chain, have been reported to occur exclusively in rodents following the administration of the lactone form of the statin derivatives.[166,167] Carboxylic acid metabolites shortened by two or four carbon units, resulting in pentanoate or propionate derivatives, respectively, have been observed *in vivo* for the



Scheme 3.8 β-Oxidation of fatty acids.

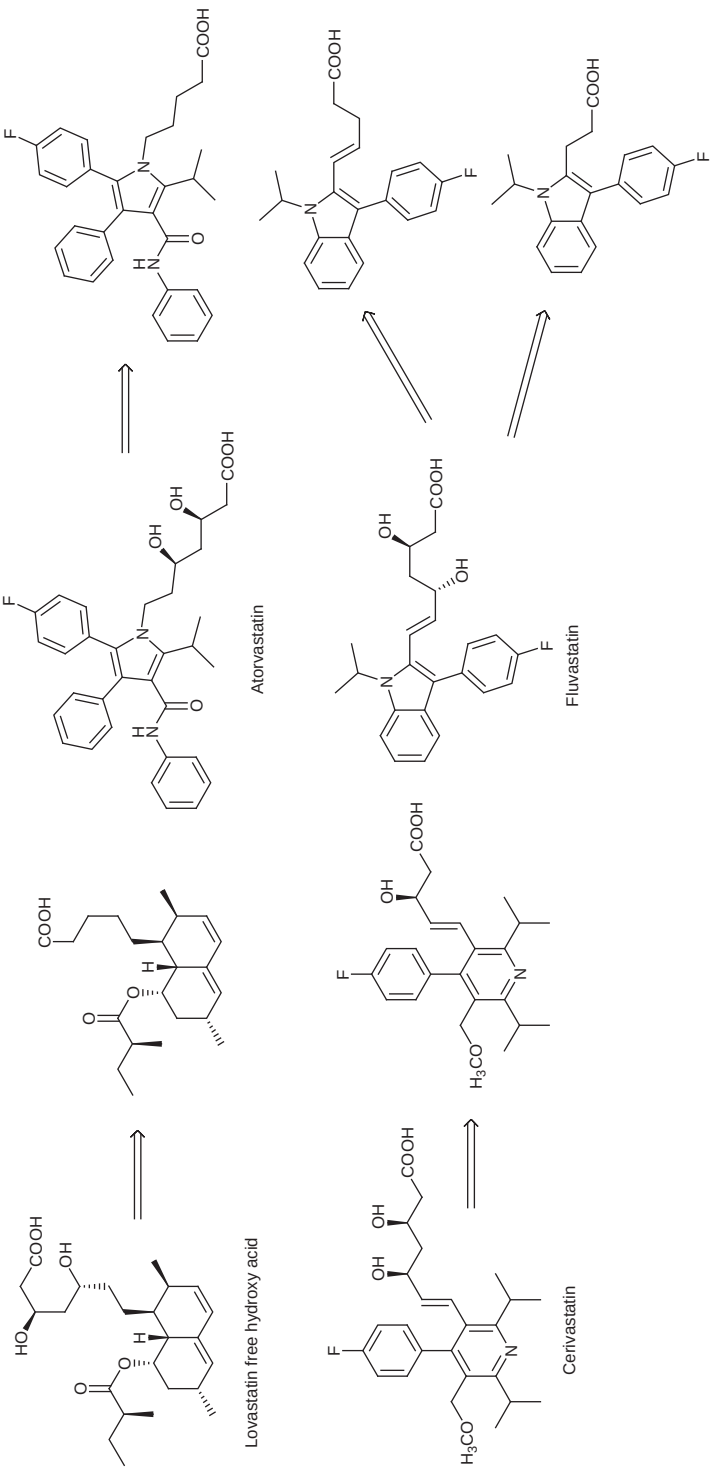


Figure 3.21 β-Oxidation of statins.

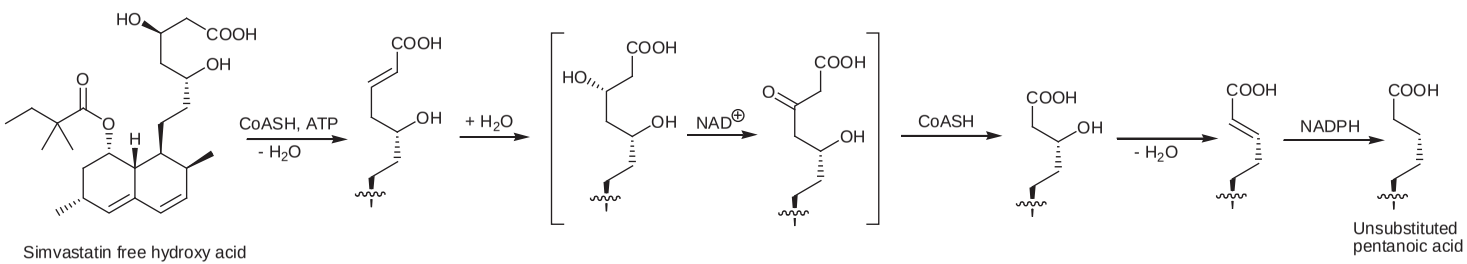
dihydroxyheptanoic acid derivatives atorvastatin and pravastatin primarily in rodents and minimally in humans (Figure 3.21).[168–170] Analogous metabolites have also been described for cerivastatin and fluvastatin, both of which contain the dihydroxyheptanoic acid moiety (see Figure 3.21).[171,172] Statins that form propionic acid metabolites (loss of four carbon unit) are believed to undergo two cycles of β-oxidation.[172]

Furthermore, since all statins possess a D-β-hydroxy configuration, an epimerisation to the L-configuration is needed for the β-oxidation cycle to occur. The mechanism(s) for the formation of *unsubstituted* pentanoic acid metabolites of statins proceeds *via* the initial β-oxidation cycle, which yields a D-β-hydroxypentanoic acid derivative, followed by fatty acid biosynthetic processes involving dehydration of the remaining D-hydroxyl group, followed by hydrogenation to form the unsubstituted pentanoic acid metabolites as shown in Scheme 3.9 for simvastatin free acid.[167,173]

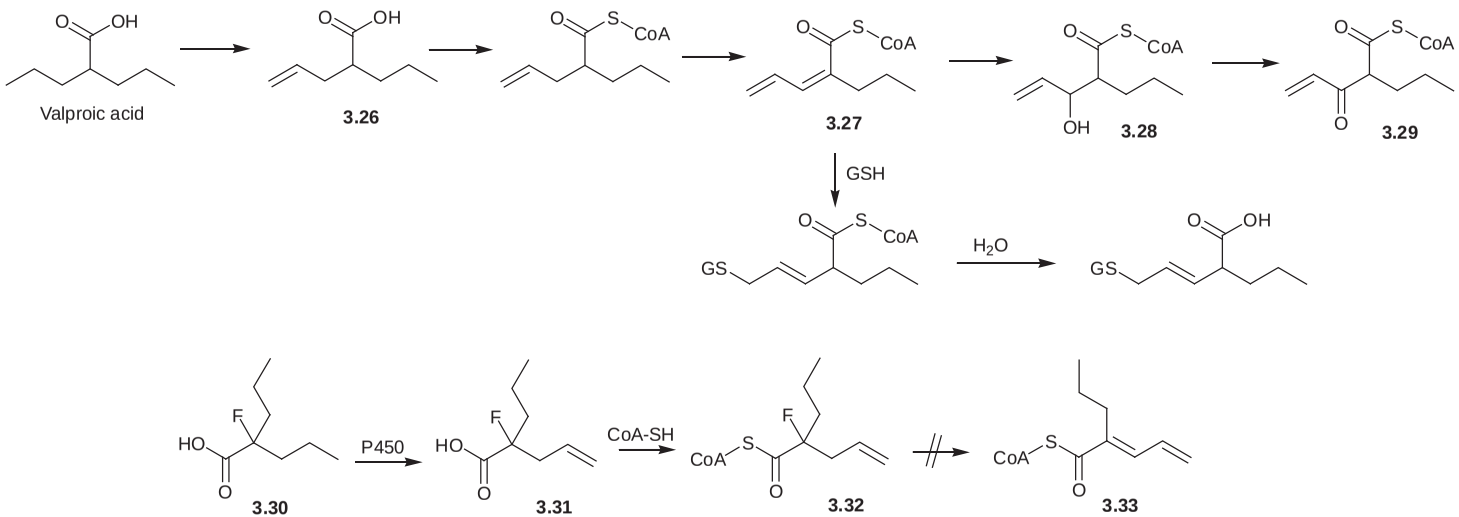
3.8.3.9 β-Oxidation of Valproic Acid to Reactive Metabolites

Valproic acid (Scheme 3.10) is an anticonvulsant agent first introduced in France in 1967 for the treatment of epilepsy.[174] Although, valproic acid has been shown to be effective against a broad spectrum of seizure types, its usage has been associated with a rare but serious effect involving irreversible liver failure (usually characterised by hepatic steatosis with or without necrosis).[175–178] The biochemical mechanisms that underlie valproic acid hepatotoxicity are not clearly understood, although a number of hypotheses have been advanced, including a role for toxic valproate metabolites. The involvement of toxic and potentially reactive metabolites was first suggested by Gerber *et al*. based on structural analogy with the known hepatotoxin 4-pentenoic acid, which is associated with mitochondrial damage and impairment of fatty acid oxidation.[179] The line of reasoning was developed further by Zimmerman and Ishak, who proposed that the terminal olefin metabolite of valproic acid [*i.e*. Δ^4-valproic acid (**3.26**), Scheme 3.10], might be the responsible hepatotoxin.[180] Interestingly, **3.26** was first detected as a minor metabolite in the plasma of epileptic children receiving valproic acid; much higher levels were detected in the serum of paediatric patients (the group most susceptible to valproic acid induced liver injury) than in either youths or adults.[181]

In the case of 4-pentenoic acid, β-oxidation is believed to lead to 3-oxo-4-pentenoyl-CoA, a reactive, electrophilic species that is proposed to alkylate 3-ketoacyl-CoA-thiolase, the terminal enzyme for β-oxidation, resulting in the mechanism-based inactivation of this enzyme complex.[182] Studies on the metabolism of Δ^4-valproic acid (**3.26**) in perfused rat liver or in primates indeed revealed products of β-oxidation as illustrated in Scheme 3.10.[183–185] Following conversion of **3.26** to its CoA derivative, sequential steps of β-oxidation lead to 2(*E*)- $\Delta^{2,4}$-valproic acid (**3.27**), 3-hydroxy-Δ^4-valproic acid (**3.28**) and 3-oxo-Δ^4-valproic acid (**3.29**) as the corresponding CoA derivatives. The diene **3.27** and



Scheme 3.9 Proposed mechanism for β-oxidation of statins leading to unsubstituted pentanoic acid metabolites: simvastatin as an example.



Scheme 3.10 β-Oxidation of valproic to reactive, electrophilic intermediates: effect of 2-fluorine substitution.

the allylic alcohol **3.28** intermediates (free acid forms) have been identified as metabolites in perfused rat liver and in primates *in vivo*. The 3-oxo-Δ^4-valproic acid (**3.29**) is believed to be the reactive, electrophilic species that binds covalently to the ketoacylthiolase protein resulting in its inactivation, while adducts derived from the reaction of GSH and *N*-acetylcysteine with the diene **3.27** in preclinical species and humans suggest a role for this reactive metabolite in the hepatotoxic event.[186–189]

Of much interest in this aspect is the finding that substitution of the methine hydrogen atoms on the C2 position in valproic acid with a fluorine atom yields a non-heptatotoxic compound **3.30** that retains anticonvulsant activity of the parent drug in mice.[190] The fluorine atom prevents oxidation of the 4-ene-2-fluoro valproic acid CoA intermediate **3.32** to the diene **3.33**,[191] although subsequent experimental work argued that failure to form the acyl CoA intermediate **3.32** prevents 4-ene-2-fluoro valproate from undergoing β-oxidation (see Scheme 3.10).[192]

3.8.4 P450 Isozymes Involved in the Oxidative Metabolism of Carboxylic Acid Derivatives

P4502C9 exhibits selectivity for the oxidation of relatively small and structurally diverse lipophilic carboxylic acid derivatives such as NSAIDs, fibrates and even some statins.[193–198] The structural and molecular basis for P4502C9 selectivity for carboxylic acids is evident in the P4502C9 structure co-complexed with the NSAID flurbiprofen, which is known to undergo 4′-hydroxylation by the isozyme.[199] The co-crystal structure highlights the importance of several amino acid residues in the binding site that are likely to be important for binding lipophilic carboxylic acids. In particular, the Arg^{108}, Asn^{289} and Asp^{293} residues were flagged as potential substrate recognition moieties; data which are in agreement with site-directed mutagenesis studies on P4502C9 catalysed oxidations.[200–202] Some of these residues have also been noted as important from in the various *in silico* approaches,[203–206] highlighting the utility of the pharmacophore-based models to predict P450 oxidation in general.

The gene encoding for P4502C9 carries numerous inherited polymorphisms. Those coding for R144C (*2) and I359L (*3) amino acid substitutions have both significant functional effects and appreciable high population frequencies.[207,208] Consequently, drugs that are metabolised by P4502C9 are prone to considerable interindividual variability in pharmacokinetics. For example, mean CL_p in homozygous carriers of the *3 allele were below 25% of that of the wild-type for several P4502C9 substrates including warfarin, tolbutamide, glipizide and fluvastatin.[207,208] It is of interest to note that P4502C9 is not the exclusive P450 isoform responsible for the oxidative metabolism of carboxylic acids. Highly lipophilic carboxylic acids such as the statins and/or some PPAR-α agonists also tend to be undergo oxidative metabolism by P4503A4 (in addition to P4502C9).[209,210]

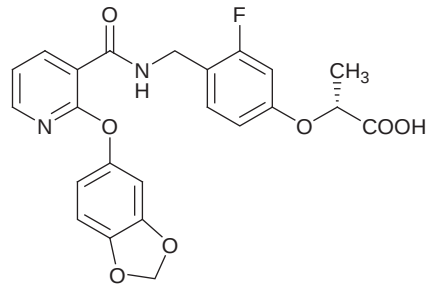
3.8.5 Hepatobiliary Disposition of Carboxylic Acids

Over the past 15 years, a number of important human drug transporters have been identified that are expressed at the apical or basolateral side of the epithelial cells in various tissues. Most drug transport proteins, which catalyse cellular uptake and efflux belong to two super-families namely the SLC (solute-linked carrier) and the ABC (ATP-binding cassette) transporters, respectively.[211,212] The combination of organic anion transporting polypeptides (OATPs) and multidrug resistance-associated protein 2 (MRP2), which represents two classes of transporters from the SLC and ABC super-family, respectively, play an important role in the hepatobiliary transport of organic anions including carboxylic acid derivatives at the sinusoidal and canalicular membranes. In the human liver, OATP1B1 (also known as OATP2 or OATPC), OATP1B3 (OATP8) and OATP2B1 (OATPB) are predominant transporters responsible for the hepatic uptake of a variety of organic anionic compounds.[213] Once taken up into hepatocytes, anionic compounds and/or metabolites derived from phase II glucuronidation can undergo MRP2-mediated biliary excretion.[214] Besides MRP2, multidrug resistance 1 (MDR1, P-glycoprotein) protein and breast cancer resistance protein (BCRP), which are located on the bile canalicular membrane of the liver, can also be involved in the active efflux of organic anions into bile.[215] Uptake *via* OATPs followed by excretion *via* MRP or other efflux transport proteins from the ABC family constitutes vectorial transport for the hepatobiliary excretion of several carboxylic acid based drugs.

An example of this phenomenon is evident with the selective histamine H_1-receptor antagonist and carboxylic acid derivative fexofenadine (see Scheme 3.2). Hepatic metabolism is of minimal importance in the elimination of fexofenadine in rodents and human; this lipophilic carboxylic acid is predominantly eliminated *via* biliary excretion in the unchanged form.[85] Biliary excretion of fexofenadine in humans is mediated by MDR1.[216] In addition, fexofenadine also functions as a substrate for hepatic uptake by human and rat OATPs.[216,217] In the case of fexofenadine, inhibition/stimulation of these uptake/efflux processes is known to lead to significant DDIs in humans.[218,219]

The role of transporters in the disposition of a carboxylic acid containing phosphodiesterase-4 inhibitor CP-671 305 (Figure 3.22) has also been examined in great detail.[220] Like fexofenadine, CP-671 305 is resistant to metabolism by either phase I or phase II drug metabolising enzymes in liver microsomes and hepatocytes from preclinical species and human; these findings are supported by the lack of detectable metabolites in pooled plasma, urine and/or bile from rats, dogs and monkeys following CP-671 305 administration.[221]

Preliminary investigations into the clearance mechanism in rats revealed that the compound undergoes substantial biliary excretion in the unchanged form.[221] In bile duct exteriorised rats, a 7.4-fold decrease in the half-life of CP-671 305 was observed implicating enterohepatic biliary circulation of the parent drug. A statistically significant difference in CP-671 305 pharmacokinetics was also discernible in cyclosporin A- or rifampicin-pretreated rats as reflected from



CP-671,305

Figure 3.22 Chemical structure of a novel PDE4 inhibitor and carboxylate analog which undergoes active hepatobiliary transport.

a four-fold decrease in clearance and a four-fold increase in the area under the curve (AUC).[220] Given the ability of cyclosporin A and rifampicin to inhibit multiple drug transporters, the interactions of CP-671 305 with the major human hepatic drug transporters, MDR1, MRP2, BCRP and OATPs, were evaluated *in vitro*.[220] CP-671 305 was identified as a substrate of MRP2 and BCRP, but not MDR1. CP-671 305 was a high affinity substrate of human OATP2B1, but not a substrate for human OATP1B1 or OATP1B3. Examination of the hepatobiliary transport of CP-671 305 in sandwich-cultured hepatocytes indicated active uptake into hepatocytes followed by efflux into bile canaliculi, consistent with the results from *in vitro* transporter studies. The role of rat Mrp2 in the biliary excretion was also examined in TR^- (Mrp2-deficient) rats, and the observations that CP-671 305 pharmacokinetics were largely unaltered in TR^- rats were consistent with the finding that compromised biliary clearance of CP-671 305 was compensated by increased urinary clearance.[220] As such, these *in vitro* and *in vivo* studies, which suggest an important role for transport proteins in the hepatobiliary disposition of CP-671 305 in rat and human, could be valuable in the design of clinical DDI studies.

3.8.5.1 Hepatobiliary Transport of Statins

Statins are avidly taken into hepatocytes by active uptake transporters, among which OATP1B1 appears to be the most important.[213,222,223] Other hepatic uptake transporters that can transport statins are OATP1B3, OATP2B1, OATP1A2 and sodium-dependent taurocholate co-transporting polypeptide (NTCP). The carrier-mediated hepatic uptake process not only represents the first step of hepatic drug elimination, but is also an active drug delivery system for many statins to the liver as a target organ.[224] As all statins are eliminated mainly by the liver, their active hepatic uptake, metabolism by P450 isozymes and biliary excretion *via* MDR1, MRP2, BCRP and bile salt export pump (BSEP) can regulate their total clearance.[213,223,225]

To treat patients with dyslipidemias resistant to diet or single agent pharmacotherapy, combination therapies of statins with other drugs are widely used, which can result in DDIs. An increase in the plasma concentration of many of the currently used statins can cause severe side effects such as muscle toxicity or even rhabdomyolysis. Cerivastatin was withdrawn from the market after combinations of the statin derivative with the fibrate gemfibrozil[226,227] or the immunosuppressant cyclosporin A[228] led to marked increases in its systemic exposure resulting in severe muscle toxicity.

The observed DDIs are thought to arise from the inhibition of P4502C8-mediated cerivastatin oxidative metabolism by gemfibrozil[229,230] and/or an inhibition of the OATP1B1-mediated hepatic statin uptake into the liver by gemfibrozil and its glucuronide conjugate.[230,231] In addition, daily use of gemfibrozil also increases the AUC of simvastatin and lovastatin free acid derivatives and pravastatin by 2–3 fold.[232,233] It is interesting to note that neither fenofibrate nor bezafibrate increase the AUC of simvastatin, lovastatin or pravastatin, which indicates that DDI with statins is not a group effect of the fibric acid derivatives.[234,235] Apart from DDI with ceravastatin, cyclosporin A, which inhibits numerous membrane transporters including OATPs and MDR1, also increases the AUCs of simvastatin, lovastatin and pravastatin about 10-fold.[236–238] Cases of rhabdomyolosis have occurred during concomitant use of cyclosporin A and different statins.[239–241] Polymorphisms of *SLCO1B1* (encoding OATP1B1) and *ABCC2* (encoding MRP2) can cause considerable interindividual variability in plasma concentrations of statins.[242–247] Common variants in SLCO1B1 have also been associated with an increased risk of myopathy with certain statins such as simvastatin.[246,247]

3.9 ADME Profile of Tetrazoles

Structural diversity within the tetrazole-based AT_1 receptor antagonists results in subtle differences in physiochemical properties,[248] which then influences: (a) binding affinity to the AT_1 receptor, (b) aqueous and lipid solubility, and (c) pharmacokinetic profile (*e.g.* absorption, distribution, clearance and routes of elimination). Table 3.5 lists key physiochemical attributes of representative members of the tetrazole-based AT_1 receptor antagonists.

The acidic nature of the tetrazole moiety can provide a convenient means for preparation of salt forms to improve aqueous solubility (*e.g.* losartan is

Table 3.5 Some key physiochemical properties of tetrazole-based AT_1 receptor antagonists.

AT_1 receptor blocker	*MW*	*cLogP*	*Log $D_{7.4}$*	*f_u (% bound)*	*Vd_{ss} (L/kg)*
Losartan	422	4.1	1.7	98.6–98.8	0.49
Irbesartan	428	6.0	1.8	90	0.75–1.3
Candesartan	440	5.4	−0.79	>99	0.12
Valsartan	435	4.9	−1.3	94–97	0.24

commercially sold as the potassium salt). Candesartan and olmesartan, which contain a carboxylic acid group (in addition to the presence of the tetrazole motif), are administered as the corresponding carboxylic acid ester derivatives to increase oral absorption (see Figure 3.17). Candesartan cilexetil (prodrug form) is rapidly and completely activated *via* ester hydrolysis during absorption from the gastrointestinal tract to active candesartan. Even upon administration in the prodrug form, the absolute oral bioavailability of candesartan is low ($\sim$15%), a likely characteristic of its high polarity despite the 'prodrug' handle. Consistent with this hypothesis, food with a high fat content does not have any effect on candesartan absorption or bioavailability.

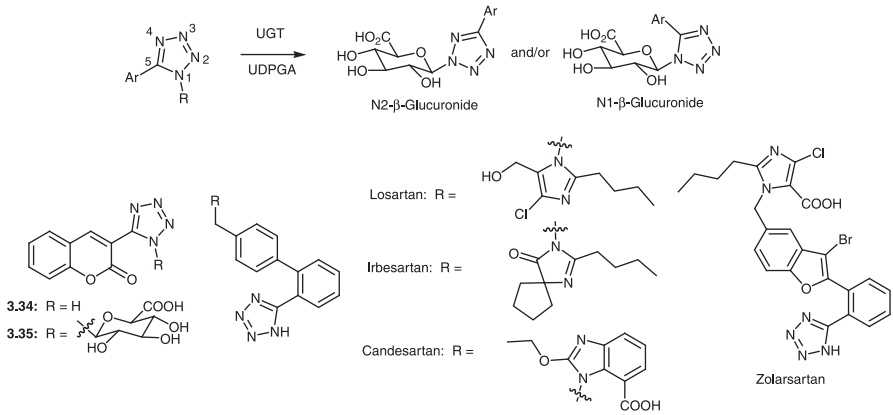
Because of the bioisosteric relationship, the tissue distribution pattern is similar for drugs that contain the either the carboxylate or tetrazole functionalities. For example, tetrazoles, like their carboxylic acid counterparts, are heavily bound to plasma proteins (mainly to albumin; a small proportion of binding to α_1-acid glycoprotein has also been noted) (see Table 3.5). Overall, the extensive plasma protein binding is also reflected in a low Vd_{ss} for the tetrazole AT_1 receptor antagonists in humans.[249–253] Furthermore, as seen in Table 3.6, subtle differences in log D also impacts CL_p and $T_{1/2}$ of the tetrazole-based AT_1 receptor antagonists in humans.[249]

The elimination pathways of tetrazole-based AT_1 receptor antagonists involve phase I/II metabolism and/or non-metabolic (biliary and urinary) excretion, a phenomenon that has some commonality with the excretion pattern discerned with carboxylic acid containing drugs.[254–258] For example, mass balance studies on irbesartan in humans reveal that $\sim$9% of the orally administered dose is metabolised *via* oxidative and conjugation pathways catalysed by P450 and UGT isozymes; $\sim$80% of the administered dose is excreted in the faeces *via* the bile and the remainder of the dose appears in the urine.[254] In the case of losartan, oxidative metabolism of its primary alcohol motif by P450 results in the formation of the active carboxylic acid metabolite EXP3174. After oral administration to humans, $\sim$14% of the losartan dose is converted to EXP3174; faecal and renal elimination account for the remainder of the losartan dose. EXP3174 is $\sim$10- to 40-fold more potent than the parent compound and it appears that most of the *in vivo* pharmacological activity of losartan is derived from the metabolite.

Table 3.6 Human pharmacokinetics of tetrazole-based AT_1 receptor antagonists.

AT_1 receptor blocker	*Dose (mg)*	*CL_p*[a]	*$T_{1/2}$ (h)*	*Tmax (h)*	*F (%)*
Losartan	50–100	8.6	1.5–2.0	1.0	33
Irbesartan	150–300	2.2	13	0.3	60–82
Candesartan[b]	4–32	0.4	6.0–13	2.0–5.0	15
Valsartan	80–320	0.5	6.0–10	2.0–4.0	19 (10–35)

[a] $mL\,min^{-1}\,kg^{-1}$.
[b] Administered as the ester prodrug (candesartan cilexetil).



Scheme 3.11 *N*-Glucuronidation of tetrazoles.

3.9.1 Metabolism of the Tetrazole Motif

In contrast to carboxylic acids, tetrazoles are resistant to metabolic pathways involving β-oxidation and amino acid conjugation. However, β-*N*-glucuronidation has been shown to be an important clearance pathway of tetrazole-containing compounds in a similar as that discerned with the carboxylic acid moiety. The biotransformation reaction results in the formation of *O*-β-glucuronides. Tetrazole glucuronidation can occur on the *N*-1 or the *N*-2 nitrogen as shown in Scheme 3.11. Nohara and co-workers were the first to identify a tetrazole-*N*-1-glucuronide (**3.35**) in urine of animals dosed with 6-ethyl-3-(1*H*-tetrazol-5-yl)chromone (**3.34**) (Scheme 3.11).[259] Glucuronide **3.35** was identified as the exclusive isomer by chemical synthesis and NMR studies. Recent studies with biphenyltetrazole derivatives, however, have indicated the *N*-2-glucuronide conjugate to be the preferred metabolite over the *N*-1-glucuronide based on NMR and X-ray crystal structure characterisation.[260,261] In fact, several *N*2-tetrazole glucuronide conjugates have been reported as metabolites of tetrazole-based angiotensin II receptor antagonists losartan, irbesartan, candesartan and zolarsartan in animals and/or humans.[262–266] The optimal pH value for the transformation of tetrazoles to their respective *N*2-glucuronide conjugates correlates very well with the reported pKa of tetrazole (pKa = 4.9).[267] Recent studies have also shown that UGT1A3 is highly selective towards tetrazole-*N*2 glucuronidation in losartan, candesartan and zolarsartan.[268]

3.9.1.1 Role of P4502C9 in the Oxidative Metabolism of Tetrazole Derivatives

Considering the bioisosteric relationship between the carboxylic acid group and the tetrazolyl moiety, it is not surprising that the polymorphic P4502C9 is also

involved in the oxidative metabolism of many of the tetrazole-based AT_1 receptor antagonists. This attribute often results in pharmacokinetic interactions with other P4502C9 substrates or inhibitors in a manner analogous to the situation with carboxylate-containing drugs.[249,269] The substituent attached to the biphenyl tetrazolyl scaffold in these compounds is usually the site of oxidation. As described earlier, the conversion of losartan to its pharmacologically active metabolite EXP3174 is principally mediated by the action of P4502C9 (with some contribution from P4503A4).[112,270,271] Variability in losartan metabolism to EXP3174 has been discerned in individuals with different P4502C9 genotypes.[272,273] P4502C9 also catalyses the oxidative metabolism of irbesartan, candesartan and valsartan to some degree; the metabolism usually results in weakly active or inactive compounds.[274–276] Telmisartan and olmesartan are generally resistant to oxidation by P450 isoforms; telmisartan is partially metabolised by glucuronidation, and olmesartan is excreted unchanged.[277,278] The ability of tetrazole-based AT_1 receptor antagonist to inhibit P4502C9 in human liver microsomes has also been examined; all compounds were shown to possess weak inhibitory activity (IC_{50} $>30\,\mu M$) against P4502C9 catalysed warfarin hydroxylation.[279]

3.9.2 Role of Transporters in the Disposition of Tetrazole-based Angiotensin II Receptor Antagonists

The hepatobiliary and urinary excretion of tetrazole-based AT_1 receptor antagonists can be subject to active transport. The findings that olmesartan is excreted in both bile and urine in the unchanged form has led to studies aimed at characterising the role of active transporters in olmesartan disposition. On the basis of *in vitro* studies as well as *in vivo* studies in Eisai hyperbilirubinemic rats, a role for MRP-2 in olmesartan biliary has been established.[280–282] Olmesartan was also shown to function as a OATP1B1, OATP1B3, organic anion transporter (OAT) 1 and OAT3 substrate.[281,282] Likewise, Yamashiro *et al.* have shown the role of OATP1B1, OATP1B3 and MRP-2 in the hepatobiliary transport of valsartan.[283] Consistent with these findings, interindividual variability in valsartan pharmacokinetics in human subjects with OATP1B1*1b alleles has been noted.[284] Finally, the finding that OATP1B3 is also involved in the hepatic uptake of the carboxylic acid based angiotensin antagonist telmisartan[285] serves to illustrate the bioisosteric relationship between the carboxylate and the tetrazole group in terms of affinity towards active transport.

3.10 ADME Profile of Thiazolidinedione Derivatives

The thiazolidinedione (TZD) class of compounds—collectively referred to as the 'glitazones'—are PPAR γ agonists used for the treatment of type 2 diabetes. The thiazolidinedione group shares a non-classical biosisosteric relationship with the carboxylic acid group owing to the acidic nature of the imide fragment

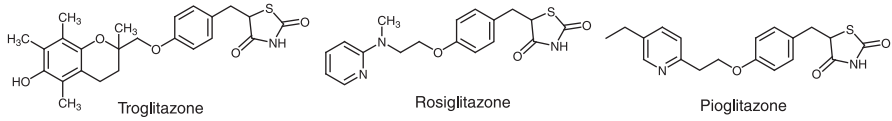


Figure 3.23 Structures of thiazolidinedione-based anti-diabetic drugs.

in the 5-membered thiazolidinedione ring system. The first commercialised TZD drug, troglitazone (Figure 3.23) was withdrawn from the US market after numerous reported cases of severe liver failures leading to liver transplantation or death; after being on the market for 17 months, the FDA received 560 reports of hepatotoxicity and 24 cases of acute liver failure.[286–288] In contrast, the related TZD analogs rosiglitazone and pioglitazone (Figure 3.23) are devoid of the hepatotoxicity associated with troglitazone.

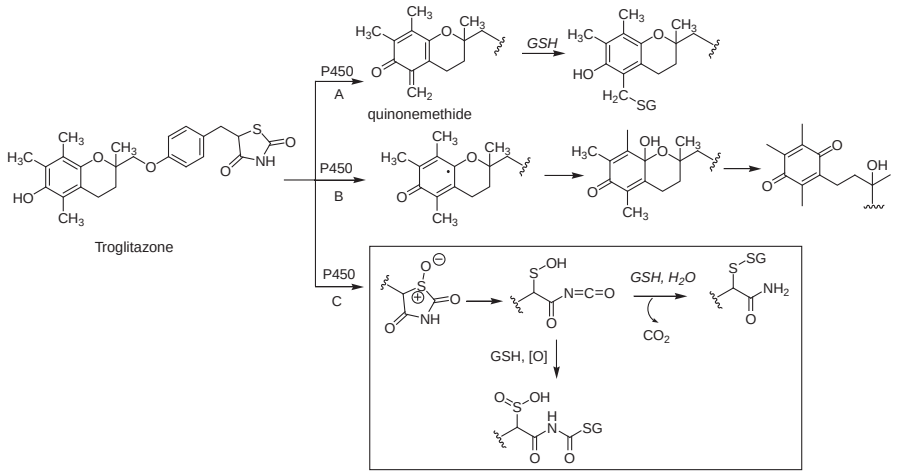
3.10.1 Clearance and Oral Bioavailability

Because they are non-classical bioisosteres of the carboxylic acid functionality, their pharmacokinetic parameters are fairly similar to those discerned with carboxylate-based drugs. For example, the human CL_p, Vd_{ss}, plasma f_u and elimination $T_{1/2}$ of rosiglitazone is 0.65 mL min^{-1} kg^{-1}, 0.20 L kg^{-1}, 0.002 and 3.9 h, respectively.[127] The oral bioavailability of rosiglitazone is nearly 100% and that of pioglitazone $>80\%$.[289,290]

3.10.2 Metabolism of the Thiazolidinedione Ring System

The thiazolidinedione ring is susceptible towards oxidative ring scission. The observation was first noted during bioactivation studies on troglitazone.[291,292] *In vitro* incubations of troglitazone in NADPH- and GSH-supplemented human liver microsomes led to the characterisation of several GSH conjugates. Based on these structures of these conjugates, the two proposed pathways for the bioactivation of troglitazone include metabolism of the chromane ring to the quinone or *ortho*-quinonemethide (Scheme 3.12, panels A and B) and oxidative cleavage of thiazolidenedione ring (Scheme 3.12, panel C). While rosiglitazone and pioglitazone do not contain the chromane ring system found in troglitazone, they do contain the thiazolidinedione scaffold. And consistent with the findings with troglitazone, both rosiglitazone and pioglitazone have been shown to undergo thiazolidinedione ring scission mediated by P450 enzyme(s) in human microsomes resulting in reactive metabolites trapped by GSH.[293]

The mechanisms of troglitazone-induced hepatotoxicity remain unclear at the present time and seem to be multi-factorial. Besides differences in metabolism (absence of quinonoid formation in rosiglitazone and pioglitazone), a comparison of the effects of TZD drugs on toxicologically relevant gene expression in primary culture hepatocytes using microarray analysis showed



Scheme 3.12 Bioactivation of the thiazolidinedione ring system in 'glitazones' to electrophilic intermediates.

that substantially higher numbers of genes were affected by troglitazone treatment when compared to rosiglitazone and pioglitazone.[294] Masubuchi *et al.*[295] have also investigated the effects of troglitazone, rosiglitazone, and pioglitazone on mitochondrial function. Troglitazone, but not rosiglitazone or pioglitazone, was shown to induce decreases in mitochondrial membrane potential and mitochondrial Ca^{2+} accumulation consistent with the induction of mitochondrial permeability transition.

3.10.3 P450 isozymes Responsible for the Metabolism of 'glitazones'–DDI Potential

P4502C8 and P4503A4 are the major P450 isozymes, which catalyse the oxidative biotransformation of troglitazone and pioglitazone, whereas rosiglitazone is metabolised by P4502C9 and P4502C8.[296–298] The major oxidative pathways of 'glitazones', however, do not involve thiazolidinedione ring scission. For instance, *N*-demethylation and pyridine ring hydroxylation are the principal metabolic pathways of rosiglitazone. For both rosiglitazone and pioglitazone, the most relevant clinical pharmacokinetic interactions have been described in healthy volunteers with rifampicin (rifampin), which results in a significant decrease of AUC (54–65% for rosiglitazone; 54% for pioglitazone), and with gemfibrozil, which results in a significant increase of AUC (130% for rosiglitazone; 220–240% for pioglitazone).[299–302] As noted earlier with carboxylic acids, DDIs between 'glitazones' and gemfibrozil stems from inhibition of the P4502C8 enzyme by gemfibrozil and its glucuronide conjugate.[303]

3.11 ADME Profile of Esters and Amides

Unlike carboxylic acids, esters and amides are neutral and more lipophilic in nature, which results in vastly different pharmacokinetic parameters for compounds containing these functional groups. Consequently, it can be debated whether esters/amides are true bioisosteres of the carboxylic acid group. Apart from the role of esters as carboxylic acid prodrugs, the utility of this functional group as a carboxylic acid replacement is limited. This is primarily due to the facile *in vivo* hydrolysis of the ester functional group by esterases in the gut, liver and plasma, resulting in less than optimal pharmacokinetic parameters. For example, the half-life for deacetylation of the acetoxy group present in aspirin is $\sim$2 h in human plasma, and reduces to 1–3 minutes for carboxylic acid ester derivatives of aspirin.[304]

Our recent study on the disposition of the non-selective COX inhibitor indomethacin and its corresponding COX-2-selective neutral amides derivatives **3.36**–**3.38** (Figure 3.24) in the rat provides a comparison of the ADME profile of the two functional groups.[305,306] Unlike the parent carboxylic acid derivative, which is resistant to metabolic turnover in liver microsomes from rat and human, neutral amide derivatives **3.36**–**3.38** were considerably less stable in this biological matrix and underwent extensive oxidative metabolism by P450, a

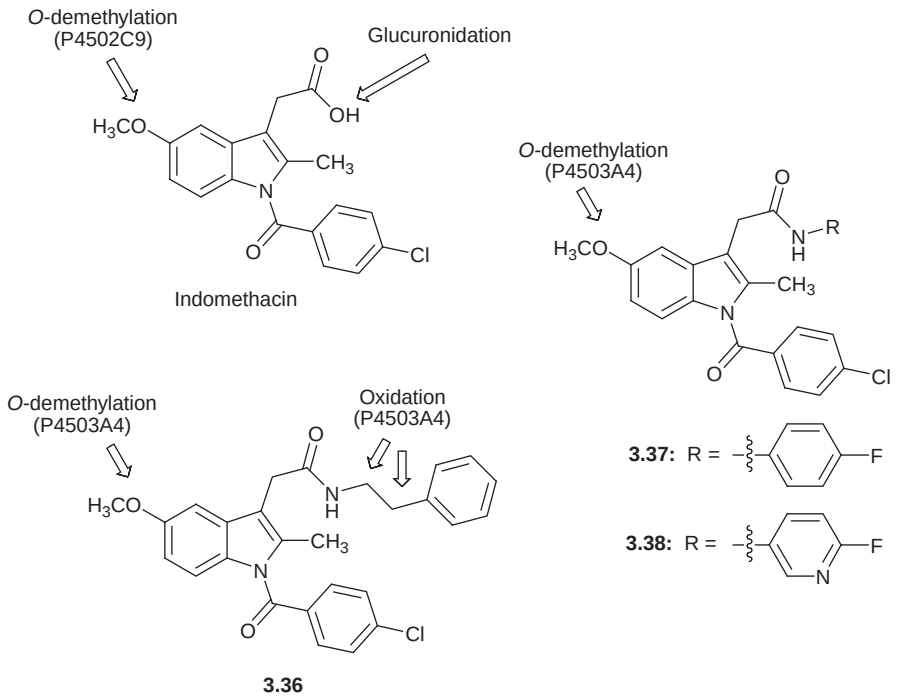


Figure 3.24 Differences in the metabolic fate of carboxylic acid derivative indomethacin and its neutral amide derivatives.

Table 3.7 Physiochemical and pharmacokinetic parameter comparison between indomethacin and some of its neutral amide derivatives.

Compound	MW	cLogP	Fraction unbound (rat plasma)	$T_{1/2}$ (min)[a]		CL_p (mL/min/kg)	Vd_{ss} (L/kg)	F (%)
				Rat	Human			
Indomethacin	357	4.18	0.03	>90	>90	0.51	0.19	98
Amide **3.36**	460	5.50	0.0045	1.0	1.5	155	5.3	<1
Amide **3.37**	450	5.85	0.0040	8.5	25	26	2.3	20
Amide **3.38**	451	5.05	0.0070	8.0	23	39	2.0	38

[a]Liver microsomes.

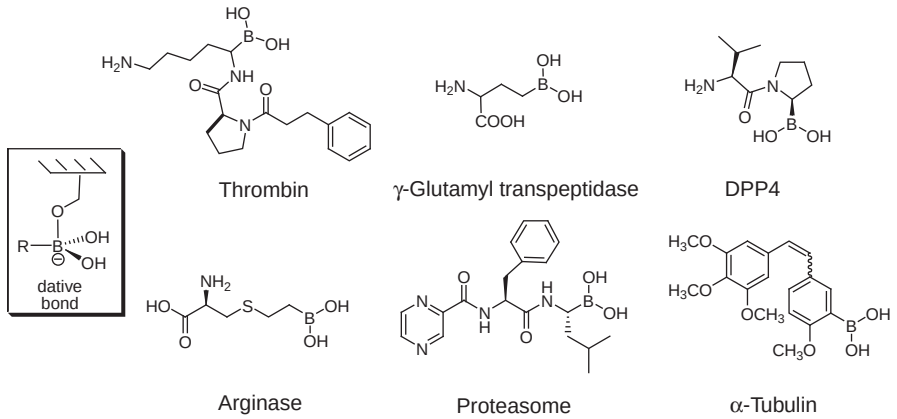
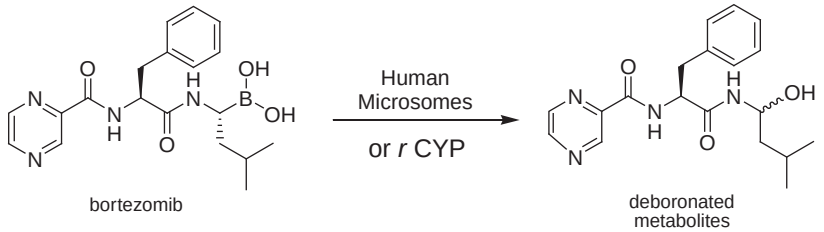


Figure 3.25 Boronic acid-containing pharmacologic active compounds.

feature that is linked to the increase in molecular weight and lipophilic character (Table 3.7). As such, the increase in molecular weight and lipophilicity also results in a decrease in plasma free fraction (see Table 3.7). A comparison of the pharmacokinetic attributes of indomethacin and neutral amides **3.36**–**3.38** is depicted in Table 3.7 and reveals a dramatic difference in CL_p and Vd_{ss} between the parent carboxylic acid and its amide derivatives; the neutral amide analogs are cleared at a more rapid rate than the free carboxylic acid derivative, a phenomenon that ultimately results in a lower oral bioavailability for the amides. Unlike indomethacin, which is susceptible to glucuronidation and P4502C9-catalysed *O*-demethylation, indomethacin amides **3.36**–**3.38** underwent oxidative *O*-demethylation and oxidation on the amide substituent, which was almost exclusively mediated by P4503A4 (Figure 3.24).[305,306]

3.12 Boronic Acid Derivatives

The boronic acid is a functional group with dual character. In addition to delivering the electronic and hydrogen-bonding requirements of carboxylate isostere (Figure 3.25, α-tubulin inhibitor), the boronate is also effective as a



Scheme 3.13 Biotransformation of the boronic acid group in bortezomib.

pharmacophore in itself. The vacant *p*-orbital of boron is amenable to dative bonding with the nucleophilic active site residues of proteolytic enzymes such as those bearing serine (*e.g.* thrombin, arginase, DPP4) and threonine (*e.g.* proteasome, γ-glutamyl transpeptidase).[307–309] This dative bonding capability of boron results in enzyme inhibition kinetics that are nearly indistinguishable from irreversible inhibitors, a hallmark trait of a transition state analog (Figure 3.25, inset).

Although 20 years of drug discovery had elapsed in the arena of boronate-mediated inhibition of proteolysis, little had been reported regarding the biotransformation pathways which characterise boronate metabolism and disposition in humans. More recently, the discovery and clinical development of the peptidyl boronate proteasome inhibitor, bortezomib, has resulted in the characterisation of the hepatic metabolism of this functional group. And while the metabolism of bortezomib did not proceed *via* prototypic carboxylic acid biotransformation pathways (*e.g.* glucuronidation), the boronate was shown to be equally labile to oxidation. Consistent with the *in vitro* metabolism appraisal in human liver microsomes, the principal route of biotransformation of bortezomib in patients was deboronation, the result of which was formation of a pair of carbinolamide metabolites (Scheme 3.13).[310] Multiple P450 enzymes including P450 1A2, 2C9, 2C19, 2D6 and 3A4 catalysed deboronation in bortezomib.[310] A subsequent investigation demonstrated that the deboronation reaction in human liver microsomes involved multiple oxidants, including both reactive oxygen species (*e.g.* $O_2^{\bullet -}$) and specific activated-enzyme oxidants (*e.g.* peroxo-iron), both generated during the CYP catalytic cycle.[311]

3.13 Concluding Remarks: Carboxylic Acid and Drug Safety

From a drug discovery perspective, the presence of the carboxylic acid functionality in drug candidates provides an interesting topic for debate. Carboxylic acid containing drugs (*e.g.* NSAIDs, β-lactam antibacterials and statins) have revolutionised drug discovery in the 20th century, yet a number of carboxylic acid containing drugs have also been withdrawn from the market due to rare but serious adverse reactions. Of 29 drugs withdrawn from the market in the

UK, Spain or USA between 1974 and 1993, nine were carboxylic acid containing drugs, making this compound class the most frequently involved in drug discontinuations in this period.[312] In a recent review by Fung *et al.*, it was found that of the 121 prescription drugs withdrawn worldwide between 1960 and 1999, 17 were carboxylic acid containing drugs.[313]

Many of the carboxylic acid containing drugs that have been associated with toxicity (idiosyncratic or otherwise) belong to NSAID class. As NSAIDs are some of the most used prescription and over-the-counter drugs, the number of patients exposed to these drugs on a daily basis may be part of the explanation why so many adverse drug reactions, including idiosyncratic ones, have been observed with this therapeutic class. Some of the more prominent cases of NSAIDs that have been withdrawn post-marketing include ibufenac and benoxaprofen (Figure 3.26); this was due to incidents of overt liver toxicity, and zomepirac, which caused anaphylactic shocks. Additional examples of carboxylic acid drugs that have been withdrawn after introduction to the market, or have otherwise been associated with liver toxicity, are shown in Figure 3.26.

In many instances, bioactivation of the carboxylic acids to electrophilic esters (*e.g.* acylglucuronides, acyl CoA thioesters) is thought to represent the rate-limiting step in the aetiology of idiosyncratic adverse drug reactions (IADRs). When such metabolites react with critical proteins, cellular functionality may be disturbed or an immune response may be induced, eliciting adverse effects that in serious cases can be fatal. For example, reported adverse effects to carboxylic acid containing drugs span from mild elevation in serum liver enzymes or jaundice over skin rash and eczema to fatal anaphylactic shock.

Although it is now generally accepted that there is a link between the formation of chemically reactive metabolites and a number of IADRs, the mechanisms by which this occurs are generally not well understood. Furthermore, several carboxylic acid-based drugs (*e.g.* diclofenac, suprofen, zomepirac) also contain additional structural alerts/toxicophores (thiophene, aniline), susceptible to P450 catalysed bioactivation.[314–316] As a consequence, it is difficult to draw conclusions on the overall contribution of carboxylic acid bioactivation *versus* P450 catalysed reactive metabolite formation towards IADR occurrence.

From an industrial perspective, there is no clear rationale for avoiding the carboxylic acid moiety in drug design especially since mammals are exposed to many carboxylic acid based compounds from dietary sources every day and there are a number of safe carboxylic acid drugs on the market. Research over the last decades has, however, revealed the bioactivation potential of the carboxylic acid moiety to protein-reactive metabolites, and structure–toxicity relationships between acyl glucuronide reactivity and IADRs have been fairly compelling in some instances (*e.g.* ibufenac *versus* ibuprofen); consequently, it is sensible to evaluate the ability of all carboxylate drugs to form electrophilic acyl glucuronides (especially the propensity to the glucuronide to rearrange).[317] Since several factors play a role in determining which metabolites are formed and to what extent, and since little is known about the mechanisms that govern

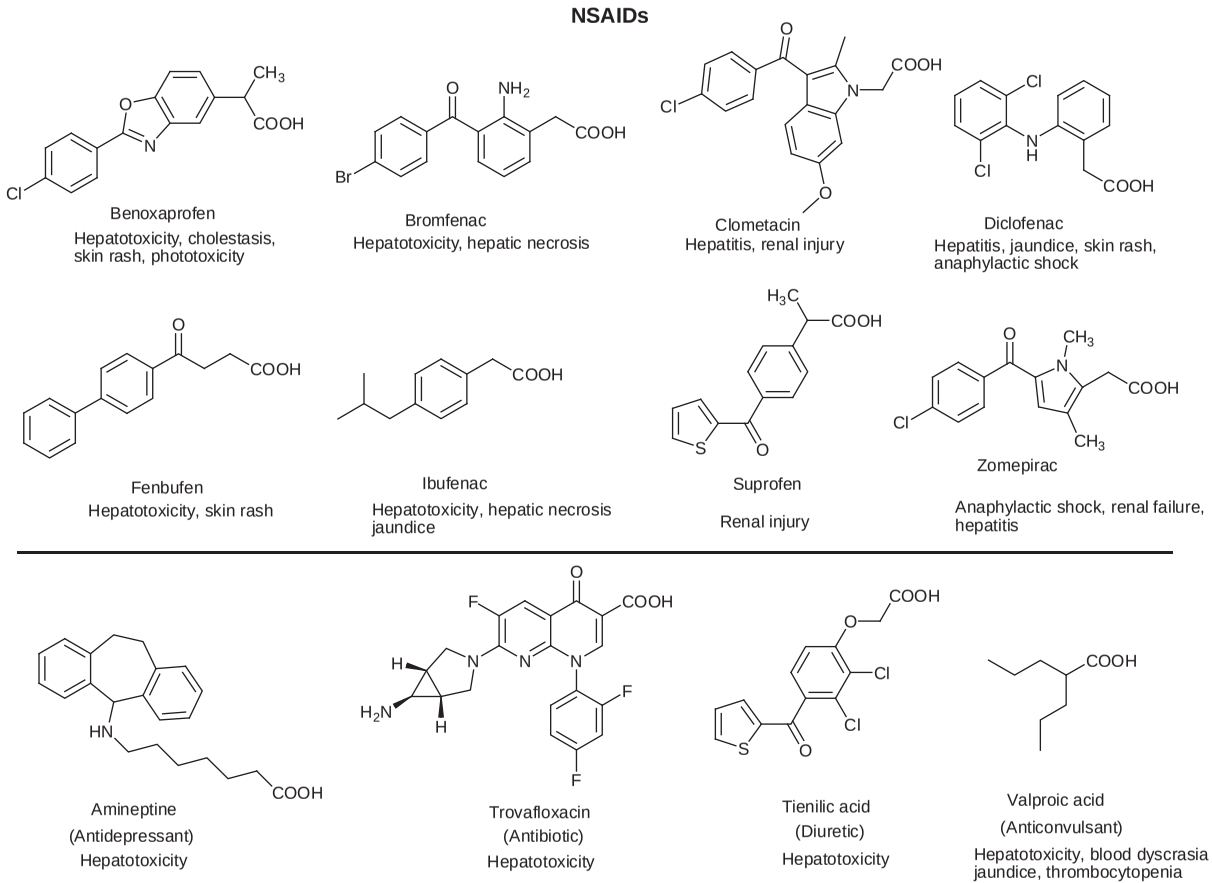


Figure 3.26 Carboxylic acid based drugs withdrawn from the market or associated with significant incidences of idiosyncratic drug toxicity.

toxicity caused by chemically reactive metabolites, it is difficult to conclude whether specific carboxylic acid metabolites will ultimately cause toxicity.

From a safety risk mitigation perspective, additional considerations such as the daily dose of the drug candidate may be a pivotal factor mitigating the risks of IADRs. Examples of low dose drugs ($<50\,mg\,day^{-1}$) that cause IADRs are rare (whether or not these agents are prone to bioactivation).[317,318] Atorvastatin serves as the ideal example of this phenomenon; despite biotransformation to acyl glucuronide metabolites; there have been no instance of IADRs with this blockbuster drug, a feature that can be linked with its low daily dose. Likewise, it is important to note the differences in daily doses of troglitazone ($200–400\,mg\,day^{-1}$) when compared with the structurally related thiazolidinedione derivatives rosiglitazone and pioglitazone ($10–40\,mg\,day^{-1}$). This feature may offset the bioactivation liability associated with the thiazolidinedione ring system in general resulting in an improved safety profile for the successor agents relative to troglitazone, which has been withdrawn due numerous cases of fatal hepatotoxicity.

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CHAPTER 4

Primary, Secondary and Tertiary Amines and their Isosteres

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4.1 Introduction

Amines are one of the most well-represented functional groups amongst small drug molecules. These compounds cover a wide range of therapeutic applications and possess a broad spectrum of physicochemical properties. In the majority of cases, the amine provides a positively charged function that is important for interaction with the target receptor and thus provides potency and selectivity. Whilst this ionic interaction may not be essential for binding to the target, it clearly represents an opportunity to provide higher affinity binding than may be achieved through weaker intermolecular interactions. There are also a number of examples where the incorporation of an amine confers pharmacokinetic advantages and some of these are discussed in this chapter.

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4.1.1 Amines that Interact with Aminergic Receptors

Foremost amongst compounds where the target receptor favours binding to an amine are the drugs targeting the aminergic receptors that play such a central role in physiologic function. The natural ligands of these receptors (see Figure 4.1) all contain amines and hence, for drugs to interact with these receptors or their re-uptake mechanisms, an amine function is clearly indicated.

Noradrenaline, serotonin (5-hydroxytryptamine), dopamine and histamine are primary amines, with adrenaline being the only secondary amine amongst the group. Drugs that interact with these targets comprise a mixture of primary, secondary and tertiary amines and are employed for a variety of pharmacological treatments. The discovery of early available antidepressants arose from chance observations[1] including the tricyclic antidepressants (*e.g.* imipramine, chlorpromazine and amitryptiline) which are, in the majority, tertiary amines and exert their pharmacological effect through interaction with multiple aminergic receptor targets.[2] Monoamine oxidase inhibitors (*e.g.* moclobemide, tranylcypromine, selegiline and indeloxazine) are also used as antidepressants and were also initially discovered by chance. These agents are inhibitors of monoamine oxidases (MAO-A and MAO-B), the enzymes responsible for the breakdown of serotonin, dopamine, adrenaline and noradrenaline. From the 1980s onward, there was a more deliberate design of antidepressants to target specific single or combined pharmacological action. Hence there are a vast number of amine containing drugs including:

- selective serotonin re-uptake inhibitors (SSRIs), *e.g.* fluoxetine, sertraline and paroxetine;
- noradrenaline re-uptake inhibitors (NSRIs), *e.g.* desipramine and reboxetine;
- 5HT antagonists, *e.g.* trazadone and nefazodone;

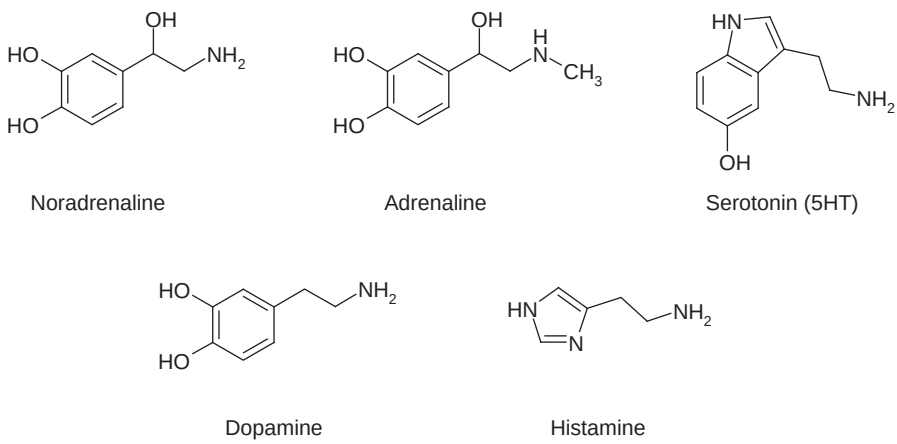


Figure 4.1 Structure of aminergic transmitter substances that are drug targets.

- serotonin and noradrenaline re-uptake inhibitors (SNRIs), *e.g.* venlafaxine;
- dopamine and noradrenaline re-uptake inhibitors, *e.g.* bupropion;
- serotonin and noradrenaline antagonists, *e.g.* mirtazapine.

Cardiac drugs that operate *via* action on aminergic receptors include β-adrenergic antagonists (*e.g.* propranolol and atenolol) and α-adrenergic antagonists (*e.g.* doxazosin and tamsulosin). β-Adrenergic agonists containing an amine function are utilised as bronchodilators in the treatment of pulmonary disease (*e.g.* salbutamol and salmeterol). Treatment of migraine is achieved by amine drugs targeting blockade of α-adrenoreceptors (*e.g.* ergotamine) and serotonin (*e.g.* sumatriptan and eletriptan). H_1-antihistamines are used for the treatment of allergies and include the amine containing drugs, loratidine and terfenadine, whilst H_2 antagonists are used to inhibit gastric acid production and include the amine containing drugs, ranitidine and famotidine.

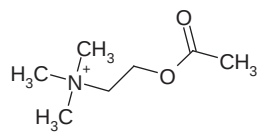
4.1.2 Amines that Interact with Acetylcholine

In addition to the aminergic drugs, there are amine containing drugs that are antagonists of acetylcholine (ACh). Acetylcholine (Figure 4.2) is a quaternary ammonium neurotransmitter, which dictates the requirement for a positively charged function within a molecule to interact with the acetylcholine receptor.

There are a set of secondary (*e.g.* terodiline), tertiary (*e.g.* atropine, oxybutynin and pirenzipine) and quaternary (*e.g.* ipratropium and oxitropium) amine containing drugs that represent the class of antimuscarinic agents as antagonists of acetylcholine. There are also a number of amines that inhibit the breakdown of acetylcholine by acetylcholinesterase—and thus potentiate its effect—including the tertiary amine, physostigmine and the quaternary amines, neostigmine and pyridostigmine.

4.1.3 Amines that Interact with Opioid Receptors

Several opioid agonists and antagonists also contain amine functions to bind to the various opioid receptors. Whilst the endogenous ligands of these receptors are peptidic in nature, the presence of a primary amine in endomorphins (*e.g.* endomorphin-1: Tyr-Pro-Trp-Phe-NH_2) demonstrates the role of the amine in the ligand–receptor interaction for this pharmacological target as exemplified in



Acetylcholine

Figure 4.2 Structure of acetylcholine.

prototypical opioid drugs such as morphine. Many of these agents (*e.g.* buprenorphine and pethidine) show mixed activity as agonists and antagonists at μ, κ and δ-opioid receptors. Opioid antagonists are used for central pharmacological effects for example in the treatment of opioid overdose (*e.g.* naloxone) or for peripheral action in the treatment of diarrhoea (*e.g.* loperamide).

4.1.4 Amines that Interact with Ion Channels

Another group of amine containing drugs are those intended to block the sodium and potassium channels, the positive charge of the amine function mimicking the positive charge of the sodium or potassium ion to elicit this effect. Amine containing sodium channel blockers include encainide and flecainide, whilst examples of potassium channel blockers include dofetilide and amiodarone.

It is the non-specific interaction of amines with a specific potassium channel that gives rise to the most common clinical safety concern amongst amine containing drugs. Cardiac arrhythmia due to blockade of the hERG channel—which conducts the repolarisation of cardiac muscle *via* the rapid delayed rectifier K^+ current (I_{Kr})—may lead to *Torsades de Pointes* and sudden cardiac death, and is a serious side effect for a number of amine containing drugs (*e.g.* cisapride, terfenadine and grepafloxacin). Whilst incidence of this adverse event is low (about 1 in 120 000 for cisapride), the potential severity of the outcome has meant that several amine containing drugs have been withdrawn from the market or relegated to restricted use. This toxicity is widespread across different drug classes[3] and reflects unusual susceptibility of the hERG channel to blockade by drug molecules.[4]

A number of calcium channel blockers also contain amine functions (*e.g.* verapamil and amlodipine), although this is far from a requirement for this drug class which contains many neutral molecules (*e.g.* felodipine, nifedipine). Amines are also represented amongst local anaesthetics, some of which function through sodium channel blockade (bupivacaine, lignocaine, prilocaine) or *via* unknown mechanisms (ketamine). Lignocaine (also known as xylocaine and lidocaine) may be used either locally as an anaesthetic or intravenously as an anti-arrhythmic agent.

4.1.5 Amine Antimalarial Drugs

Alkaloid antimalarial drugs (*e.g.* amodiaquine, chloroquine, hydroxychloroquine, halofantrine, mefloquine, primaquine and quinine) have a mechanism of action that depends on the amine function of these molecules. The drug molecule diffuses down a pH gradient to accumulate in the acidic food vacuole (pH 4.7) of the parasite. The high concentration of drug inhibits the polymerisation of haem, hence the haem that is released from the breakdown of haemoglobin builds up to toxic levels killing the parasite with its own waste product.[5]

4.1.6 Miscellaneous Amine Drugs

In addition to this substantial number of amine containing drugs where the amine can be seen to provide interaction, there are a number of miscellaneous amines including several antibiotics (enoxacin, erythromycin, azithromycin, clarithromycin, aclarubicin, rifampicin, tetracycline, arbekacin and tobramycin), an antifungal (terbinafine) and an antiparasitic (diethylcarbamazine). Amines are also represented amongst oestrogen antagonists (clomiphene and tamoxifen) and antitussive agents (dextromethorphan and pholcodine). Amines in other drug classes include almitrine (respiratory stimulant), ticlopidine (antithrombotic agent), sildenafil and vardenafil (PDE5 inhibitors), maraviroc, aplaviroc and vicriviroc (CCR5 antagonists), pinacidil (potassium channel opener) and vinblastine and vincristine (tubulin inhibitors).

4.1.7 Amine Isosteres

Amidine and guanidine functions (see Figure 4.3) are present in a number of drug molecules as alternatives to simple primary, secondary or tertiary amines and provide an equivalent basic function. Drugs that contain a guanidine function include the adrenergic blocker, debrisoquine, the antiseptic, chlorhexidine and the H_2-antagonist, cimetidine. Amidine containing drugs include the antiparasitic agent diminazene. Related to amidine is the imidazoline function, a heterocycle derived from imidazole and containing an imine bond. The α-adrenergic agonist, naphazoline, is an example of an imidazoline containing compound.

Hydrazine provides another alternative chemical function in place of simple primary secondary or tertiary amines. There are a number of drugs containing hydrazine functions including the antituberculosis compound, isoniazid, the antihypertensive, hydralazine, and the MAO inhibitor, phenelzine.

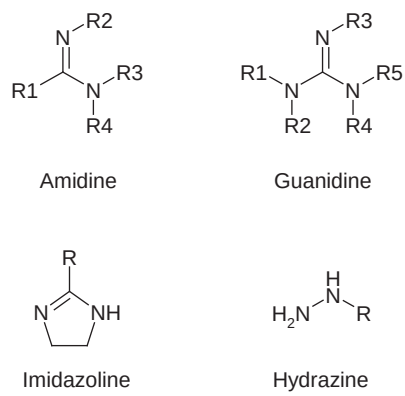


Figure 4.3 General structure of amine isosteres.

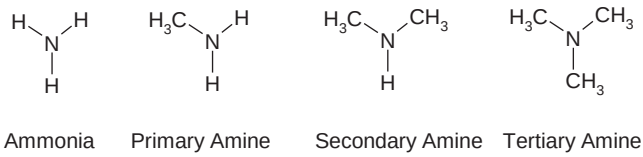


Figure 4.4 Structures of ammonia and primary, secondary and tertiary amines.

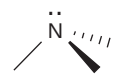


Figure 4.5 Chirality of trimethylamine.

4.2 Physicochemical Properties of Amines

Amines are hydrocarbon derivatives of ammonia consisting, in the neutral state, of a nitrogen atom that forms three bonds and a lone pair of electrons. Amines can be classified as primary, secondary and tertiary depending on the degree of hydrocarbon substitution (Figure 4.4).

Amines are sp^3 hybridized and thus are chiral (Figure 4.5), although rapid pyramidal inversion means that the individual enantiomers are not usually separable (resolvable).

The primary physicochemical properties of importance of the amino group are polarity (see Section 4.2.1) and basicity (see Section 4.2.2). As a result of these properties, amino groups are incorporated into drug molecules to facilitate solubility (in water of other vehicles for drug administration) and to enhance binding to many drug targets including receptors and enzymes (see Section 4.1).

4.2.1 Polarity of Amines

Due to the electronegativity of the nitrogen atom, C–N and N–H bonds possess polarity with the partial negative charge situated on the nitrogen. Thus, most amine containing compounds will have a dipole which will facilitate aqueous solubility *via* dipole–dipole interactions with water molecules (Figure 4.6). The impact of amino groups on solubility and hence on oral absorption is discussed further in Section 4.3.1.

The holistic lipophilicity of a compound, defined by the partition coefficient (P, normally presented as log P) between organic and aqueous solvents is clearly dependent on the relative polarity of the functional groups present in the molecule. The introduction of a primary amine group into a molecule lowers its log P by 1.23, reflecting an approximately 17-fold change in the partitioning coefficient itself.[6] It is of course important to remember that whilst amines

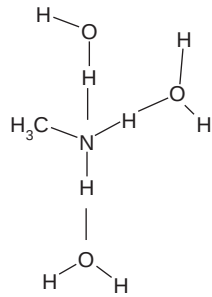


Figure 4.6 Hydrogen bonding interactions between methylamine and water.

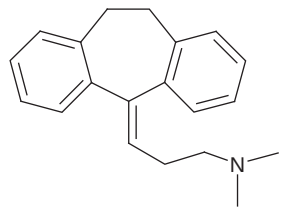


Figure 4.7 Structure of amitriptyline.

confer polarity to a molecule, their incorporation will not inevitably result in a molecule of high aqueous solubility. As a simple example, the tricyclic antidepressant drug amitriptyline. (Figure 4.7), which contains a tertiary amine group, is extremely lipophilic with a log P of 4.9 due to the large number of non-polar functional groups.

4.2.2 Basicity of Amines

The second key physicochemical property of amines is their basicity, defined as the ability to donate a pair of electrons and hence accept a proton. The relative basicity of amine functional groups is defined by the dissociation constant (Ka), typically described using a log scale (pKa) such that, the higher the pKa value, the greater the basicity. As compounds are essentially fully ionised when the pH is 2 or more units below the pKa, then the basicity of a molecule determines its ionisation at physiological pH which in turn affects many aspects of molecular behaviour, including target binding, distribution, absorption and solubility.

4.2.2.1 Factors Affecting Basicity

The basicity of amine groups depends on the ability of the amine functionality to donate its lone pair which in turn is dependent on the chemical environment of the nitrogen atom. One factor that affects basicity is induction, whereby

Table 4.1 pKa values for some simple amines.

	Amine	*pKa*
	Ammonia	9.21
Primary amines	Methylamine	10.62
	Ethylamine	10.63
	Benzylamine	9.34
	Ethanolamine	9.50
	Hydrazine	8.10
	Hydroxylamine	5.97
	Acetamidine	12.52
	Guanidine	13.71
Secondary amines	Dimethylamine	10.64
	Diethylamine	10.98
	Benzylethylamine	9.68
	Morpholine	8.36
	Pyrolidine	11.27
	Piperidine	11.22
Tertiary amines	Trimethylamine	9.76
	Triethylamine	10.65
	Dimethylethylamine	9.99
Anilines	Aniline	4.62
	o-Hydroxyaniline	4.72
	m-Hydroxyaniline	4.17
	p-Hydroxyaniline	5.50
	o-Chloroaniline	2.62
	m-Chloroaniline	3.32
	p-Chloroaniline	3.81
	o-Methylaniline	4.38
	m-Methylaniline	4.67
	p-Methylaniline	5.07

proximal electron-donating groups (such as alkyl groups) increase electron density on the nitrogen and hence increase basicity whilst electron-withdrawing groups tend to reduce basicity. For this reason, secondary amine groups tend to be more basic (higher pKa) than primary amines (Table 4.1).

In theory, tertiary amines should be more basic still. However, tertiary amines introduce steric factors, which may hinder the ability of the amine group to donate its lone pair. Thus, trimethylamine (pKa 9.76) is in fact less basic than dimethylamine (pKa 10.64) or methylamine (pKa 10.62) (Table 4.1).

The third factor that affects basicity is resonance, whereby nitrogen lone pairs are delocalised across more than one functional group, thereby reducing the ability of the amine to donate its lone pair and hence reducing basicity. This explains why aromatic amines (anilines) are significantly less basic than aliphatic amines (Figure 4.8) and also why amide functional groups have low basicity.

The basicity (pKa values) of a selection of simple amines is shown in Table 4.1. Additional reference data on amine basicity are available from a number of sources.[7–11]

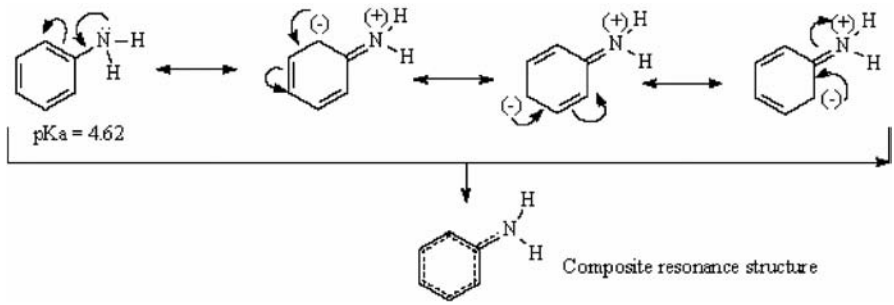


Figure 4.8 Electron delocalisation of aromatic amines.

4.3 Absorption Properties of Amine Containing Drugs

The majority of drugs are administered by the oral route and hence absorption from the gastrointestinal tract is needed for these agents to reach the systemic circulation and exert their pharmacological effects. The physicochemical properties of a drug that influence absorption are solubility, particle size, crystal form, lipophilicity, dissociation constant and molecular weight. The presence of an amine function within a drug molecule has direct influence on solubility, lipophilicity and dissociation constant and thus impacts on the process of gastrointestinal absorption.

For absorption to occur it is first necessary for the drug molecule to be in solution. There are then essentially two pathways by which drug can cross the gastrointestinal membrane and enter the circulation. These are the transcellular pathway where drug passes through the membranes and across the cells of the gut wall and the paracellular pathway where drug passes through the tight junctions between the cells. Transcellular transport includes active carrier mediated processes; however, the majority of drug absorption occurs by the passive transcellular pathway.

The weakly basic nature of primary, secondary and tertiary amines has two fundamentally opposing effects on oral absorption *via* the transcellular route. On the one hand, the presence of a polar and ionisable function enhances aqueous solubility in the gastrointestinal fluid whilst, on the other hand, the same function can hinder diffusion across the membrane which is largely restricted to the non-ionised form of the drug and is enhanced by lipophilicity.

4.3.1 Solubility and Absorption

The benefit that an amine can provide in terms of facilitating aqueous solubility is illustrated in the case of the HIV-1 protease inhibitor, indinavir. Early chemical leads in this series such as L-685434 (Figure 4.9) were shown to be potent inhibitors of HIV-1 protease but possessed poor oral bioavailability.

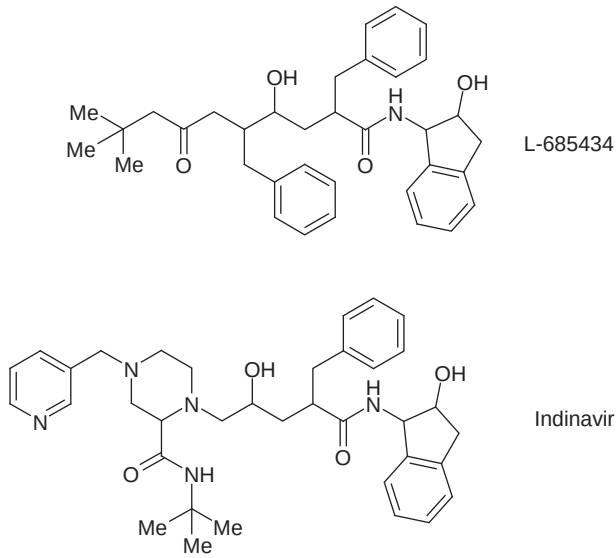


Figure 4.9 Structure of the protease inhibitors L-685434 and the amine containing indinavir.

Only through incorporation of the basic amine (piperazine) was adequate solubility achieved and oral bioavailability realised.[12] It should be noted that whilst the amine constituent of indinavir provided adequate solubility, the intrinsic solubility of the molecule remains relatively low and exhibits marked pH dependency with solubility of only $0.03\,\mathrm{mg\,ml^{-1}}$ at pH 6 rising to $>100\,\mathrm{mg\,ml^{-1}}$ at pH below 3.5. This results in pH dependent oral absorption of the compound[13] and is a source of pharmacokinetic variability and sensitivity to food intake.[14]

4.3.2 Membrane Permeability and Absorption

The pH of the stomach is typically acidic with a pH range of 1.4 to 2.1 in the fasted state and pH 3.0 to 7.0 in the fed state.[15] Amine containing drugs, or salts of these compounds, will be expected to exhibit their maximum solubility at these pH values[16] and thus will most easily enter solution in the upper regions of the gastrointestinal tract. However, the ionised form of a drug is less lipophilic than the unionised form and is thus less able to diffuse across the lipoidal membrane. Hence quaternary ammonium compounds generally have extremely poor oral absorption due to their permanently charged nature compared with partially ionised tertiary amine analogues as illustrated by atropine and ipratropium bromide (Figure 4.10). The tertiary amine, atropine exhibits 90% absorption[17] whilst the quaternary ipratropium shows low absorption and only 2% oral bioavailability.[18]

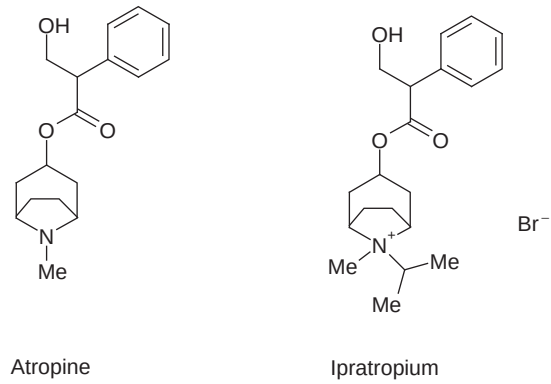


Figure 4.10 Structure of the tertiary and quaternary amine containing antimuscarinic agents, atropine and ipratropium bromide.

The rate of absorption of an amine containing drug is thus dependent upon the concentration of the unionised species at the site of absorption. The fraction in unionised form (f) is predicted by the Henderson–Hasselbalch equation and is dependent upon the pKa of the compound and the pH of the medium.

$$f = \frac{1}{1 + 10^{(pKa - pH)}} \tag{4.1}$$

Thus for an amine with a pKa of 8.0, the fraction in the unionised form in the stomach (pH $\sim$2.0) will be only 0.0001% compared to about 25% in the ileum (pH $\sim$7.5). Based on the theory that only the unionised form is able to penetrate biological membranes (pH partition theory), it can be expected that amine drugs are better absorbed in the lower regions of the gastrointestinal tract with higher pH values when a significant proportion will always be present in the unionised form. This theory is nicely illustrated by the example above featuring atropine; no compound is absorbed from the stomach whereas absorption reaches 90% during passage to the distal part of the jejenum.[17]

The limitation to this theory derives from an assumption that only unionised drug is able to be absorbed by passive diffusion when in fact the ionised form of drug is able to be absorbed by the paracellular route, albeit at a relatively slow rate. Paracellular absorption is particularly effective for small drug molecules (MW $<$ 250) and can be the major route for some low molecular weight hydrophilic compounds. Hence for a small, quaternary ammonium compound such as pyridostigmine, which is permanently charged but is a very small molecule (MW = 181), oral bioavailability is as high as 15%;[19] this is presumed to be due to paracellular absorption. The clear difference in terms of physicochemical properties between the two quaternary ammonium compounds, pyridostigmine and ipratropium, is molecular size (MW values of 181 *versus* 332) and is reflected in the markedly higher oral bioavailability of the former

due to its ability to pass through the restricted access afforded by the aqueous pores (diameter typically 3–6 Å in humans).

Effective transcellular absorption requires molecules to possess sufficient lipophilicity to partition into and through the lipophilic environment of the cell membranes that line the gastrointestinal tract. The distribution coefficient (D) is a convenient parameter for describing the lipophilicity of a molecule and reflects the contribution of the degree of ionisation at the pH at which the determination is carried out to the overall expressed lipid affinity of a molecule.[20] Generally speaking, a distribution coefficient greater than 1 (log $D > 0$) will indicate adequate lipid affinity for a compound to diffuse into and through cell membranes.[21]

Examination of the absorption properties of a series of β-adrenoceptor antagonists demonstrates a number of factors that influence absorption. Figure 4.11 shows the relationship between lipophilicity and the estimated oral absorption for 16 β-adrenoceptor antagonists ranging from log $D_{7.4}$ values of -1.9 (atenolol) to 2.0 (carvedilol). The majority of these compounds show high and essentially complete (>80%) absorption with the exception of atenolol, nadolol, xamoterol and talinolol. Absorption of the hydrophilic agents, sotalol and practolol, is complete despite their log $D_{7.4}$ values of −1.4 and −1.3, respectively, and indicates that these molecules are sufficiently small (MW 272 and 266, respectively) to pass through the aqueous pores. Three other hydrophilic members of this series show incomplete absorption in man with values of 9% for xamoterol, 30% for nadolol and 44% for atenolol. For these three compounds, the increase in molecular size from atenolol (MW = 266) to

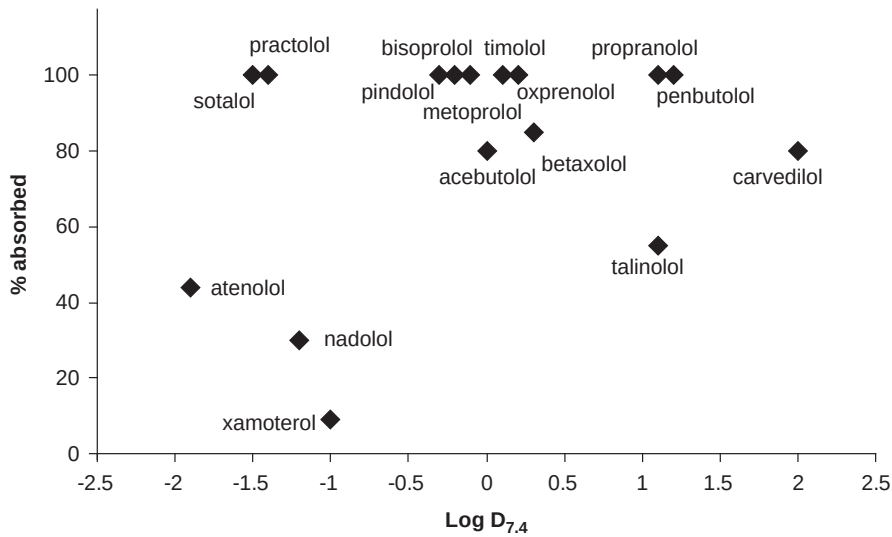


Figure 4.11 Relationship between lipophilicity (Log $D_{7.4}$) and extent of absorption in man for a series of 16 β-adrenoceptor antagonists.

nadolol (MW = 309) to xamoterol (MW = 339) would appear to correlate with their ability to enter the systemic circulation *via* the paracellular route. It should be recognised that *in vitro* systems for permeability studies, such as Caco-2 cell monolayers, may not provide permeability assessments that are representative of *in vivo* permeability for compounds which are absorbed *via* the paracellular pathway. For example, sotalol displays low permeability in the Caco-2 cell line despite its greater than 90% absorption *in vivo*.[22] This *in vitro*/*in vivo* difference is considered to be due to differences between the tightness of the intercellular junctions.

4.3.3 Impact of P-glycoprotein on Absorption

The other β-antagonist to show incomplete absorption is talinolol, which despite its high lipid affinity (log $D_{7.4} = 1.1$), does not appear to show good membrane permeability. The lipophilicity of talinolol is similar to other β-antagonists that have complete absorption (*e.g.* propranolol, log $D_{7.4} = 1.1$) and, whilst its molecular weight (339) is toward the high end for this series, it is certainly not of a size that would be expected to restrict transcellular permeation. Several studies have shown that the incomplete absorption of talinolol is a result of the compound being a substrate for the efflux transporter P-glycoprotein.

Preclinical and clinical studies have demonstrated that P-glycoprotein acts to prevent the compound from traversing the intestinal membrane by active efflux back into the gut lumen. The P-glycoprotein inhibitor, verapamil, has been shown to increase oral bioavailability of talinolol in rats[23] and also to decrease the intestinal secretion of intravenously administered talinolol in humans.[24] The affinity of talinolol for this efflux transporter would appear to be due to the additional hydrogen bonding functionality within this molecule which is imparted by the urea function, with hydrogen bond acceptor functions having been identified as important for P-glycoprotein mediated efflux.[25] However, several other β-adrenoceptor antagonists, including propranolol, have similar affinity for P-glycoprotein[26] without suffering from incomplete absorption. Thus it is likely the combination of P-glycoprotein affinity and low intrinsic membrane permeability that results in the relatively poor absorption of talinolol.[27]

Whilst affinity for P-glycoprotein and a resultant effect on absorption is not directly attributed to an amine function, there are several other amine containing compounds that have been shown to exhibit dose dependent absorption potentially due to P-glycoprotein affinity including maraviroc,[28] the NK2 antagonist, UK-224 671[29] and the PDE5 inhibitor, UK-343 664.[30] For each of these three examples, physicochemical properties are less than ideal for good membrane permeability[31] with molecular weight values above 500 and relatively high numbers of hydrogen bond acceptors. These features result in borderline membrane permeability and the molecules are thus susceptible to P-glycoprotein mediated efflux.

4.4 Systemic Behaviour of Amine Containing Drugs

4.4.1 Tissue Affinity and its Impact on Distribution

It is well recognised that the physicochemical properties of drug molecules have a fundamental impact on their distribution throughout the body and thus are essential considerations in drug design. Such distribution properties impact the pharmacological activity of drugs as a result of their affect on pharmacokinetics and also the ability of compounds to reach intended cellular targets by crossing cell membranes. The extent to which a drug is distributed outside of the circulation is dependent on the relative affinity of the compound for tissue proteins relative to plasma proteins and this determines the observed volume of distribution according to eqn (4.2):

$$Vd = V_p + V_t.f_u/f_{ut} \tag{4.2}$$

where Vd is the distribution volume , V_p is the volume of plasma, V_t is the volume of tissues, f_u is the unbound fraction in plasma and f_{ut} is the unbound fraction in tissues.

Hence the higher the tissue binding (and smaller the free fraction in tissues) relative to plasma binding, the larger will be the apparent volume of tissues in which the molecule resides.

4.4.2 Distribution and Duration

A key component of the systemic behaviour of amine containing drugs is the high affinity with all types of tissue resulting from the electrostatic interaction between the basic amine group of the drug and the anionic functionalities of the phospholipid membranes. As a result, whilst compounds of all physicochemical classes demonstrate an increase in volume of distribution (Vd) with increasing lipophilicity (log D), basic compounds show higher Vd values than acids or neutral molecules of comparable lipophilicity—depending on the basicity of the amine (as defined by its pKa; see Section 4.2.2).

Furthermore, it is important to recognise that free (unbound) volumes of distribution are also higher for basic compounds, demonstrating that holistic affinity for tissue membranes is a more important factor than increased interactions with plasma proteins.[21] The general principle of high volumes of distribution for basic drugs is exemplified by the SSRIs paroxetine, sertraline and fluvoxamine, all of which have large volumes in humans and animals.[32,33] As a component of drug design, therefore, the addition of a basic centre will often result in an increase in Vd and concomitant increase in elimination half-life ($T_{1/2} = 0.693 \times Vd/Cl$) as highlighted by the pharmacokinetics properties of antibacterial agents based on the rifamycin structure (Figure 4.12).

The original agents (*e.g.* rifamycin SV; Figure 4.12) had short elimination half-lives and low oral bioavailability.[34] Incorporation of a basic centre in rifampicin (Figure 4.12) increased volume of distribution[35] and further

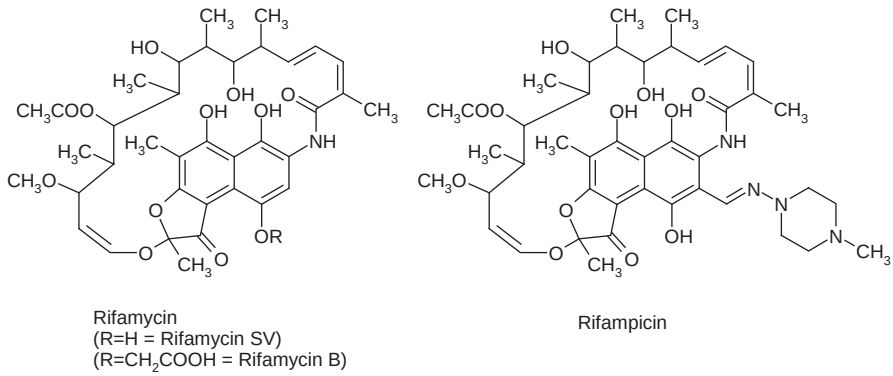


Figure 4.12 Structure of rifamycin and rifampicin.

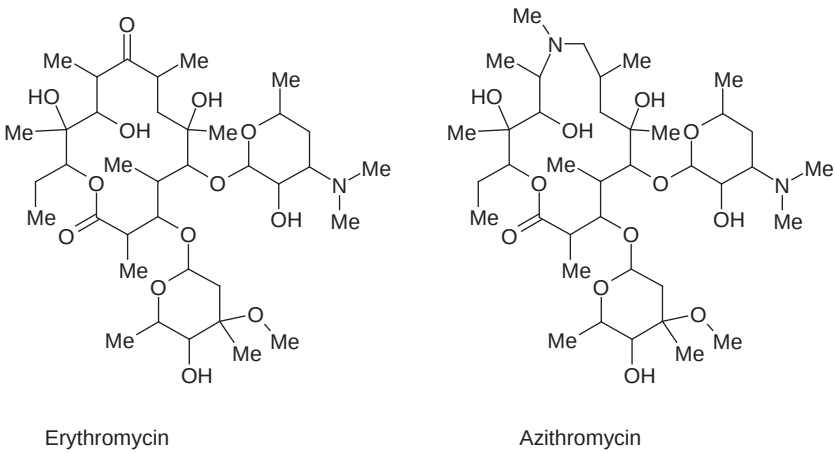


Figure 4.13 Structures of monobasic erythromycin and dibasic azithromycin.

modifications to the basic centre in rifabutin and rifapentine have contributed to increased distribution volumes, longer elimination half-lives and extended duration of action.[36]

Further impact of a basic centre on volume of distribution is shown by the dramatic increase in Vd observed for the macrolide antibiotic, azithromycin, when compared to the first generation drug, erythromycin (Figure 4.13). Erythromycin contains one basic centre and has a Vd of $0.5\,L\,kg^{-1}$ in humans and a half-life of about three hours. Introduction of a second basic centre in azithromycin increases the volume to $23\,L\,kg^{-1}$ and half-life to 48 h, leading to a significant prolongation of pharmacological action.[37,38]

As well as adding a basic centre to a molecule to affect distribution volume, there is also evidence that manipulation of the distribution volume can be achieved by altering the precise nature of the basic centre, as shown by a series of

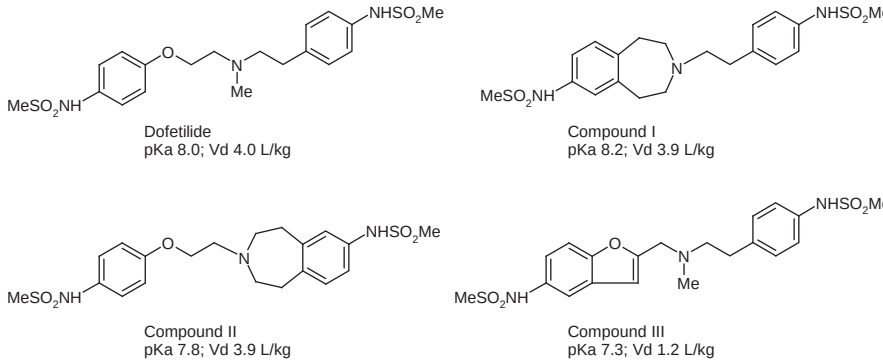


Figure 4.14 Structures of four potassium channel blockers with varying pKa values and volumes of distribution.

four potassium channel blockers, including the anti-arrhythmic drug dofetilide (Figure 4.14). Whilst three amines with pKa values in the range 7.8–8.2 show very similar volumes in dog (3.9–4.0 L kg^{-1}), the fourth compound, with a lower pKa of 7.3 (due to delocalisation of the tertiary amine) has a Vd of only 1.2 L kg^{-1}.[39] This reduced volume reflects the increased plasma protein binding of the less polar analogue restricting compound distribution out of the circulation.

4.4.3 Additional Specific Interactions Enhancing Tissue Affinity

Whilst generically high tissue affinity explains to a large extent the high volumes of distribution for amine containing compounds, specific interactions between the basic centre and tissue membranes can result in further increases in the volume of distribution. An extreme example of this principle is the calcium channel blocker, amlodipine, where the long plasma half-life (35 h) in humans is largely driven by a Vd of 21 L kg^{-1}. That the basicity of the drug is the key driver for the large Vd is demonstrated by the fact that the neutral analogue, felodipine, which has a higher intrinsic lipophilicity than amlodipine (log $D_{7.4}$ of 4.8 compared to 1.8), has a markedly lower Vd and thus a shorter elimination half-life of 10 h. Further studies have shown that amlodipine binds in the phospholipid bilayer in a manner that enables additional electrostatic interactions which contribute to the large Vd.[40,41]

Another example is provided in the long duration of β-agonist activity for salmeterol[42,43] compared to many other β-agonists such as fomoterol. This is thought to result from the interaction between the drug and a specific 'exosite' on or in the vicinity of the membrane-bound receptor in the lung.[44,45]

4.4.4 Distribution Dependent on pH

As well as tissue affinity resulting from electrostatic interactions between amines and membranes, the basicity of amines can be responsible for other

observed distribution phenomena. Most notably, basic compounds are known to migrate to acidic environments *in vivo*, driven by pH gradients, rationalising such effects as passive gut secretion. A recent example is provided by the CCR5 inhibitor maraviroc, which possesses a tertiary amine basic centre and was shown to distribute 15% of the total dose into the gastrointestinal tract following intravenous administration to bile duct cannulated rats.[28] Although postulated as direct secretion into the gut, this phenomenon could also be facilitated by active transport as has been observed for the beta-blocker talinolol, where P-glycoprotein is known to be responsible for excretion of the compound into the gut.[46] The potential exploitation of the pH gradient phenomenon has also been demonstrated for amine containing chemotherapeutic agents such as mitoxantrone, where systemic alkalinisation of mice with sodium bicarbonate increases ion trapping of the weak base within tumour tissue due to the increased pH gradient.[47]

4.4.5 Plasma Protein Binding

The observation that many amine containing compounds exhibit large volumes of distribution reflects the fact that the general affinity of amines for tissues outweighs specific interactions with plasma proteins. However, it is important to consider such interactions to fully understand the impact of the basic centre on drug disposition. Whilst lipophilic acids show high affinity for human serum albumin (HSA), amine containing drugs have a tendency to bind strongly with the other most abundant plasma protein, α1-acid glycoprotein (AAG). Although lipophilic amines will also bind to albumin *via* non-specific binding effects, this specific interaction with AAG explains to a large extent the high protein binding of many amine containing compounds such as the antiarrhythmic drug amiodarone, which is 96.3% bound in human plasma,[48] and most notably the antimuscarinic agent zamifenacin, which demonstrates binding in excess of 99% in all species.[49]

An important consideration regarding the high affinity of amine containing compounds for AAG is the lower concentration of this protein (10–30 μM) compared to HSA (640 μM).[50] In theory, this reduced concentration increases the potential to saturate protein binding of amine containing compounds. However, given the significant tissue distribution of these compounds and the fact that the AAG concentration provides a molar equivalent concentration of $4\,mg\,ml^{-1}$ for a compound of molecular weight 250, protein binding saturation is rarely observed in practice. Saturation of binding to AGP has been shown for the I_f channel inhibitor YK754 *in vitro* with an estimated Bmax value of $7.8\,mg\,ml^{-1}$ (MW = 469).[51] This concentration is vastly in excess of plasma concentrations observed in animal studies.

An important consideration for basic drugs is the variations in AAG concentration within the human population. AAG is an acute phase protein such that several inflammatory states (*e.g.* infections, rheumatic disorders and surgical injury) and pathological conditions (*e.g.* myocardial infarctions,

malignancies and nephritis) can raise its serum concentration by up to three- or four-fold. Furthermore, pregnancy and various disease states can affect the concentration of AAG.

Thus, it can be important to understand the affinity of potential drugs for AAG and HSA, and the impact of disease and/or special population on AAG concentration such that the effect on the degree of protein binding can be taken into account. To illustrate this phenomenon, the free concentration of propranolol and chlorpromazine were shown to be inversely correlated to AAG concentration in studies with plasma from patients with Crohn's disease, inflammatory arthritis and renal failure as well as healthy control samples.[52] Furthermore, the higher incidence of toxic side effects of bupivicaine in pregnant women may be explained by the increased free exposure resulting from saturation of AAG.[53]

4.4.6 Brain Distribution

Although the same principles of interactions between basic centres and cell membranes apply equally well to all tissue types, it is important to acknowledge that differences exist between the blood–brain barrier and the gastrointestinal tract. Thus, whilst many CNS drugs contain basic centres (*e.g.* tricyclic antidepressants and SSRIs), good oral absorption does not necessarily correlate with good brain penetration. Whilst lipophilicity impacts tissue affinity, there is evidence to suggest that the blood–brain barrier is more sensitive to log D changes than the gastrointestinal tract. Indeed, it has been proposed that polar surface area (PSA) should be considered in early discovery as a potential means of separately impacting oral absorption and brain penetration.[54]

An example of using lipophilicity to reduce brain penetration for amine containing drugs is atenolol, which is a less penetrant β-antagonist than propranolol due to its lower log D, and thus has a reduced side effect profile but maintains high absorption following oral administration.[55,56] Increased size may also reduce brain penetration, as evidenced by maraviroc[57] where low permeability of the blood–brain barrier in rats was observed. The low penetration of this compound is likely a result of P-glycoprotein affinity which is often encountered with large amine molecules containing polar functionalities (see Section 4.3.3).

4.5 Clearance of Amine Containing Drugs

4.5.1 Metabolic Clearance

Amine containing compounds may undergo a number of biotransformations, of which the most common phase I pathways are *N*-oxidation and *N*-dealkylation, with deamination and methylation occurring less frequently. Amines may also undergo the phase II metabolic clearance routes of glucuronidation, sulfation and acetylation. The ability for an amine to be cleared by a particular

metabolic route depends on the enzymes present in the tissues to which it is exposed, the propensity of the compound to be metabolised at sites other than the amine, and variation in the expression of the metabolic enzymes due to gender, species, age or polymorphism.

4.5.2 Phase 1 Metabolism

4.5.2.1 N-Oxidation and N-Dealkylation

Two groups of enzymes, with overlapping substrate specificities, catalyse the formation of *N*-oxide metabolites. The cytochrome P450 (CYP 450) enzymes have a broad range of substrates with diverse metabolic products, while flavin monooxygenases (FMOs) are more specific in their substrates, being involved in oxidation of soft nucleophiles such as nitrogen and sulfur (and also selenium and phosphorus). Both enzymes are NADPH-dependent and membrane-bound, being located within the endoplasmic reticulum.

The mechanism by which the P450s and FMOs form *N*-oxides differ in that CYP450 employs two sequential stage one electron oxidations whilst FMOs utilise a single two electron oxidation directly on the nitrogen. It is difficult to predict if an amine will be the substrate for P450s or for FMOs, but it does appear that primary amines and charged species seem to be poor substrates for FMOs.[58,59]

Isoforms of FMO differ in their distribution between species. FMO1 is the major hepatic isozyme in most mammalian species (including rodents), whereas FMO3 is the major form in adult human liver and hence is responsible for most of the FMO-mediated drug metabolism. Therefore caution should be applied when using animal metabolism data to extrapolate to man.

FMOs are not induced or inhibited (with the exception of FMO2),[60] reducing the propensity for drug–drug interactions in cases where the drug is mainly an FMO substrate. For example, itopride and cisapride are both used in the treatment of gastro-oesophageal reflux disease. Cisapride is metabolised by CYP3A4. Co-administered drugs that inhibit CYP3A4 lead to an elevated plasma level of cisapride and hence to adverse events such as arrhythmia. Itopride, however, is metabolised by FMO3 and so is not subject to such drug-drug interactions.[61]

FMO3 exhibits genetic polymorphism, which can effect metabolism and hence the efficacy of drugs.[62,63] This is particularly important as a significant proportion of the population are at least mildly FMO deficient.[64] Benzydamine, a non-steroidal anti-inflammatory (NSAID), is almost fully *N*-oxidised by FMO3 such that greater than 94% is excreted as the *N*-oxide in human urine in the majority of the population. In subjects with severe FMO3 deficiency, however, less than 36% *N*-oxide is found in urine.[65] This translates to a 10% increase in the Cmax of circulating benzydamine in deficient subjects relative to the control population.

As FMOs and CYP450 have overlapping substrates, many substrates are metabolised by both enzyme systems. Moclobemide (Figure 4.15) is a

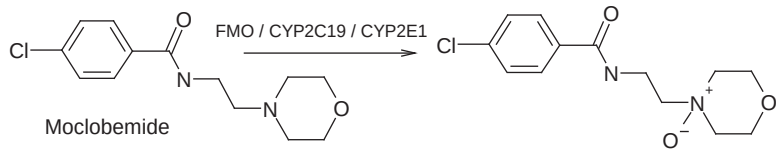


Figure 4.15 Oxidation of moclobemide by cytochrome P450 and FMO.

monoamine oxidase-A inhibitor prescribed for the treatment of depression. Although predominately metabolised by FMO, it is also a substrate for CYP2C19 and CYP2E1.[66] Similarly, the tricyclic antidepressant clozapine is *N*-oxidised by both FMO and CYP3A4.[66,67]

P450s are responsible for both *N*-oxidation and *N*-dealkylation of amines. Usually, *N*-dealkylation takes precedent over *N*-oxidation in molecules where an abstractable (highly acidic) hydrogen on the α-carbon is available. Abstraction of this hydrogen results in formation of an intermediate carbon radical which rearranges to give the aminium radical, which is stabilised by the lone pair of electrons on the adjacent nitrogen. There are exceptions to this 'rule' with examples of amines that are *N*-oxidised *via* P450s even though an abstractable hydrogen on the α-carbon is available. These exceptions include methamphetamine and the alkaloid, senecionine, both of which are *N*-oxidised in preference to being *N*-dealkylated.[68] In general, *N*-oxidation prevails over *N*-dealkylation where the aminium ion can be stabilised within the active site of the P450.[58,59]

Physicochemical properties of the molecule dictate its potential to interact with and hence to be metabolised *via* P450. Basicity does have an effect, but pKa is not the predominate factor in active site binding and the relationship between binding to P450 and basicity is not straightforward. A series of benzphetamines analogues show increase affinity for CYP2B4 with decreasing basicity.[69] A similar observation was made for propranolol and its fluorinated analogues, which showed increased interaction with CYP2D6 with decreasing pKa.[70] However, benzodiazepines (diazepam, medazepam and flurazepam, Figure 4.16) exhibit an increased rate of *N*-dealkylation with increasing basicity of the *N*-alkyl nitrogen.[71]

Lipophilicity has a major effect in the ability of a substrate to be metabolised by P450s, with increasing lipophilicity often leading to increased metabolic clearance. This has been clearly shown for the substrates of CYP2B6.[72] Additional examples include sodium channel blockers such as lidocaine, tocainide and mexilitene, which exhibit decreasing *N*-dealkylation by CYP3A4 with decreasing lipophilicity.[71] An analogous observation was made for the *N*-dealkylation of propranolol analogues by CYP1A2,[70] where the enzyme affinity increased with increasing lipophilicity. In contrast, however, these same propranolol analogues exhibited reduced affinity for CY2D6 with increasing lipophilicity. This results from the differences in binding mechanism between CYP2D6 and other P450s such as 3A4 which rely on hydrophobic interactions

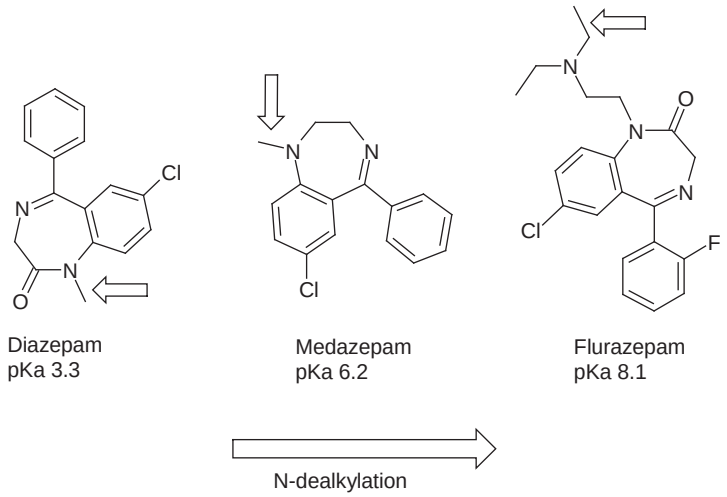


Figure 4.16 Structures of benzodiazepines, diazepam, medazepam and flurazepam showing increased rate of metabolism with increasing basicity.

for enzyme affinity; CYP2D6 binding is *via* ionic interactions such as ion pairing or hydrogen bonding, and is not predominately driven by lipophilicity.[21]

It has been reported that CYP2D6 is involved in metabolism of 20% of all drugs and its substrate binding specificity, with the requirement of a positively charged centre, means that amine containing drugs are often substrates.[73] CYP2D6 is polymorphic with approximately 10% of caucasians and less than 1% of asians being poor metabolisers, while 5% of caucasians are ultra-rapid metabolisers. Therefore, the genotype of subjects dosed with CYP2D6 substrates has a major impact on how they respond to therapy and if they experience adverse events. For example, the tricyclic antidepressant amitriptyline is hydroxylated by CYP2D6. Poor metabolisers exhibit elevated plasma levels of amitriptyline and may be at risk of adverse effects such as nausea or hypotension.[74] In general, the development as medicines of compounds that are highly dependent on clearance by CYP2D6 is avoided in order to reduce the risks associated with significant inter-subject variability. For example, development of the calcium channel antagonist UK-84 149, which is a CYP2D6 substrate, was abandoned because the variability in drug exposure between poor and extensive metabolisers was considered too great.[75]

4.5.2.2 Deamination

Amines may be oxidatively deaminated to aldehydes or ketones by P450s or amine oxidases. The aldehyde or ketone formed is often not the final metabolic product as further oxidation to the corresponding alcohol or acid may follow. The best characterised of the amine oxidases are the monoamine oxidases

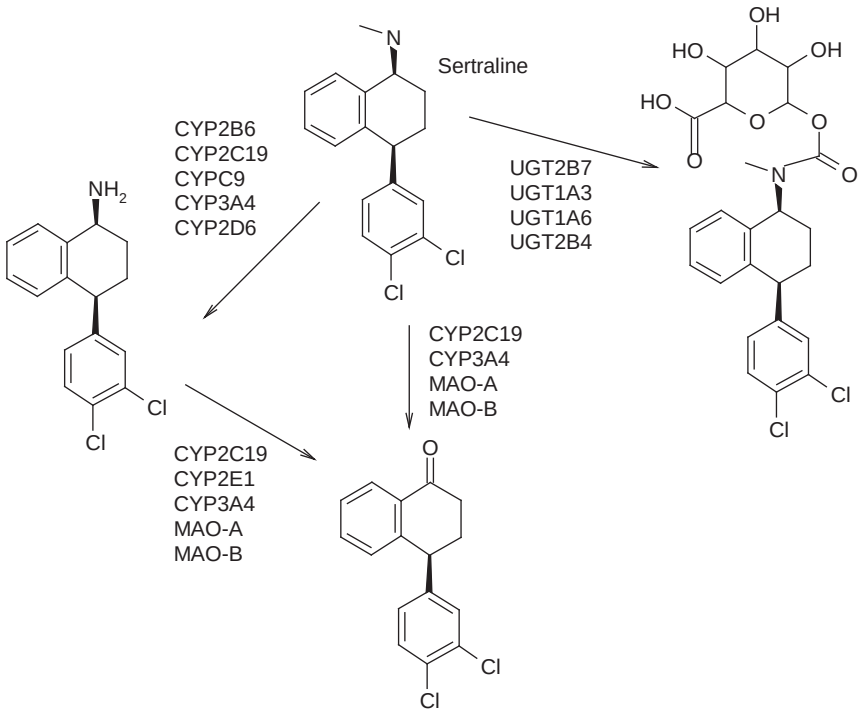


Figure 4.17 Metabolism of sertraline by MAO and cytochrome P450.

(MAOs). MAOs are located in the outer surface of the mitochondrial membrane and are found in most mammalian tissues. In humans, the highest MAO levels are found in liver and lowest levels in the spleen. The two major forms of MAO are MAO-A and MAO-B.

MAOs have affinity for polar substrates such as the endogenous neurotransmitters, dopamine and serotonin, which are outside the lipophilicity range of many P450 enzymes. To be a substrate for MAO, there must be an available hydrogen on the α-alpha to the amine and thus aniline is not a substrate. There is some overlap in specificity between MAOs and P450s, however, as exemplified by sertraline (Figure 4.17), which is deaminated to sertraline ketone by CYP3A4, 2C19 and MAO-A and MAO-B.[76] Other compounds such as sumatriptan are deaminated by MAO-A only with no P450 involvement.[77]

P450-mediated oxidative dealkylation can activate secondary and tertiary amines towards deamination by MAO. For example, zolmitriptan (Figure 4.18) is converted to its indole acetic acid metabolite *via* a P450-mediated secondary amine intermediate.[78]

The role of other mammalian amine oxidases in xenobiotic clearance is less well characterised. For example, SSAO (semicarbazide-sensitive amine oxidase) is a copper containing enzyme found predominantly in the plasma, aorta, lung and duodenum, but exhibiting low hepatic activity.[79] SSAO displays different

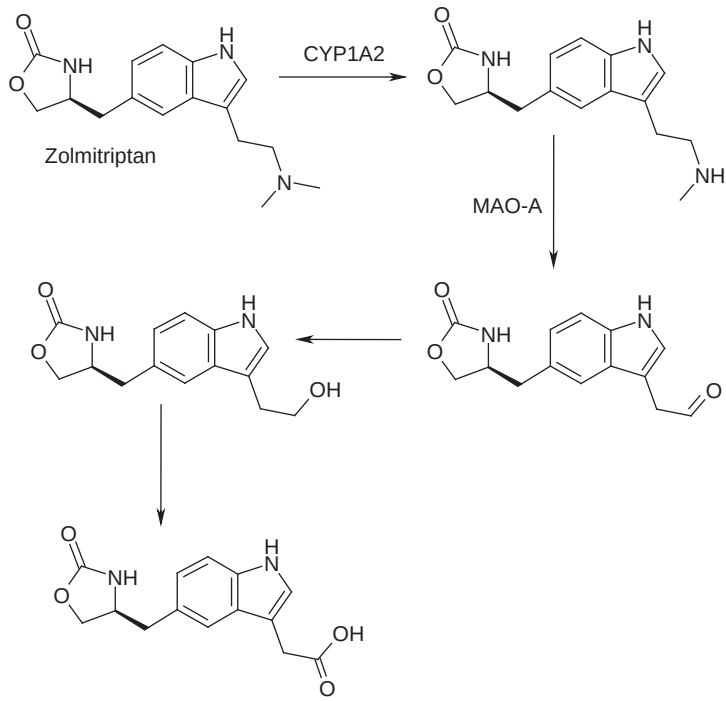


Figure 4.18 Sequential metabolism of zolmitriptan by P450 followed by MAO.

substrate specificities from MAO; for example, methylamine is a substrate for SSAO but not for MAO.[80] There have been few studies of the involvement of SSAO in metabolism of pharmaceuticals, the exception being amlodipine[81] (Figure 4.19), which is extensively deaminated in the dog but not the rat. This reflects the high SSAO activity levels in dog plasma relative to that in rat.

4.5.2.3 N-Methylation

Amine *N*-methyltransferases are responsible for the biotransformations of endogenous substrates; for example, the methylation of norepinephrine to epinephrine and histamine and glycine to their methylated forms. They are also involved in the metabolism of xenobiotics. This initially seems an illogical metabolic route in that methylation often results in a less polar product reducing the metabolites propensity to be excreted. When methylation introduces a quaternary ammonium ion, however, the metabolite is more polar than the parent. The methylation of nicotine (Figure 4.20) is an example of this.

The tricyclic antidepressants, imipramine and amitriptyline, are metabolised by demethylation to give the active metabolise desipramine and nortriptyline (Figure 4.21). However, a recent study has shown that 9–15% of subjects showed methylation of desipramine and nortriptyline back to their parent

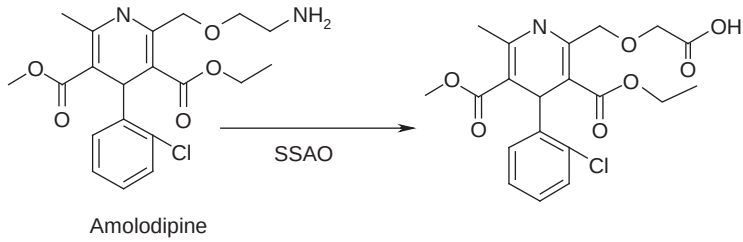


Figure 4.19 Metabolism of amlodipine by SSAO in dog plasma.

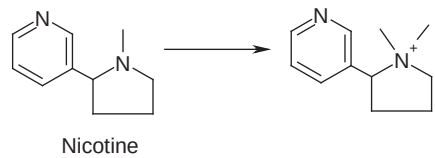


Figure 4.20 Methylation of nicotine by amine *N*-methyltransferase to yield a more polar quaternary amine.

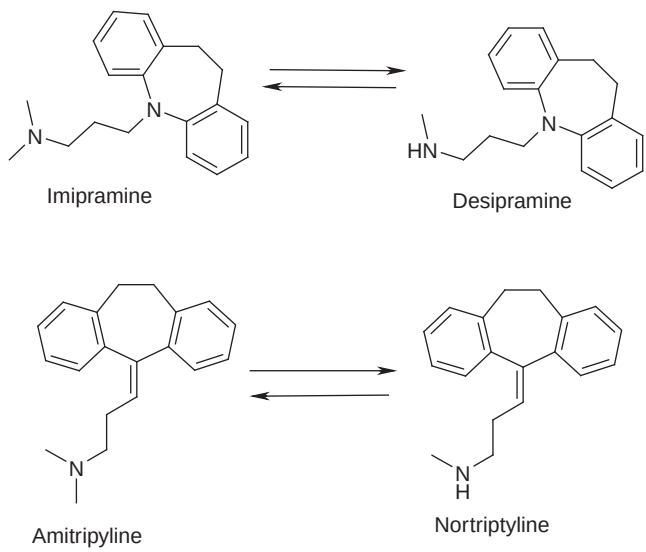


Figure 4.21 Reversible metabolism of imipramine and amitrryptyline to their *N*-desmethyl metabolites involving P450 and N-methyl transferase.

compounds, imipramine and amitriptyline.[82] The *N*-methylation observed in a minority of subjects may possibly result from polymorphism of the amine *N*-methyltransferases. However, polymorphism is known for other methyltransferases but has not been reported for amine *N*-methyltransferase.

An alternative hypothesis is that these tricyclic antidepressants are also substrates for CYP2D6 and CYP2C19, which are known to exhibit polymorphism. In poor metabolisers, it may be that the low rate of turnover by CYP2D6 and CYP2C19 gives the *N*-methyltransferase an opportunity to re-methylate the secondary amine. This cyclic metabolism has implication in adjusting doses and avoiding toxicity.

4.5.3 Phase 2 Metabolism

4.5.3.1 N-Glucuronidation

N-glucuronidation is catalysed by the enzyme UDP-glucuronyltransferase. This enzyme is located in the endoplasmic reticulum but its cofactor, UDP glucuronic acid, is found in the cytosol; thus, glucuronidation is not observed in microsomal incubations unless the cofactor is added. Two groups of compounds are *N*-glucuronidated:

- sulfonamides, aryl amines, amides, and acyclic and cyclic amines which give secondary or tertiary amine glucuronides;
- tertiary amines which give rise to quaternary *N*-glucuronides.

Man and the common preclinical species (rat, dog rabbit, guinea pig, rabbit and non-human primates) exhibit the ability to form secondary and tertiary *N*-glucuronides, the species differences tending to be quantitative rather than qualitative.[83] Sulfadimethoxine (Figure 4.22) is *N*-glucuronidated in primates to the extent of 4–27% of the administered dose and 1–6% in non-primates.[84]

Glucuronidation of tertiary amines is seen in non-human primates and man, but occurs to a lesser extent in other preclinical species. For example, chlorpromazine is *N*-glucuronidated extensively *in vivo* in man, but not in rat or dog.[85]

Where there are two possible sites of *N*-glucuronidation, both of similar basicity, glucuronidation often occurs on the nitrogen with the least bulky

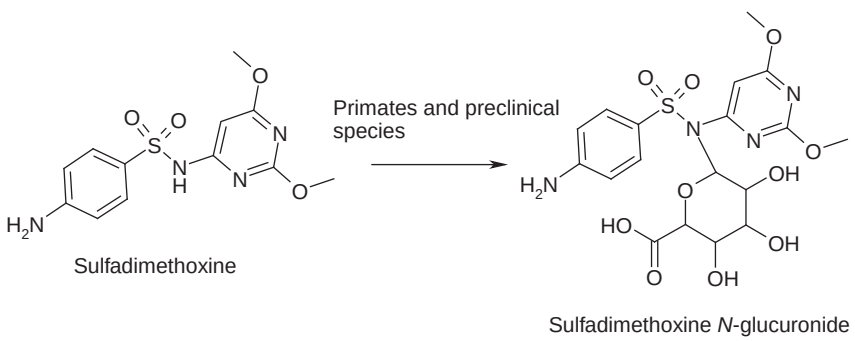


Figure 4.22 *N*-Glucuronidation of the sulphonamide of sulphadimethoxine.

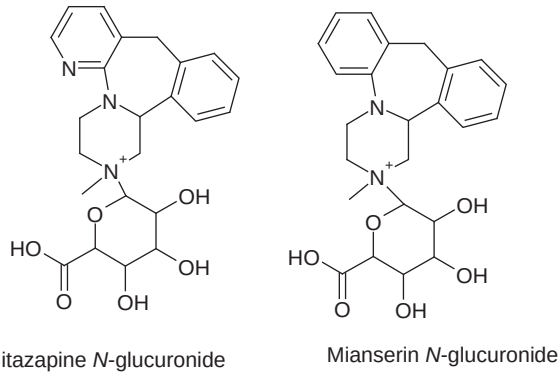


Figure 4.23 *N*-Glucuronides of mitazapine and mianserin.

substituents.[86] Otherwise the propensity for one amine to be glucuronidated over another can be difficult to rationalise.

N-Glucuronides can undergo enterohepatic recirculation which can have significant clinical implications. Both the antihistamine, cyclizine,[86] and the anticonvulsant, *N*-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester,[87] undergo enterohepatic recirculation resulting in a prolonged half-life. The tricyclic antidepressant, mirtazapine is converted to its quaternary *N*-glucuronide in the gastrointestinal tract. This acts as a prodrug as the N^+-glucuronide is better absorbed than the parent drug, resulting in a doubling of bioavailability (after deconjugation) of mirtazapine relative to the related compound mianserin (Figure 4.23) which is *N*-glucuronidated to a lesser extent.[88]

4.5.3.2 N-Sulfation

N-sulfates are formed by the cytosolic enzyme *N*-sulfotransferase (NST), which transfers the sulfate group from its cofactor, 3′-phosphoadenosine 5′-phosphosulfate, to the substrate. A wide range of amines such as aniline and octylamine[89] have been shown be sulfated by NST *in vitro*. Reports of *in vivo* xenobiotic substrates are limited, but desipramine (Figure 4.24) and 4-phenylpiperazine,4-phenyl-1,2,3,6-tetrahydropyridine are both *N*-sulfated *in vivo*, rat and man.[90,91]

4.5.3.3 N-Acetylation

The enzyme arylamine *N*-acetyltransferases (NAT) are cytosolic enzymes that catalyse the transfer an acetyl group from acetyl coenzyme A to the nitrogen or oxygen of primary aromatic amines, aryl hydroxyl amines or hydrazine. Examples of *N*-acetylation of drugs in each these categories include hydralazine, *N*-biphenyl-4-yl-hydroxylamine and procainamide.

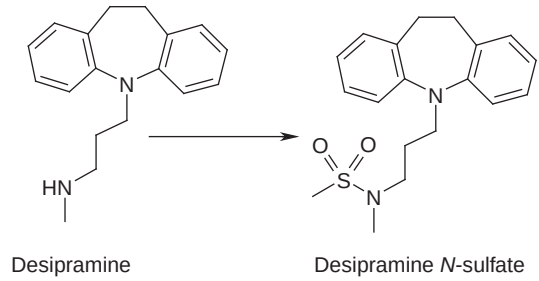


Figure 4.24 *N*-Sulphation of desipramine occurs in rat and man.

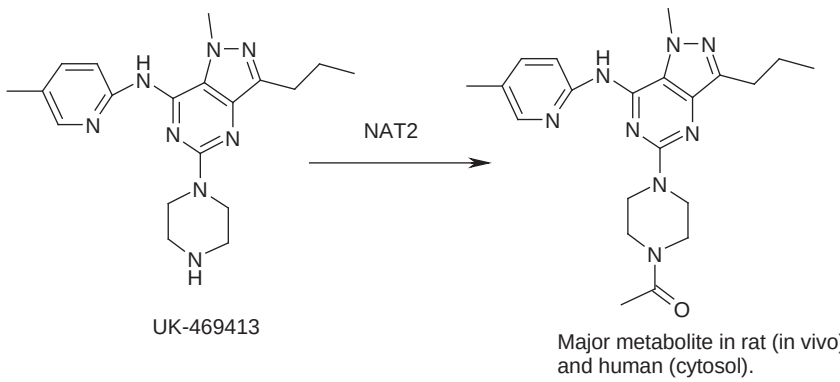


Figure 4.25 *N*-Acetylation of UK-469413 by NAT2 as a major route of clearance.

Recently unsubstituted piperazines have also shown to be substrates, *e.g.* UK-469 413,[92] where the *N*-acetylation was the major route of clearance (Figure 4.25). This was unexpected as acetylation of aliphatic amines has previously been reported to be a minor route of metabolism. The piperazine nitrogen must be unhindered for *N*-acetylation to occur as the introduction of a methyl group adjacent to the piperazine nitrogen blocks *N*-acetylation.

Partial charge on the nitrogen influences whether or not a substituted aniline will be *N*-acetylated,[93] with the introduction of electron-withdrawing groups *para* to the amino group resulting in decreased *N*-acetylation.

NAT has been known to be polymorphic since 1954 when a proportion of the population (50% of caucasians and 10% asians) were observed to be slow acetylators of the anti-tubercular drug isoniazid, leading to adverse events.[94]

There are two major isozymes in humans, NAT-1 and NAT-2. Slow acetylation, at least partially, results from reduced expression of NAT-2. NAT-1 is also polymorphic but this is less well characterised.[95] NAT1 and NAT2 have overlapping specificities; NAT-1 preferentially acetylates *p*-aminobenzoic acid, sulfamethoxazole and sulfanilamide, whilst isoniazid, hydralazine, procainamide, dapsone and sulfamethazine are better substrates for NAT-2.[92]

4.5.4 Non-metabolic Clearance

Amines may be cleared unchanged in urine and faeces (bile), and less commonly in saliva, sweat, expired air and breast milk.

4.5.5 Renal Clearance

Renal clearance is regulated by the kidney by passive and active processes. Passive clearance involves filtration and reabsorption. Polar compounds are readily cleared in the kidney because they can diffuse freely across the membrane in the glomerulus and cannot diffuse back despite the concentration being in favour of reabsorption. Conversely, lipophilic compounds are extensively reabsorbed.

This relationship between lipophilicity and renal clearance of amines has been exemplified for the β-adrenergic antagonists with polar compounds such as atenolol being mainly cleared by renal clearance and lipophilic compounds such as propranol showing negligible renal clearance.[21] Urine pH can vary widely between pH 4.5 to 8.0 and this can have a marked affect on the renal excretion of amines. For example, when flecainide is co-administered with sodium bicarbonate to make the urine alkaline, only 7% of the dose is renally cleared compared with 45% in acidic urine over 32 h, increasing the exposure (AUC) by 70% at the higher urinary pH. Similarly, only low levels of amphetamine are excreted unchanged in alkaline urine but more than 50% is excreted in acidic urine.

Active secretion by the kidney involves transport proteins in the proximal tubule that can actively secrete cations such as pramipexole, dofetilide and cimetidine. These transporters include the organic cation transporter, OCT2, which is involved in the renal secretion of metformin used in the treatment of type 2 diabetes. Orally administered metformin is renally eliminated (80% of dose) without any significant metabolism. Expression of the transport protein is polymorphic and, in individuals with reduced OCT2 activity, there is reduced renal clearance and increased circulating plasma levels of metformin, impacting on the control of plasma glucose levels.[96]

4.5.6 Biliary Clearance

Amine containing drugs often undergo a degree of biliary excretion, although it is generally not a major clearance pathway, as may be the case for some acidic compounds. Excretion of xenobiotics into the bile is *via* active transport. Hepatocytes display a variety of active transport proteins that may be responsible for the transport of amines. For example, in humans OCT1 is a cation uptake transporter which is involved in the extraction of small hydrophilic molecules such as cimetidine and verapamil[97,98] from the blood into the liver. MDR1 and MDR3 (also known as P-glycoproteins or P-gp) and MRP2 are ATP-dependent efflux pumps that transport amphiphilic cations and neutral compounds such as tamoxifen, vincristine and ceftriaxone[99] from the liver

to the bile. Transport proteins may be inhibited and so may be subject to drug–drug interactions. Co-administration of digoxin, a P-gp substrate with other P-gp substrates or inhibitors, including amines such as quinidine or verapamil, can reduce biliary excretion.[100,101]

4.6 Amines as Toxicophores and Toxicity of Amine Containing Drugs

Structural alerts are a means to identify substructures that are considered to pose a toxicity risk and are thus seen as undesirable groups for drug discovery programmes. A number of amine containing structural alerts have been identified and are listed in Table 4.2.

Toxicity of small pharmaceutical molecules may arise from accumulation of the unchanged compound in tissue. This is particularly true for very lipophilic compounds including the amine, amidarone, which accumulates in the lung[109] leading to toxicity.

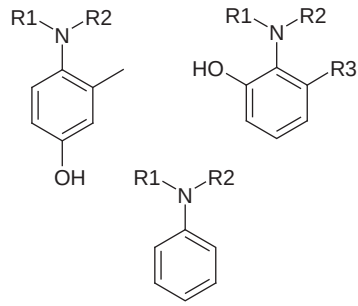
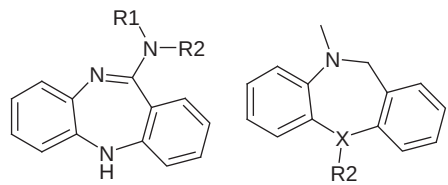
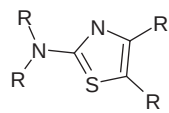
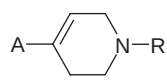
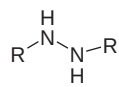
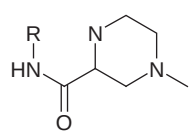
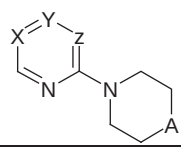
The ability of a compound to form a reactive metabolite does not in itself mean that it will give rise to adverse reactions in clinical use. The amount and activity of toxic species formed relative to its extent of removal (detoxification) dictates whether or not toxicity will be observed. In particular, consideration should be given to the size of the administered dosed. Low dose drugs are significantly less likely to give rise to adverse reactions. For example, procainamide gives raise a series of idiosyncratic toxicities as a result of formation of a reactive nitroso metabolite formed from an *N*-hydroxy intermediate. Metoclopramine forms a similar nitroso metabolite, but does not display the same range of adverse reactions as procainamide because it is dosed at $10\,mg\,day^{-1}$ rather than the gram quantities at which procainamide is dosed.[110] The nitroso metabolites (Figure 4.26) of both molecules go on to react with the tripeptide glutathione, which acts to detoxify the molecule. At doses in excess of $1\,g\,day^{-1}$ of procainamide, there is insufficient glutathione to mop up the reactive nitroso species.

Rigid planar primary amines (*e.g.* 2-aminofluorene) may be metabolised to carcinogenic species by *N*-oxidation catalysed by CYP1A. This appears to be specific to these flat structures, which can interchelate with DNA, and does not occur with flexible molecules which may be similarly metabolised.

In other situations, *N*-oxidation may represent a detoxification pathway. For example, tamoxifen (used in the treatment of breast cancer) is α-hydroxylated by CYP3A4 which is then sulfated to form a species that binds to DNA. The *N*-oxide of tamoxifen, formed by FMO1 or FMO3, is not reactive. This has been linked to the observation that tamoxifen toxicity is lower in kidney where FMO1 expression is high relative to CYP3A4 than in the liver where CY3A4 activity is higher.[111]

In addition to dose considerations, the observation of adverse drug reactions also depends on the balance between generation of the toxic (reactive) species and its removal by various detoxification mechanisms. One route of

Table 4.2 Structural alerts featuring amines and their associated toxicities.

Substructure		*Toxic effects*
Aniline *ortho*- or *para*-hydroxyanilides/anilines, *e.g.* procainamide, propanil		Methaemoglobinemia Agranulocytosis Aplastoc anaemia Hepatotoxicty Skin hypersensitivity Carcinogenic[102]
Dibenzazepines, *e.g.* clozapine, mianserin		Agranulocytosis[103]
Aminothiazoles or thiazoles, *e.g.* cefepime, pramipexole		Hepatotocicity Neurotoxicity[104]
4-Substituted-*N*-alkyl-tetrahydropyridine, *e.g.* MPTP		Neurotoxicity[105]
Hydrazines, hydrazides, *e.g.* phenelzine, procarbazine		Hepatotoxicity Carcinogenic[106]
1,3-Disubstituted piperazine, *e.g.* MB243		Microsomal binding[107]
Diazine-piperazine/morpholine, *e.g.* BAY-41-8543		Mutagenic[108]

detoxification is *via* the endogenous tripeptide glutathione, which can react with and clear electrophiles, as exemplified previously with metoclopramine. Another mechanism of detoxification is further metabolism of reactive species to remove the toxicophore. For example, *N*-hydroxy amphetamine is toxic

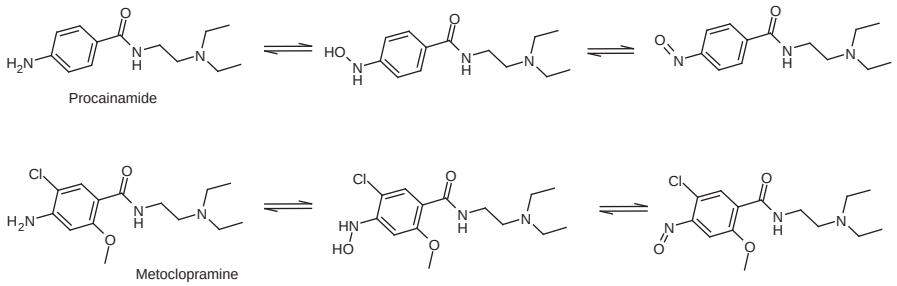


Figure 4.26 Formation of nitroso metabolites of procainamide and metoclopramine.

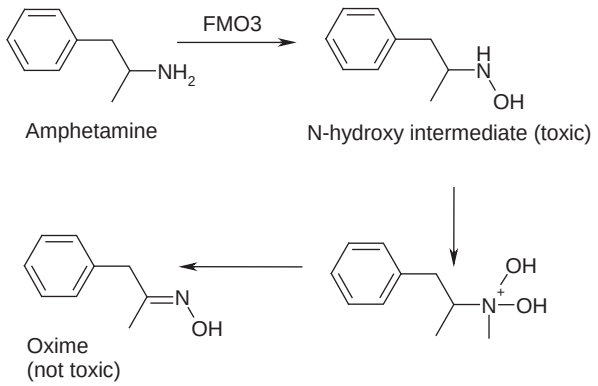


Figure 4.27 Formation of the toxic *N*-hydroxy metabolite of amphetamine.

(Figure 4.27), capable of modifying biomolecules, but can be further metabolised to form the non-toxic oxime.[112]

Conjugates are often considered innocuous in that they rarely give rise to active metabolites and the increased solubility resulting from conjugation often increases renal clearance. This is not always the case however. For example, sulfation of 2-aminofluorene *N*-oxide, leads to an unstable *N*-*O*-sulfates, which may decompose to a reactive species that can adduct to DNA and proteins.[113] Another example is amitriptyline, which forms a quaternary *N*-glucuronide that has been linked to flushing and tachycardia in man.[86]

N-Acetylation reactions are involved in the detoxification/activation of several amine containing xenobiotics. In the case of hydralazine, *N*-acetylation represents a detoxifying route as it reduces the amount of parent available to undergo an alternative metabolic route that results in the formation of a reactive species involved in the development of the autoimmune condition lupus. Thus, the incidence of lupus is higher in slow acetylators on chronic treatment than in fast acetylators.[114]

Less commonly, acetylation may be involved with the creation of a toxicophore. An example of this is that of 2-aminofluorene (Figure 4.28), where

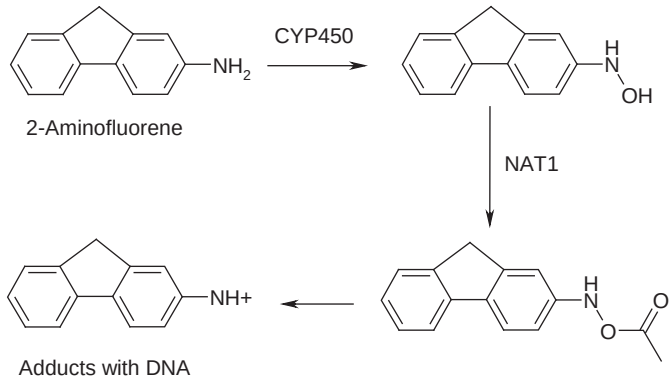


Figure 4.28 Formation of a mutagenic cation from 2-aminofluorane.

the parent molecule is first *N*-hydroxylated and then *O*-acetylated as an intermediate in the formation of a mutagenic cation.

Avoiding cardiotoxicity is a major concern when developing compounds as potential medicines. This concern arises from the need to withdraw a number of drugs from market on the basis of cardiotoxicity, due to the prolongation of ventricular repolarisation, or as commonly referred to QT prolongation.[115] QT prolongation may lead to arrhythmia and possibly to *Torsade de Pointes,* a form of arrhythmia that can cause sudden death. Not all drugs that cause QT prolongation result in either of these adverse effects, but development of medicines that show QT effects is preferably avoided and causes complexity and delay.

Repolarisation of cardiac tissue is controlled by potassium channels, known as rectifier K^+ channels or I_{Kr} channels. Drug-induced QT prolongation results from blockage of the I_{Kr} channels, changing the distribution of potassium ions across the membrane. Because of the potential toxicity of these potassium channel blockers, considerable effort has gone into modelling what structural aspects of compounds give rise this form of cardiotoxicity, highlighting the particular risk of amine containing compounds. One model suggests that a pyramidal structure with four hydrophobic groups and a basic centre at the apex is required for channel blocking.[116] An example of this is astemizole which consists of three aromatic rings and a basic nitrogen which make up the four point of the pyramid.[117] Other drugs that cause QT prolongation include dofetilide, terfenadine, erythromycin and ketoconazole.

4.7 Zwitterions

Combining basic amine and carboxylic acid functions in the same molecule provides zwitterions which have very different physicochemical properties to molecules containing either functional group alone. These properties of

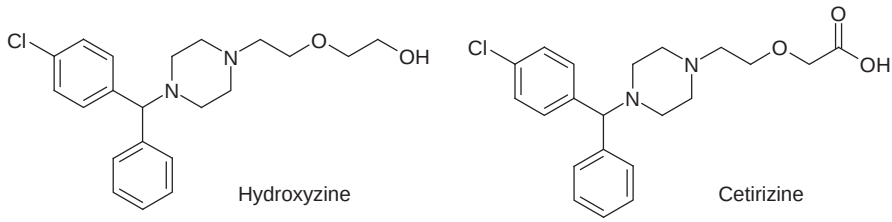


Figure 4.29 Structures of the *H*1 antihistamines, the basic molecule hydroxyzine and its zwitterionic metabolite cetirizine.

zwitterions may be worthy of medicinal chemistry design considerations when working with acid, basic or neutral leads.

The change from a basic molecule to a zwitterionic molecule has significant impact on pharmacokinetic characteristics. This is exemplified by the H_1 antagonist class of compounds. First generation H_1 antihistamines were lipophilic basic molecules such as hydroxyzine (Figure 4.29), which penetrated the brain and caused CNS side effects such as drowsiness. Many of the second generation H_1 antihistamines were zwitterions, which maintained the pharmacologically important amine, but were more polar due to the presence of a carboxylic acid function and showed low CNS penetration and hence improved side effect profiles. These agents include cetirizine (Figure 4.29), the carboxylic acid metabolite of hydroxyzine. Cetirizine has a log $D_{7.4}$ value of around 1.5 compared with 3.1 for hydroxyzine;[118] this reduction in lipophilicity is less than for other zwitterionic compounds, such as acrivastine, and reflects the ability of hydroxyzine to form an internal hydrogen bond between the acid and amine functions. In addition to the improved CNS side effect profile of cetirizine, the physicochemical profile confers additional advantages such as slow receptor dissociation and negligible cytochrome P450 interaction.[119]

Another example which contrasts the pharmacokinetic properties of a basic compound and zwitterion is the fibrinogen receptor antagonist L-767 679 and its carboxyl ester prodrug.[120] Whilst the zwitterion, L-769 679 is highly polar (log $P < -3$), it is absorbed *via* the paracellular route. Attempts to improve absorption by the carboxyl ester prodrug found that this basic compound was a substrate for P-glycoprotein—a property not shared with the zwitterion. Most P-glycoprotein substrates are identified as moderately lipophilic bases and hence raising lipophilicity of zwitterions by a prodrug approach may not always result in improved absorption.

4.8 Prodrugs of Amines to Change Physicochemical Properties

Prodrugs are employed to enable more effective delivery of a pharmacological active molecule by overcoming one or more barriers to drug delivery through alteration of physicochemical properties. Ideally, prodrugs are pharmacologically

inactive, but once they have overcome the delivery barrier for which they are intended, they should be rapidly transformed into the drug molecule. On this basis there are a number of potential applications of prodrugs to facilitate improved drug therapy:

(1) Improved solubility to enhance absorption through incorporation of polar functionality
(2) Improved permeability to enhance absorption through increased lipophilicity
(3) Targeted delivery to tissues or organs through tissue specific delivery or cleavage of pro-moiety.

Given that inclusion of an amine function in itself benefits the aqueous solubility properties of a molecule, there is not surprisingly no clear benefit from masking this function with the intent of enhancing solubility. On the other hand, inclusion of an amine function may compromise membrane permeability due to the overall effect of reducing lipophilicity and the potentially large fraction that will exist in ionised form for amines with high pKa values. Consequently amine derivatives that prevent ionisation and add lipophilicity have the potential to increase membrane permeability and hence absorption.

4.8.1 Prodrugs to Enhance Absorption

The challenge for potential prodrugs of amines is the release of the active moiety once the prodrug has fulfilled its role of aiding absorption. Unlike ester prodrugs of carboxylic acid compounds, simple amides do not generally undergo rapid hydrolysis to yield the free amine. The non-enzymatic hydrolysis of amides is generally so slow under physiological conditions that such compounds may only be contemplated if enzymatic hydrolysis reactions are available to them. Species differences in hydrolytic enzyme capabilities need to be considered for such prodrugs, with generally much greater hydrolytic capability in rodents compared to larger mammalian species. Indeed a simple diacetyl prodrug of an antimalarial agent (Figure 4.30) was found to be active against malarial infection in mice but not rats and this was concluded to be a result of the inability of rats to activate the prodrug.[121]

A number of studies utilising a double prodrug approach to provide advantageous physicochemical characteristics for amines have been explored,

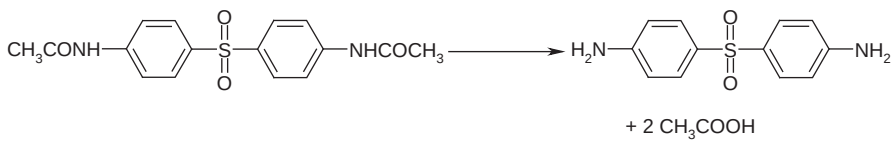


Figure 4.30 An antimalarial prodrug 4,4′-diacetylamidodiphenylsulphone that was active in mice but not rats.

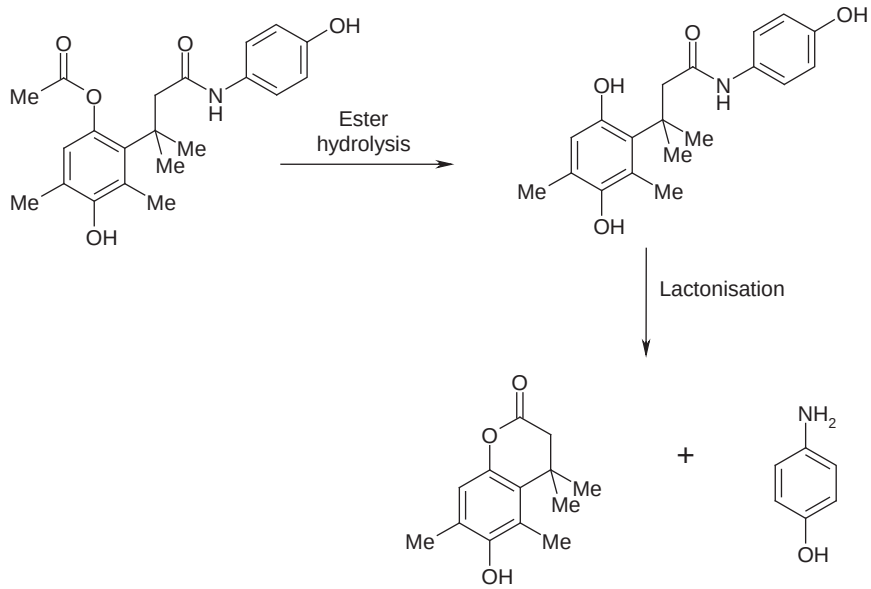


Figure 4.31 Schematic for proposed conversion mechanism for esterase and redox-sensitive double prodrugs of amines (ref. 120).

although these have not to date yielded viable agents. The strategy with such an approach is to utilise the established esterase activation as a first pro-moiety forming a chemically reactive intermediate that undergoes spontaneous conversion to the active compound. This is illustrated by the example of esters of chemically reactive hydroxy amides[122] to liberate 4-methoxyaniline (Figure 4.31). Formation of the amine was demonstrated to be mediated by enzymatic catalysis by a serine esterase.

Alternative proposals for double prodrug approaches for amines have involved *N*-acyloxyalkoxycarbonyl derivatives of primary and secondary amines which undergo enzymatic hydrolysis of the ester moiety leading to a (hydroxyalkoxy) carbonyl derivative that undergoes spontaneous decomposition to yield the parent amine *via* an unstable carbamic acid.[123]

4.8.2 Prodrugs to Achieve Tissue Specificity

As mentioned previously, another potential use of prodrugs is to achieve tissue specific delivery of the active species. A prodrug that is subject to activation by an enzyme which is restricted to the target tissue is one method by which this may be achieved. This approach has been explored for application of renal specific vasodilation using a prodrug of dopamine. The enzyme, γ-glutamyl-transpeptidase, has highest concentrations in the kidney. The hypothesis therefore was that L-γ-glutamyl dopamine would be preferentially hydrolysed in the kidney (Figure 4.32) and hence exert a local vasodilator effect; the

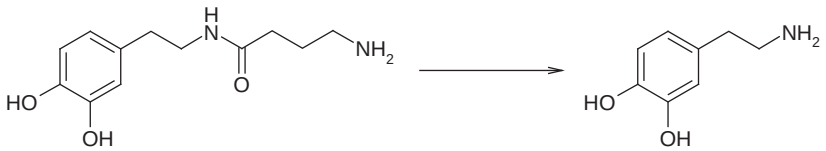
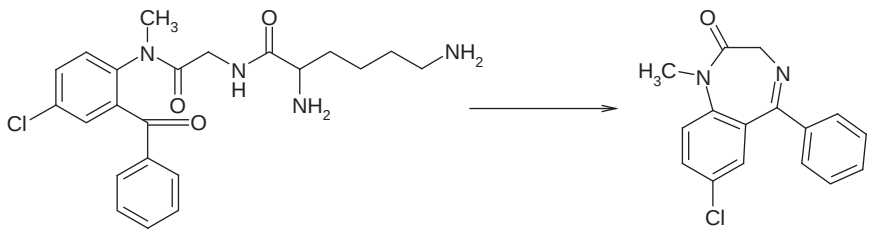


Figure 4.32 L-γ-Glutamyl dopamine, an experimental kidney specific prodrug of dopamine.



Avizafone Diazepam

Figure 4.33 Avizafone, a water soluble prodrug of diazepam.

dopamine would be rapidly metabolised and excreted without producing general systemic adrenergic stimulation.[124] This approach has shown some promise in achieving a level of kidney specificity though significant adrenergic stimulation has been observed in other tissues, suggesting that hydrolysis is not restricted entirely to the kidney.

4.8.3 Prodrugs Utilising Amine Functionality

As discussed previously, the presence of an amine function can be advantageous in enhancing the solubility of a molecule due to its hydrophilic nature. Therefore adding a pro-moiety that contains an amine can facilitate the absorption of a poorly soluble agent—provided of course that it is then readily able to release the active compound once in the systemic circulation.

An example where this has been applied is in the use of the anticonvulsant diazepam in the treatment of nerve agent poisoning.[125] Such treatment requires the rapid attainment of pharmacologically effective plasma levels in order to prevent the extreme consequences of poisoning. Drugs are therefore administered by intravenous or intramuscular injection which, in the case of diazepam, is not facilitated by its poor aqueous solubility and requirement for an organic solvent. Avizafone is a water soluble prodrug of diazepam that is hydrolysed by an aminopeptidase to liberate lysine and diazepam (Figure 4.33). The aqueous solubility is advantageous from a formulation and delivery aspect. Bioavailability of diazepam has been shown to be 62–66% from avizafone in primates,

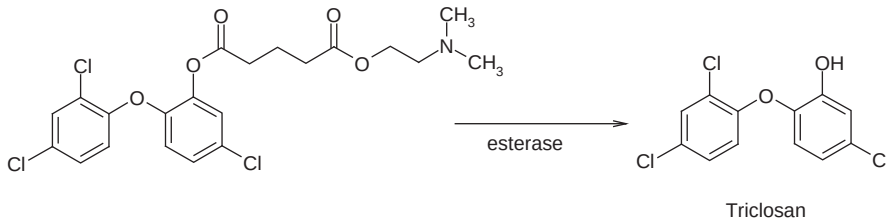


Figure 4.34 An amine containing prodrug of the antimalarial agent triclosan with enhanced cellular uptake.

indicating high but incomplete conversion of the prodrug. In addition, the prodrug provided an earlier T_{max} (35 *vs.* 55 minutes) after intramuscular administration and superior clinical efficacy.[126]

An amine prodrug of the antibacterial agent triclosan has been shown to enhance hydrophilicity of the molecule and the weakly basic nature has facilitated accumulation inside bacterial cells providing a four-fold increase in *in vitro* potency.[127] This prodrug utilised an ester linkage between triclosan and dimethylaminoethylglutaric acid (Figure 4.34). Such a prodrug approach has clear potential for a topical agent such as triclosan, but would appear more challenging for systemic application where targeted delivery will require prodrug stability in the gastrointestinal tract and circulation.

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CHAPTER 5

Sulfonamide as an Essential Functional Group in Drug Design

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5.1 Introduction

The sulfonamide, acylsulfonamide/sulfonimide and sulfonylurea functionalities are found within numerous marketed agents for a wide range of therapies. In 2008 there were 112 marketed drugs in the United States that contained a sulfonamide group (Table 5.1). The drugs differ in chemical structure, molecular weight (MW) and lipophilicity, and act at different receptors/enzymes *via* distinct biochemical mechanisms of action. In some instances, the presence of the sulfonamide fragment is merely a circumstantial occurrence but in the vast majority of cases, these drugs can be categorised into distinct groups based on the role of the sulfonamide motif in the primary pharmacology. By definition a sulfonamide is a molecule containing a sulfonyl group attached to an amine. This yields the possibility of a number of sulfonamide expressions by virtue of

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Table 5.1 Drugs containing a sulfonamide group marketed in the USA, 2008.

Acetazolamide	Clorexolone	Glyburide	Penflutizide	Sulprostone
Acetohexamide	Co-trimoxazole[a]	Glymidine	Piretanide	Sultiame
Almotriptan	Cyclopenthiazide	Hydrochlorothiazide	Piroxicam	Sumatriptan
Althiazide	Cyclothiazide	Hydroflumethiazide	Polythiazide	Tamsulosin
Amprenavir	Darunavir	Ibutilide	Probenecid	Teclothiazide
Amsacrine	Delavirdine mesylate	Indapamide	Quinethazone	Tenoxicam
Argatroban	Diazoxide	Isoxicam	Rosuvastatin	Tezosentan
Avitriptan	Dichlorphenamide	Levosulpiride	Sildenafil citrate	Thiothixene
Bemetizide	Dofetilide	Lornoxicam	Sotalol	Tipranavir
Bendroflumethiazide	Dorzolamide	Mafenide	Sulfacetamide	Tirofiban
Benzthiazide	Droxicam	Mebutizide	Sulfacytine	Tolazamide
Benzylhydrochlorothiazide	Epithiazide	Mefruside	Sulfadiazine	Tolbutamide
Bosentan	Ethoxzolamide	Meloxicam	Sulfadimethoxine	Torsemide
Brinzolamide	Fenquizone	Methazolamide	Sulfafurazole	Trichlormethiazide
Bumetanide	Flosulide	Methyclothiazide	Sulfamazone	Tripamide
Butizide	Fosamprenavir	Meticrane	Sulfameter	Valdecoxib[§]
Carbutamide	Furosemide	Metolazone	Sulfamethizole	Vardenafil
Celecoxib	Glibornuride	Mezlocillin	Sulfamethoxazole	Xipamide
Chlorothiazide	Gliclazide	Napsagatran	Sulfanilamide	Zafirlukast
Chlorpropamide	Glimepiride	Naratriptan	Sulfaphenazole	Zonisamide
Chlorthalidone	Glipizide	Nimesulide	Sulfapyridine	
Cinnoxicam	Gliquidone	Paraflutizide	Sulfasalazine	
Clopamide	Glisoxepide	Parecoxib sodium[b]	Sulfisoxazole	

[a]Co-trimoxazole is combination of the tetrahydropteroic acid synthetase and dihydrofolate reductase inhibitors sulfamethoxazole and trimethoprim, respectively.
[b]COX-2 inhibitor withdrawn in 2005 due to concerns about possible increased risk of heart attack and stroke. Parecoxib, which is a prodrug of valdecoxib, is marketed as Dynastat in the European Union; it has not been approved in the USA.

substituents attached to the sulfur and nitrogen atoms. The pivotal role of derivatives of sulfonamide containing drugs in opening up new areas of pharmacology was discussed earlier in Chapter 1. These developments are reviewed in detail in 5.1.1, 5.1.2, 5.1.3, and 5.14.

5.1.1 Sulfanilamide Antibacterial Agents

The term 'sulfanilamide' is used to describe a family of molecules containing the sulfonamide functional group attached to an aniline ring in the *para*-position. The first sulfanilamide derivative was a prodrug called prontosil red (**5.1**) and was made by Bayer Laboratories in 1932. The finding that the sulfanilamide (**5.2**) metabolite, as opposed to the parent compound **5.1** from which it was derived (Figure 5.1), was responsible for reversing streptococcal infections in mice paved the way for the antibiotic revolution in medicine.[1,2] The biochemical basis for the antimicrobial/antibacterial activity of sulfanilamides is due to competitive inhibition of bacterial tetrahydropteroic acid synthetase.[3] The enzyme catalyses the incorporation of *para*-aminobenzoic acid (**5.3**) into dihydropteroate diphosphate resulting in the formation of dihydopteroic acid, which is ultimately converted into dihydrofolic acid (Figure 5.1). Dihydrofolic acid and its two-electron reduction product tetrahydrofolic acid are essential for cell division in bacteria; inhibition of the formation of these pterin derivatives leads to antibacterial activity. Gerhard Domagk, Jacques and Therese Trefouel are generally credited with the discovery of the parent sulfanilamide as an antibacterial agent and Domagk was awarded the Nobel Prize for his work in this field.

The serendipitous finding that replacement of the carboxylic acid moiety in **5.3** with a sulfonamide group affords a compound with a significantly higher affinity for the enzyme than the natural substrate[4,5] constitutes one of the first examples of a non-classical bioisosteric replacement for the carboxylic acid moiety. In the case of **5.2** and **5.3**, the sulfonamide anion (RSO_2N^-) closely resembles the carboxylate anion ($RCOO^-$); the distance between the two oxygen atoms of the carboxylic acid and sulfonamide anions are virtually identical (2.1–2.3 Å) and, like the carboxylic acid group, the sulfonamide group has the potential for multiple hydrogen bonding interactions.[6,7] In contrast with the carboxylic acid moiety, however, unsubstituted sulfonamides are neutral at physiological pH (pKa $\sim$10.5–11.0); inclusion of appropriate substituents can ensure that the sulfonamide moiety is anionic at physiologic pH. Structure–activity relationship (SAR) studies indicate that *N*- and *S*-substituents, which influence the ionisation of the sulfonamide group, are known to increase bacteriostatic activity; the *para*-amino group, however, remains optimal for pharmacological activity. Figure 5.1 also illustrates representative sulfanilamide-based anti-bacterial agents in clinical use. The structure of dapsone, which is used in the treatment of leprosy, is distinct from other sulfonamide-based antibacterials in that a phenyl ring separates the sulfonyl and amine fragment.

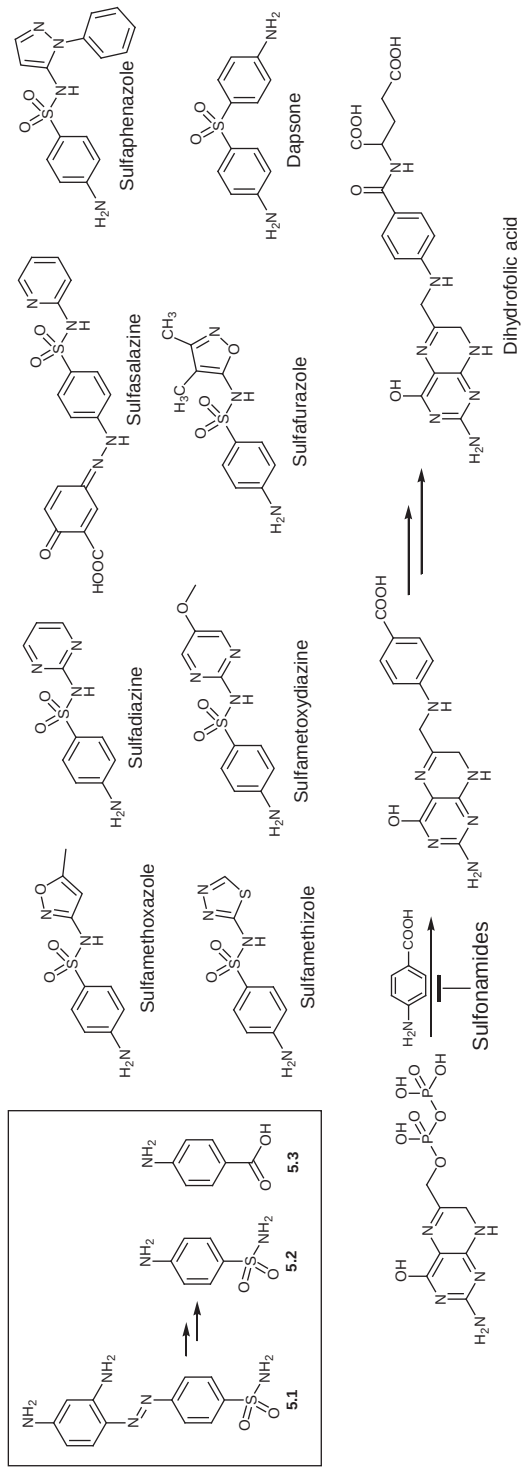


Figure 5.1 Biochemical basis for antibacterial action of sulfanilamides.

5.1.2 Sulfonamide-based Anti-inflammatory Agents

The recent discovery of the *coxibs* (celecoxib, valdecoxib and rofecoxib) as the next generation of non-ulcerogenic non-steroidal anti-inflammatory drugs (NSAIDs) provides a fascinating example of the value of the sulfonamide group in medicinal chemistry.

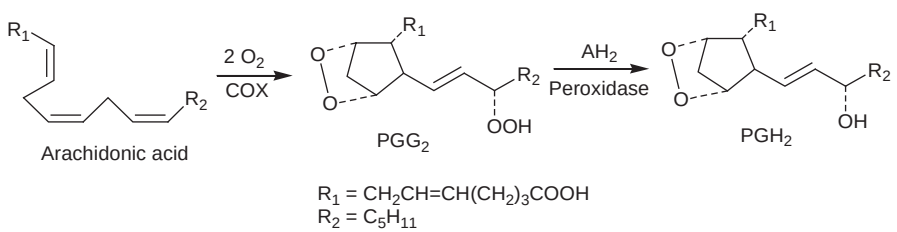
5.1.2.1 Prostaglandin Biosynthesis and Inflammation

The committed step in prostaglandin and thromboxane biosynthesis involves the conversion of fatty acid arachidonic acid to prostaglandin H_2 (PGH_2), a reaction catalysed by the sequential action of the cyclooxygenase (COX) and peroxidase activities of prostaglandin endoperoxide synthase or cyclooxygenase (PGHS or COX, EC 1.14.99.1) (Scheme 5.1).[8] COX activity originates from two distinct and independently regulated enzymes, termed COX-1 and COX-2.[9,10] COX-1 is the constitutive isoform and is mainly responsible for the synthesis of cytoprotective prostaglandins in the gastrointestinal tract. COX-2 is inducible and short-lived; its expression is stimulated in response to pro-inflammatory mediators.[11,12] COX-2 plays a major role in prostaglandin biosynthesis in inflammatory cells (monocytes/macrophages).[13,14]

These observations suggest that COX-1 and COX-2 serve different physiological and pathophysiological functions. Classical NSAIDs inhibit both COX-1 and COX-2 to varying extents; inhibition of the former isozyme is thought to be responsible for the gastrointestinal liabilities.[15] The differential tissue distribution of COX-1 and COX-2 provided a rationale for the development of selective COX-2 inhibitors as anti-inflammatory and analgesic agents that lack the side effects exhibited by currently marketed NSAIDs. This hypothesis was validated in animal models and has led to the introduction of celecoxib, valdecoxib (sulfonamides) and rofecoxib (methylsulfone) into the marketplace.[16]

5.1.2.2 Mode of Inhibition of COX Isozymes by NSAIDs and Coxibs

Traditional NSAIDs and the selective COX-2 inhibitors bind in the COX active site but not the peroxidase active site of the isozymes. Kinetic analysis indicate



Scheme 5.1 Oxygenation of arachidonic acid by COX enzymes.

that most COX inhibitors are slow, tight-binding inhibitors that conform to the minimal two-step mechanism depicted in eqn (5.1).[17,18] The first step involves the formation of a rapidly reversible (E · I) complex leading to competitive inhibition. The second step is the time-dependent conversion of the initial (E · I) complex to one, [E · I]*, in which the inhibitor is bound more tightly. Formation of the [E · I]* complex occurs in seconds to minutes and is thought to reflect the induction of a subtle protein conformational change. It is of great importance to note that all of the selective COX-2 inhibitors are actually competitive inhibitors of both COX-1 and COX-2, but exhibit selectivity for COX-2 in the time-dependent step by binding tightly at the active site and causing a conformational change in the isozyme structure.[18]

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (E{\cdot}I) \xrightarrow{k_2} (E{\cdot}I)^* \qquad (5.1)$$

5.1.2.3 *Origins of the Coxibs–the Diarylheterocycle Class of Selective COX-2 Inhibitors*

The chemical structures of diarylheterocycles can be traced back to clinical candidates discovered over 30–40 years ago.[19] Examination of the structures of diarylheterocycle-based selective COX-2 inhibitors (*e.g.* celecoxib, valdecoxib and rofecoxib) reveals a striking resemblance to the older COX inhibitor phenylbutazone (Figure 5.2).[20] The exceptional anti-inflammatory properties of phenylbutazone undoubtedly provided much of the impetus that led chemists to exploit this chemotype.[21]

The 4-methylsulfonyl (CH_3SO_2)-diarylheterocycle combination, which is a critical component for potent and selective COX-2 inhibition, was first discerned in the structure of the 2,3-diarylthiophene, DuP 697 (Figure 5.2), disclosed in the late 1980s as a non-ulcerogenic anti-inflammatory agent.[22] The COX-2-selective inhibitory properties of DuP697 were established by Copeland *et al.* in the early 1990s[18] and reaffirmed the COX-2 selective inhibition hypothesis for designing safer NSAIDs. Studies on methylsulfone replacements in DuP 697 indicated that only the 4-sulfonamido ($-SO_2NH_2$) group maintained COX-2 inhibitory potency and selectivity observed with Dup 697.[23,24] Incorporating the 4-sulfonamido group also results in increased COX-2 inhibition, albeit with a loss of COX-2 selectivity. This SAR trend appears to be a common theme in all of the diarylheterocycles studied to date and is illustrated in Figure 5.2 with the 1,5-diarylpyrazole-based methylsulfone SC 58125 (COX-1/COX-2 selectivity >1000) and the sulfonamide celecoxib (COX-1/COX-2 selectivity ~ 325).[25]

The oxidation state of the sulfur in methylsulfones is crucial for selective COX-2 inhibition; its reduction to sulfoxide or sulfide reverses isozyme selectivity. For instance, methyl-sulfone-containing 4,5-diarylthiazole (SC-8092) is a selective COX-2 inhibitor, whereas the corresponding methylthioether derivative (SC-8076) exhibits COX-1-selective inhibition (Figure 5.2).[26] Furthermore, *N*-methylation or *N*,*N*-dimethylation of the sulfonamide group or reversal of

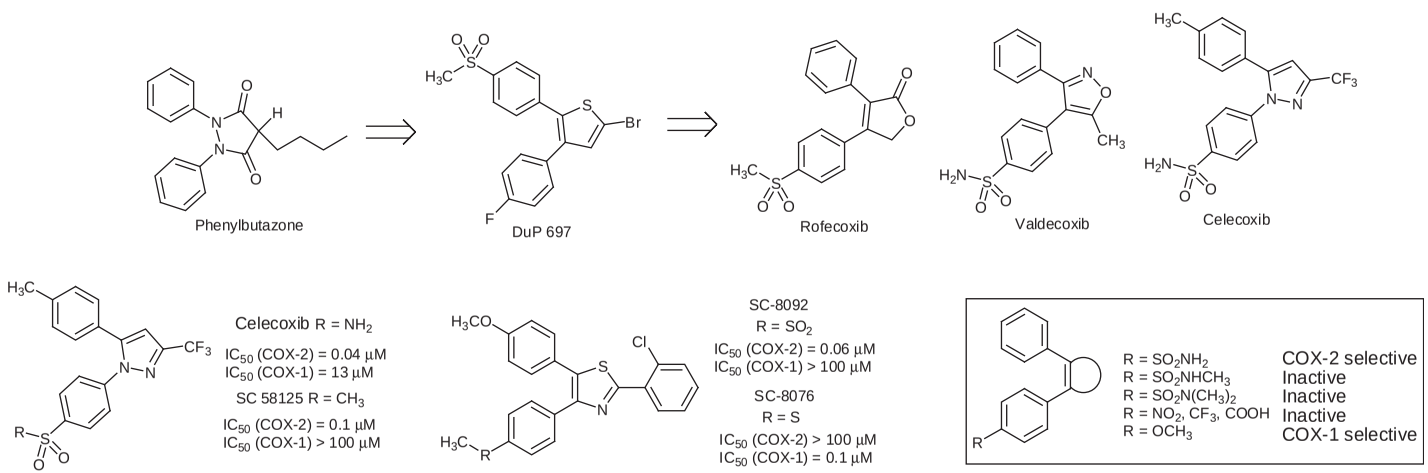


Figure 5.2 Pivotal role of the methylsulfone/sulfonamide group in selective COX-2 inhibition by diarylheterocycles.

the sulfonamide group to a methanesulfonamido moiety in celecoxib results in inactive compounds (Figure 5.2).[25] Likewise, bioisosteric replacement of the methylsulfone or sulfonamide groups with nitro, trifluoromethyl, methoxy or carboxylic acid substituents either reverses isozyme specificity or results in inactive compounds (see Figure 5.2).[25,27]

Crystal structures of complexes of sheep COX-1, mouse COX-2, and human COX-2 with non-selective and selective inhibitors have been solved at 3–3.5 Å resolution.[28,29] Most traditional NSAIDs (*e.g.* diclofenac, ibuprofen, aspirin and indomethacin) contain a free carboxylic acid group, which ion pairs to an active site Arg^{120} residue. The Arg^{120} residue also ion pairs with the carboxylate of the fatty acid substrate arachidonic acid. Site-directed mutagenesis of the arginine residue in COX-1 to glutamine or glutamate renders the protein resistant to inhibition by carboxylic acid containing NSAIDs.[30,31] Arg^{120} is part of a hydrogen bonding network with Glu^{524} and Tyr^{355} which stabilises substrate–inhibitor interactions and closes off the upper part of the COX active site from the spacious opening at the base of the channel referred to as the lobby. Disruption of this hydrogen bonding network opens the constriction and enables substrate–inhibitor binding and release to occur.

Co-crystal structures of COX-2 with methylsulfonyl- and/or sulfonamide-diarylheterocycles have shown that the selective inhibitors bind to regions accessible in COX-2 but not COX-1. For instance, solution of the COX-2 crystal structure co-complexed with the celecoxib derivative SC-558 (the 4-methylphenyl group in celecoxib is replaced with the 4-bromophenyl substituent in SC-558) demonstrates that the sulfonamide moiety wedges into a hydrophobic 'side pocket' of COX-2 bordered by a valine residue (Val^{523}) and hydrogen bonds with a neighbouring arginine residue (Arg^{513}) and the peptide bond of Phe^{518}.[29] A similar hydrophobic side pocket off the main channel in COX-1 is not accessible because of the presence of an isoleucine instead of valine at position 523, which sterically hinders inhibitor approach. The COX-2 mutant V523I is resistant to time-dependent inhibition by diarylheterocycles but not carboxylic acid type NSAIDs.[32,33] Conversely, the COX-1 mutant I523V is sensitive to time-dependent inhibition by diarylheterocycles.[33] Movement of Val^{523} and insertion of the sulfonamide or methylsulfone moiety into the side pocket is thought to contribute to the time dependence of inhibition by diarylheterocycles.

5.1.2.4 N-Alkylsulfonanilide Class of Selective COX-2 Inhibitors

As was the case with diarylheterocycles, investigations on the anti-inflammatory properties of *N*-alkylsulfonanilides (Figure 5.3) such as the marketed NSAID nimesulide also began in the 1960s.[34–36] In the mid-1990s, the observations on the potent and selective COX-2 inhibition by the structurally related sulfonanilide analog NS-398[11,37] provided a biochemical rationale for the anti-inflammatory effects of alkylsulfonanilides like nimesulide[38] and flosulide,[39] and led to a general resurgence in this class of compounds. Upon re-evaluation

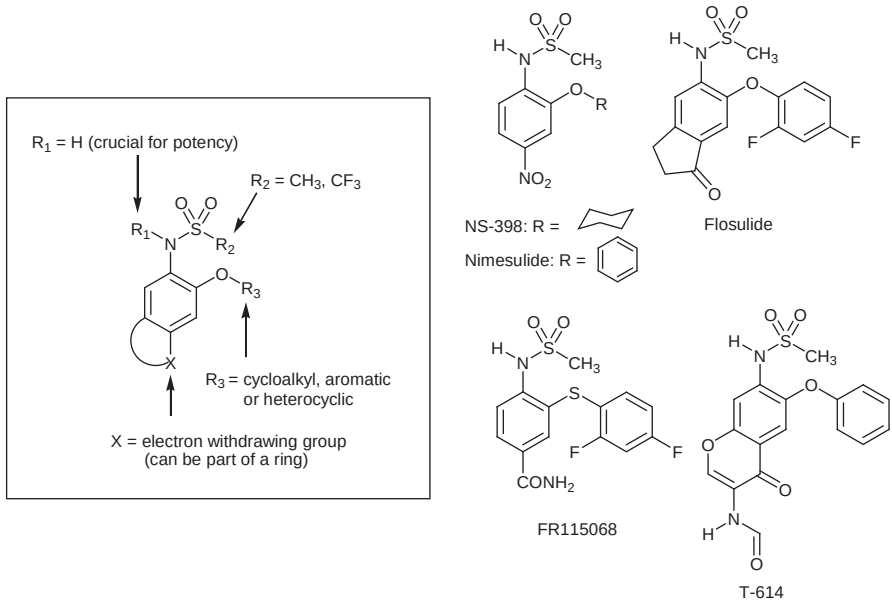


Figure 5.3 SAR on *N*-alkylsulfonanilides as selective COX-2 inhibitors.

for COX inhibition, both nimesulide and flosulide revealed their selective COX-2 inhibitory properties.[40]

The common structural features of the alkylsulfonanilides are depicted in Figure 5.3. The alkyl substituent is typically methyl, but halogenated methyl substituents such as trifluoromethyl have been reported.[41] The *ortho*-anilide substituent typically includes aryl, heterocyclic or cycloalkyl ethers and thioethers. The *para*-anilide substituent invariably bears an electron-withdrawing group that may be incorporated as part of a ring; *absence* of the electron-withdrawing group results in inactive compounds. A variety of methanesulfoanilides with different *para*-electron-withdrawing groups have been evaluated as selective COX-2 inhibitors and as orally active anti-inflammatory agents. Substituents include *para*-acetyl, *para*-cyano, *para*-carboxamido (FR115068), nitro (NS-398 and nimesulide) and *para*-sulfonamido.[42] Another structural variant is the incorporation of the *para*-electron-withdrawing group as part of a ring system as seen in flosulide and the sulfonanilide analog T-614.[43] The phenyl ring in the alkylsulfonanilide scaffold can be replaced with the electron-withdrawing pyridine ring.[42,43]

The crucial requirement of the electron-withdrawing group in COX-2 inhibition by alkylsulfonanilides is most likely related to a lowering of the pKa of the sulfonamide moiety in the range of the carboxylic acid group (the pKa of nimesulide is $\sim$6.4–6.8, whereas that of the corresponding pyridinyl derivative is $\sim$2.98[44,45]), which then allows for an efficient ion pairing interaction with a complementary active site amino acid residue(s). This is demonstrated in the

NS-398-COX-2 crystal structure complex wherein the sulfonamide group ion pairs to Arg^{120} in a manner similar to carboxylic acid containing NSAIDs rather than inserting into the Val^{523} side pocket like the diarylheterocycles.[46] Consistent with the crystal structure findings are the SAR findings that *N*-methylation of the sulfonamide nitrogen in alkylsulfonanilides generates inactive compounds.[41] Unlike the methylsulfone- or sulfonamide-based diarylheterocycles, the structural basis for COX-2 selectivity by NS-398 is not evident from the COX-2 co-crystal structure.

5.1.3 Sulfonamide-based Carbonic Anhydrase Inhibitors

Carbonic anhydrases (CAs, EC 4.2.1.1) are ubiquitous zinc enzymes which are encoded by three distinct, evolutionarily unrelated gene families; in humans, 16 different CAs isozymes (α-CAs) or CA-related proteins have been described with very different sub cellular localisation and tissue distribution.[47] The isozymes catalyse a very simple physiological reaction, *i.e.* the introversion between carbon dioxide and the bicarbonate ion—see Eqn (5.2). Because CO_2 is generated in high amounts in all living organisms, CAs are involved in crucial physiological processes connected with respiration and transport of CO_2/bicarbonate between metabolising tissues and lung, pH and CO_2 homeostasis, electrolyte secretion in a variety of tissues/organs, biosynthetic reactions (*e.g.* gluconeogenesis, lipogenesis and ureagenesis), bone resorption, calcification, tumorigenicity, and many other physiologic and pathologic processes.

(5.2)

5.1.3.1 Mode of Inhibition of CA by Sulfonamides

X-ray crystal structure has been determined for several α-CA isozymes.[48–55] The metal ion (which is Zn^{2+} in all α-CAs) is essential for catalysis.[47] Crystallographic analysis indicates that the active site metal ion is situated at the bottom of a 15Å long active site cleft and is coordinated to three histidine residues (His^{94}, His^{96} and His^{119}) and a water molecule/hydroxide ion. The zinc-bound water is also hydrogen bonded with the hydroxyl group of Thr^{199}, which in turn is bridged to the carboxylic acid moiety of Glu^{106}; these interactions enhance the nucleophilicity of the zinc-bound water molecule, and orient the substrate (CO_2) in a favourable position for nucleophilic attack by the hydroxide ion bound to Zn^{2+} (Figure 5.4).

Primary (and in some cases secondary) sulfonamides attached to an aromatic or heterocyclic scaffold are potent inhibitors of CA enzymes. X-ray crystallographic structures are available for many adducts of sulfonamide inhibitors with CA isoforms.[48–55] In all cases, sulfonamides bind in a tetrahedral geometry of the Zn^{2+} ion (see Figure 5.4) in a deprotonated state, with the sulfonamide nitrogen coordinated to Zn^{2+} and an extended network of hydrogen bonds,

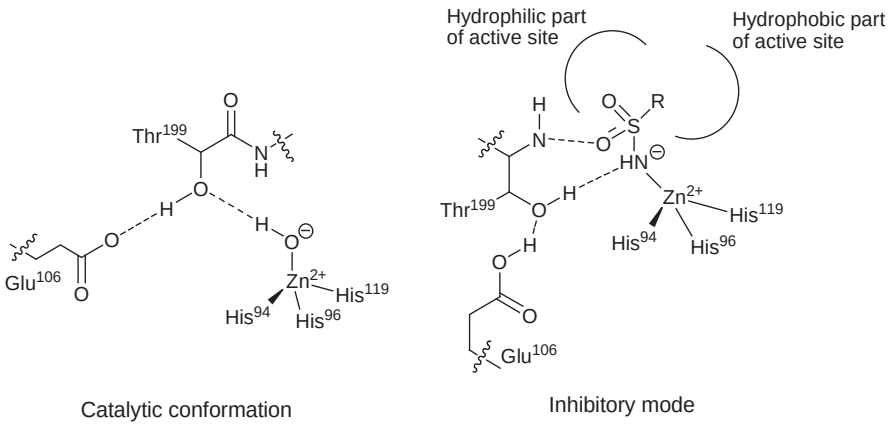


Figure 5.4 α-CA inhibition by primary aromatic and heterocyclic sulfonamides.

involving residues Thr^{199} and Glu^{106}. In such a conformation, the ionised sulfonamide NH group displaces the zinc-bound hydroxide ion. The aromatic/heterocyclic portion of the sulfonamide molecule interacts with hydrophilic and hydrophobic amino acid residues within the active site cavity. This exquisite network of interactions explains why sulfonamides selectively interact with CAs, in contrast to other functional groups (*e.g.* carboxylic acid, hydroxamic acid, phosphates and thiols) that are also capable of coordinating with zinc metal in other enzymes. Quantitative SAR (QSAR) for CA inhibition by sulfonamides has been studied in detail by Clare & Suparan.[56] They concluded that effectiveness of ionisation of the SO_2NH_2 group itself is a key contributor to inhibitory potency. The more easily the functional group can ionise the more potent the inhibitor. Otherwise lipophilicity is a modifying influence, as is the nature of the aromatic ring system if present in the molecule.

5.1.3.2 Clinical Applications of CA Inhibitors

There are over 20 clinically used primary sulfonamide drugs that possess significant CA inhibition. The structures of representative compounds are shown in Figure 5.5. In addition, several novel chemotypes that contain sulfonamide, sulfamate or sulfamide groups also have been reported to inhibit CA isozymes.[57–62]

Many CA inhibitors such as acetazolamide, methazolamide, ethoxzolamide, sulthiame and dichlorophenamide have been used in the clinic for decades as antiglaucoma agents. They were initially developed in the search for novel diuretic, antihypertensives or antiepileptic agents in the 1950s and the 1960s. Their discovery stemmed from the observation of metabolic acidosis (lowered blood pH due to excess production of H^+ or inability of the body to form HCO_3^-) as a side effect of sulfanilamide therapy, which then led to the

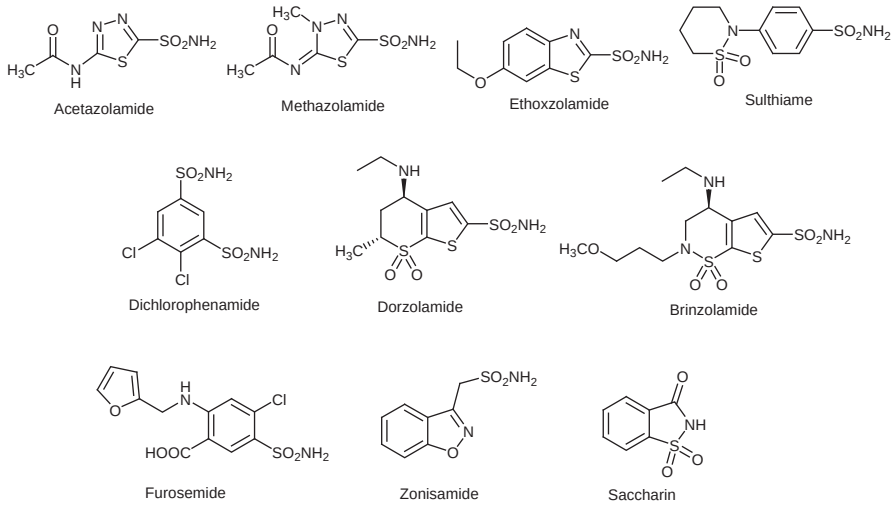


Figure 5.5 Sulfonamide-based inhibitors of CA enzymes.

synthesis of additional sulfonamide derivatives to exploit and maximise this effect as a potential therapy. By producing bicarbonate-rich aqueous humor secretion (mediated by ciliary CA isozymes) within the eye, CAs are involved in vision, and their malfunctioning leads to high intraocular pressure (IOP) and glaucoma.[63] It is well-established that effective reduction of IOP can be achieved by the systemic administration of CA inhibitors, which act by reducing the rate of aqueous humor secretion. However, systemic therapy, which requires large dosages of CA inhibitors to obtain reductions in IOP, concomitantly evokes a wide array of undesirable side effects resulting in poor patient compliance.[64,65] The hypothesis that the undesirable side effects occur *via* CA inhibition in extraocular tissues led to the search for topical CA inhibitors with direct ocular administration. In the 1990s two novel compounds, dorzolamide and brinzolamide (see Figure 5.5), were developed for topical application for glaucoma.[66] Additional examples of drugs that inhibit CA enzymes include the diuretic furosemide, the anticonvulsant zonisamide, and even the artificial sweetener and cyclic acylsulfonamide, saccharin.[67–69] All these drugs contain the sulfonamide motif required for binding to the metal ion in the CA isozymes.

Of late, selective COX-2 inhibitors celecoxib and valdecoxib (but not rofecoxib) have been shown to function as potent CA inhibitors.[70] The solution of the crystal structures of these coxibs with CA indicates that the mode of inhibition is identical to that discerned with other sulfonamides.[71,72] As such, the inhibition of CA isozymes by sulfonamides are diverse and non-specific, which may provide a rationale for the different clinical applications of the CA inhibitors ranging from diuretics and antiglaucoma agents, and more recently as anticancer and anti-obesity drugs.[73]

5.1.4 Sulfonylurea-based Hypoglycemic Agents

Sulfonylurea derivatives are a class of orally active hypoglycemic drugs that have been used in the management of diabetes mellitus type 2 ('adult onset') for several decades. They act by increasing insulin release from the β cells in the pancreas.

The discovery of sulfonylureas as hypoglycemic agents stemmed from a serendipitous clinical finding in the 1940s by Janbon and Loubatières who reported the blood sugar-decreasing effect of a sulfanilamide derivate, sulfoisopropyl thiadiazole, while treating bacterial infections.[74,75] Although this sulfonamide derivative was not proven to be useful in the treatment of diabetes, the information was exploited further in the discovery/development of novel sulfonamide derivatives, which eventually led to the discovery of carbutamide (Figure 5.6) as the first orally active sulfonylurea derivative for the treatment of hypoglycemia.[76] The structural similarity of carbutamide with prototypic sulfanilamide derivatives is obvious.

5.1.4.1 Pharmacological Mechanism of Action

Sulfonylureas bind to the complex of the ATP-dependent K^+ (K_{ATP}) ion and sulfonylurea receptor (SUR1) on the cell membrane of pancreatic β-cells. This results in the inhibition of a tonic, hyperpolarising efflux of potassium, thus causing the electric potential over the membrane to become more positive. This depolarisation opens voltage-gated Ca^{2+} channels.[77,78] The rise in intracellular calcium leads to increased fusion of insulin granulae with the cell membrane and therefore increased secretion of (pro)insulin. There is some evidence that sulfonylureas also sensitise β-cells to glucose, limit glucose production in the liver, decrease lipolysis (breakdown and release of fatty acids by adipose tissue) and decrease clearance of insulin by the liver. Recently, evidence has been presented which demonstrates a weak peroxisome proliferator-activated receptor γ agonism by some sulfonylurea-based drugs.[79,80]

5.1.4.2 SAR Relationships for the Hypoglycemic Activity

All sulfonylureas contain a central *S*-phenylsulfonylurea scaffold with *para*-substitution on the phenyl ring and various groups terminating the urea nitrogen end group. Sulfonylureas are classified into first- and second-generation compounds (Figure 5.6). The drugs of these two generations have in common the sulfonylurea group; they differ mainly by the potency of their insulin-releasing and anti-diabetic actions, the second generation compounds being the more potent.

The observation that the sulfonylurea group is not essential for drug activity was first demonstrated with the sulfonylurea glibenclamide; replacement of the sulfonylurea motif with a carboxylic acid bioisostere led to the discovery of meglitinide, a second generation hypoglycemic agent without the sulfonylurea group.[81] Non-sulfonylurea derivatives also display insulin-secreting and anti-diabetic actions.[82] These drugs (glinides) act on the same cellular targets as do

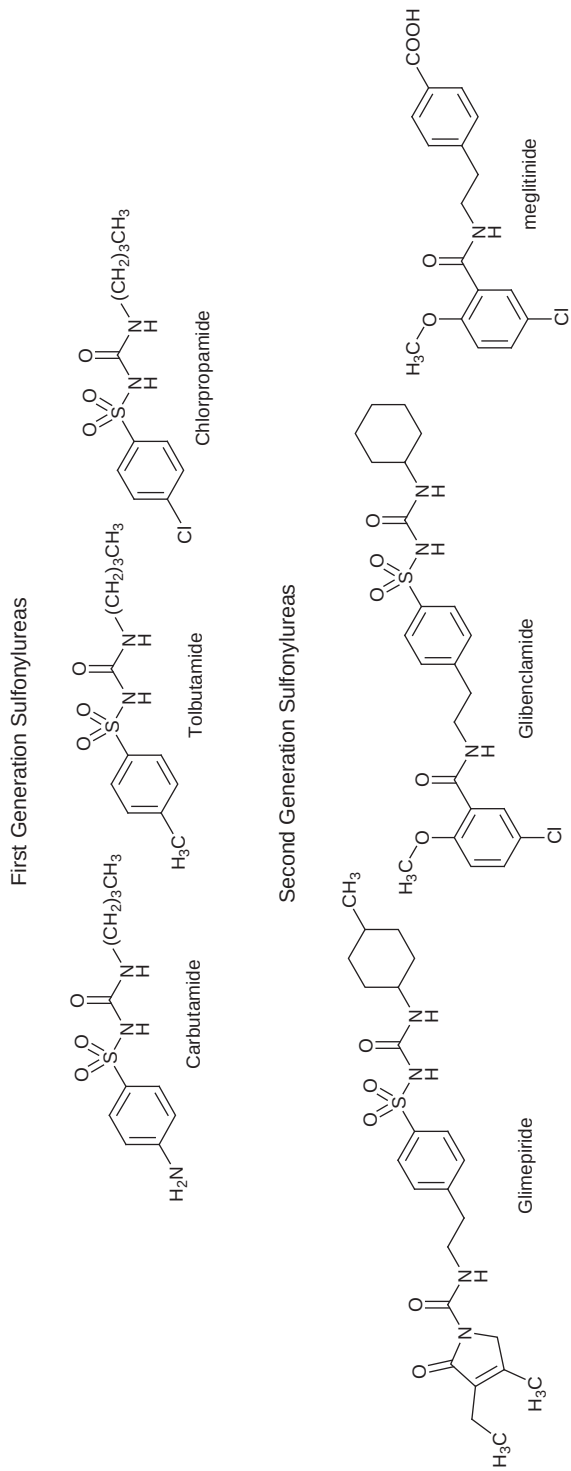


Figure 5.6 Representative examples of first and second generation sulfonylurea-based hypoglycemic agents.

the sulfonylureas, namely they close the KATP channels, although they are presumed to act in different regions of the SUR1 receptor.

5.1.5 Miscellaneous Applications of the Sulfonamide Group in Medicinal Chemistry

5.1.5.1 Sulfonamides as Phenol Bioisosteres

The alkysulfonamide group is commonly used as a non-classical bioisostere for the phenol substituent as it has similar pKa values ($\sim$8.0) to that of the phenolic hydroxyl group. For instance, during the lead optimisation of a gonadotropin-releasing hormone antagonist, methanesulfonamide replacement of the phenol in compound **5.4** resulted in bioisostere **5.5**, with a four-fold increase in binding affinity (Figure 5.7).[83] However, the lack of generality when transferring a certain type of bioisosteric transformation between lead compounds for different therapeutic targets becomes apparent in the next example. When the methanesulfonamide replacement was applied to the opioid antagonist naltrexone (**5.6**) and its receptor agonist **5.7**, the corresponding sulfonamide derivatives **5.8** and **5.9**, respectively, were totally inactive *in vitro*, despite having similar pKa values (see Figure 5.7).[84] The lack of binding affinity has been attributed to the steric bulk of the sulfonamide group, which makes this motif, an unsuitable bioisostere in this particular example.

5.1.5.2 Sulfonamides as α-Ketoamide Bioisosteres

Sulfonamides also appear to function as α-ketoamide isosteres based on a recent study dealing with the optimisation of azaindole derivatives as human

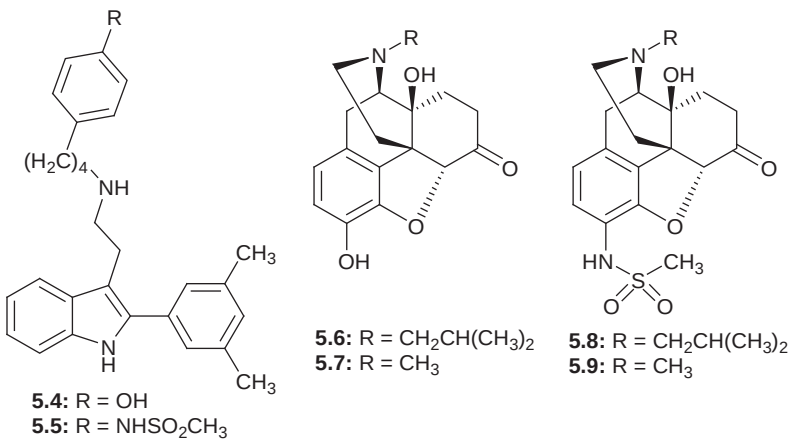


Figure 5.7 Successes and failures in the utility of the methanesulfonamide group as a phenol bioisostere.

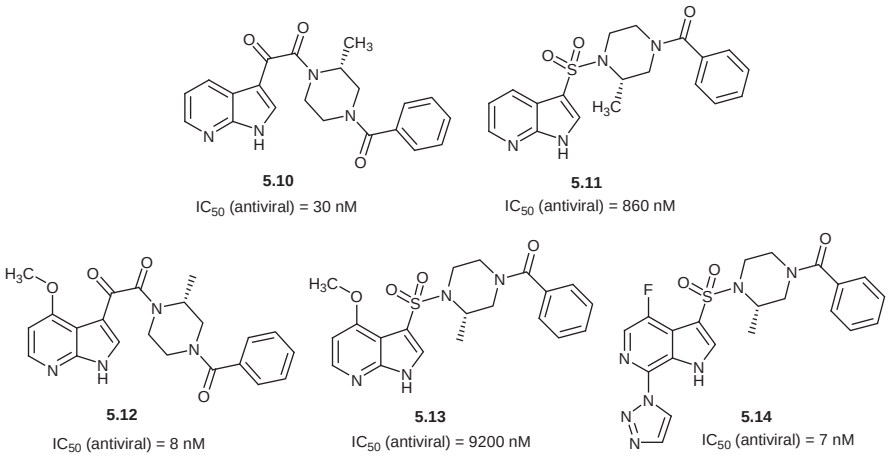


Figure 5.8 The sulfonamide group as an α-ketoamide bioisostere.

immunodeficiency virus entry inhibitors.[85] Flexible overlay calculations on α-ketoamide and sulfonamide derivatives, **5.10** and **5.11** (Figure 5.8) revealed that the sulfonamide group was in close proximity to the ketoamide group, with one sulfonamide oxygen tightly overlaid on the amide nitrogen. The other sulfonamide oxygen lies ~1.1Å from the ketone oxygen. These results also suggest that energetically accessible conformations of α-ketoamide and sulfonamide have similar dispositions of potentially pharmacophoric aromatic, lipophilic and H-bond acceptor groups. Although direct replacement of the α-ketoamide group in the azaindole derivative (*e.g.* compound **5.12**) with a sulfonamide (*e.g.* compound **5.13**) led to a loss in potency, subsequent SAR studies led to sulfonamide derivative **5.14** that possessed pharmacology comparable to **5.12** (see Figure 5.8).

5.1.5.3 Acylsulfonamides (Sulfonimides)

Replacement of the hydroxyl moiety of a carboxylic acid with a phenylsulfonamide results in the formation of a sulfonimide derivative. The estimated pKa values for sulfonimides are similar to that of an aryl carboxylic acid (pKa ~3.5–4.0). An example of the utility of this bioisostere becomes evident in the case of the indole-based leukotriene antagonists where there was little difference between sulfonamide **5.16** and its parent compound **5.15** (Figure 5.9) with respect to their ability to antagonise the cysteinyl leukotriene (cysLT) receptor.[86] Likewise, sulfonamide replacement of the hydroxyl group in carboxylic acid derivatives and serine protease inhibitors **5.17** and **5.18** led to **5.19** and **5.20**, respectively, with significantly improved inhibitory potency (K_i values of 0.2–0.6 μM for the sulfonimides *versus* 1.8–1.9 μM for the parent acids) against the hepatitis C virus (HCV) NS3 protease.[87] A similar observation was noted in a study on CXCR2 receptor antagonists where acylsulfonamide replacement

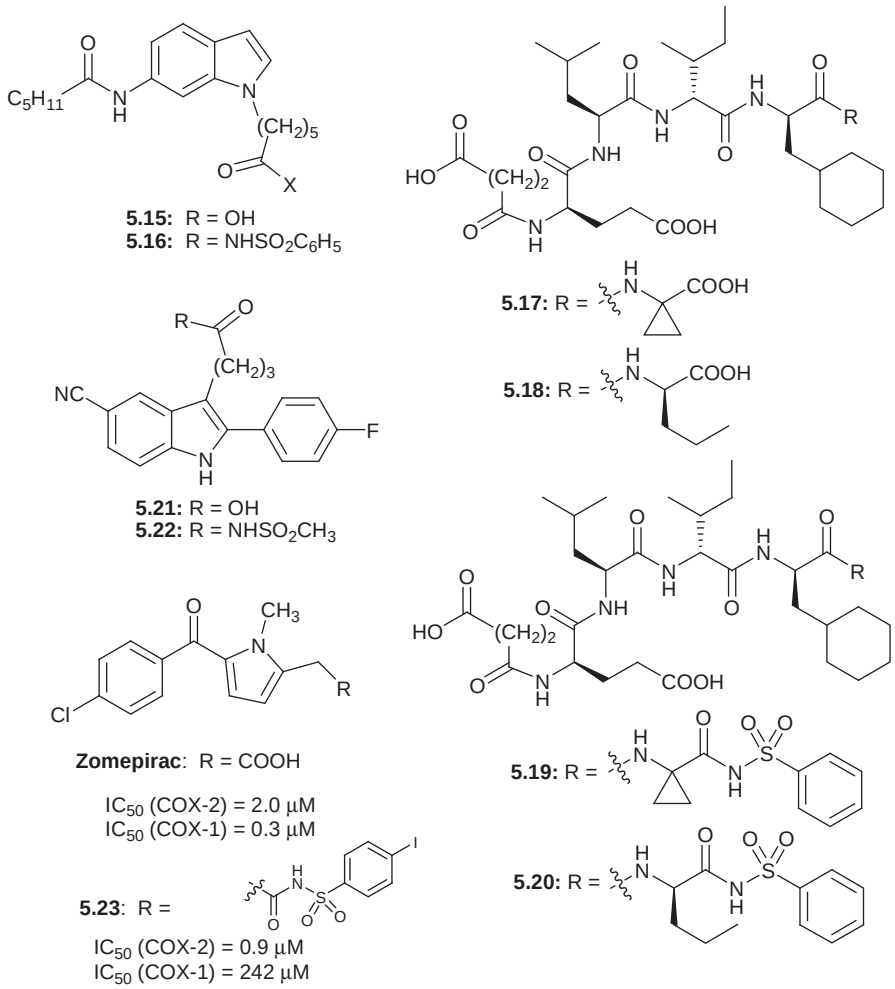


Figure 5.9 Acylsulfonamides (sulfonimides) as carboxylic acid bioisosteres.

(**5.22**) for the carboxylic acid derivative (**5.21**) and CXCR2 receptor antagonist improved antagonist potency.[88]

A noteworthy example of the impact on primary pharmacology upon bioisosteric replacement of the carboxylic acid OH group with a sulfonamide moiety was evident during studies on the COX-1-selective NSAID zomepirac. Conversion of COX-1-selective inhibitor zomepirac into a COX-2-selective inhibitor was achieved by simply replacing its carboxylic acid group with an sulfonimide bioisostere (**5.23**) (Figure 5.9).[89] Although **5.23** has been co-crystallised with COX-2, the crystal structure does not provide a rationale for COX-2-selective inhibition by the bioisostere.[89] The acylsulfonamide motif in **5.23** breeches the constriction at the mouth of the COX active site and projects

into the sterically unconjugated lobby region. The sulfonamide portion of the inhibitor hydrogen bonds to the Arg^{120}, Glu^{524} and Tyr^{355} in COX-2 in a manner similar to the carboxylic acid-based NSAIDs. Because the three amino acid residues are conserved in COX-1 and COX-2, their importance in determining the selectivity of the **5.23** for COX-2 inhibition remains uncertain. As such, replacement of the carboxylic acid moiety in several NSAIDs (*e.g.* indomethacin, sulindac, meclofenamic acid, *etc.*) with bioisosteres (*e.g.* esters and amides) has provided a facile strategy for converting non-selective or COX-1-selective inhibitors into potent and selective COX-2 inhibitors.[90–92]

5.1.5.4 Discovery of Zafirlukast–a Cysteinyl Leukotriene Receptor Antagonist and Sulfonimide Derivative

Designing antagonists of the cysLT receptors as orally active anti-asthma drugs has paid off significantly as evident from the commercial success of montelukast, zafirlukast and pranlukast (Figure 5.10). Peptidoleukotrienes LTC_4, LTD_4 and LTE_4 are potent constrictors of smooth muscle and, as such, have been implicated since the 1970s in the development of asthma.[93,94] FPL 55712, a chromane carboxylic acid, which was discovered as a prototype cysLT receptor antagonist, provided medicinal chemists with a starting point in structure-based drug design.[95] A visual examination of the structures of LTD_4 and FPL 55712 suggests that the hydroxyacetophenone region in FPL 55712 mimics the olefinic region of the leukotriene, while the chromane carboxylic acid segment mimics either the backbone C_1–C_5 carboxylic acid region or the peptidic component of LTD_4 (Figure 5.10). Exhaustive SAR studies to optimise antagonist potency of FPL 55712 led to several FPL 55712 derived compounds that were then clinically evaluated.[96] Of these, tomelukast (Figure 5.10) was arguably the most important for many reasons, foremost amongst which was the demonstration of anti-asthmatic activity in the clinic. Furthermore, from an SAR perspective, tomelukast demonstrated that substitution of the carboxylic acid motif in FPL 55712 with the tetrazole bioisostere could potentially result in compounds that exhibit increases in *in vitro* and *in vivo* potency.[97] The 600 mg B.I.D. dosing regimen required for clinical efficacy, however, suggested that even further increases in potency would be required for a low daily dose anti-asthmatic agent.[98] The search for more potent cysLT receptor antagonists led to pranlukast, the structure of which, can be considered to be a hybrid between FPL 55712 and tomelukast due to the presence of the chromane tetrazole motif (Figure 5.10).[99] Pranlukast was the first cysLT receptor antagonist approved for marketing and is currently sold in Japan.[100]

The evolution of sulfonimide derivative zafirlukast also proceeded through initial SAR analyses on FPL 55712-like compounds and led to the fabrication of an indole-containing lead compound (**5.24**) (Figure 5.11).[101] Modifications were simultaneously made on three regions of this molecule: the lipid-like tail region, the acidic head region, and the indole backbone. With regards to exploration of

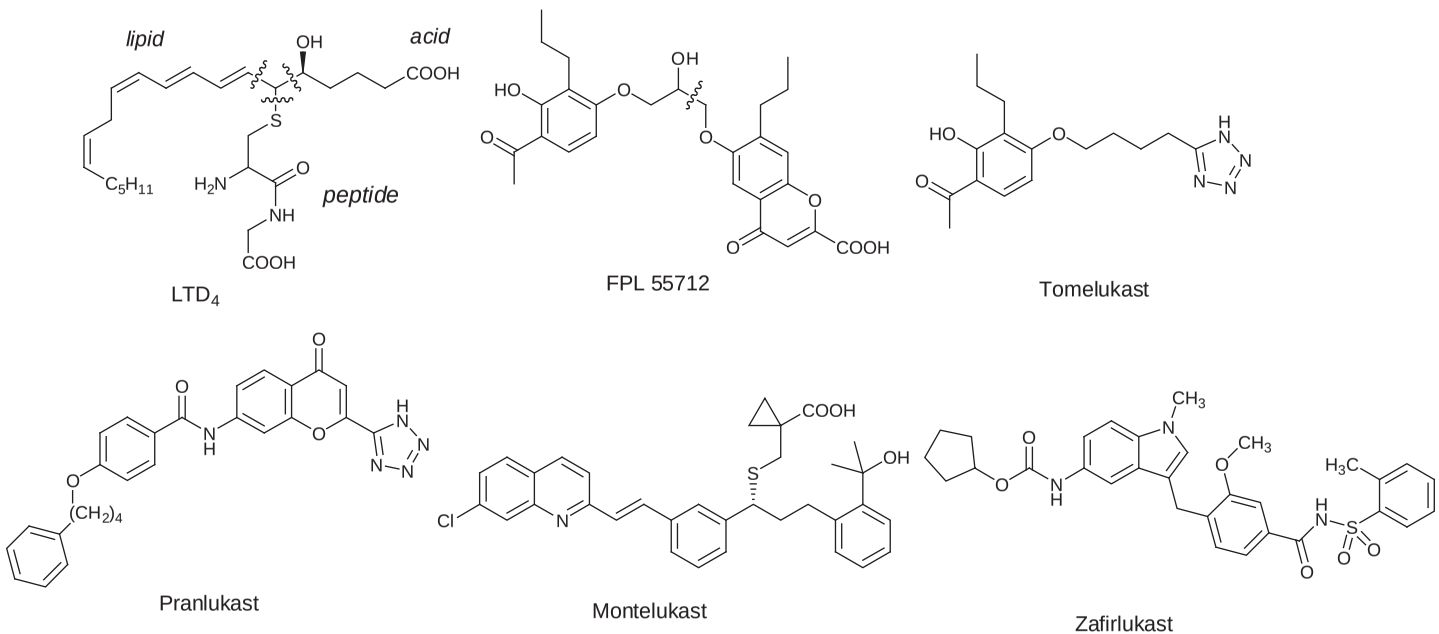


Figure 5.10 Evolution of cysteinyl leukotriene receptor antagonists for the treatment of asthma.

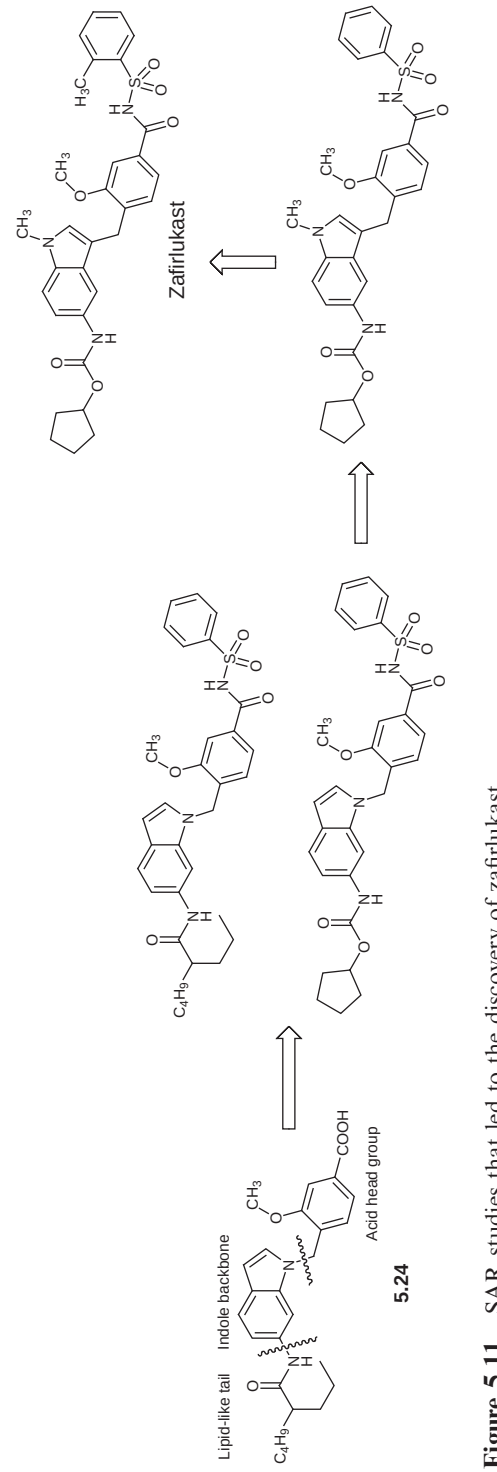


Figure 5.11 SAR studies that led to the discovery of zafirlukast.

the acidic head region, SAR studies demonstrated that a preferred linkage occurred with a 3-methoxy aryl group; however, replacement of the carboxylic acid head group with phenylsulfonimide produced an approximately 100-fold increase in potency. More importantly, this change was additive to other alterations made in the amide region of the tail with *in vitro* potency $\sim >1000$-fold relative to FPL 55712. Although a number of variations of this theme demonstrated potent *in vitro* antagonist activity, as such, these substituents had a negative impact on oral activity.[102,103] Further SAR optimisation indicated that the inverted indole backbone also maintained potent activity against the receptor, albeit with very poor rat oral bioavailability ($<1\%$); incorporation of *ortho*-tolyl sulfonamide substituent led to zafirlukast with oral bioavailability of $\sim 68\%$ and 67% in rats and dogs, respectively.[102–105]

5.2 Absorption, Distribution, Metabolism and Excretion of Sulfonamides

5.2.1 Oral Absorption

With the exception of the topical antiglaucoma agents, most sulfonamide-based drugs are administered by the oral route and hence absorption into the systemic circulation is essential for their pharmacological action in target tissue (*e.g.* kidney for the thiazides, and loop diuretics and pancreas for the sulfonylureas).

A common approach towards gaining an understanding of the balance of physiochemical properties that leads to a suitable pharmacokinetic profile after oral administration is to examine whether or not the compounds 'fit' with Lipinski's 'rule of five'.[106] Using a dataset of 2245 compounds from the World Drug Index, Lipinski found that approximately 90% of the compounds (excluding phosphates, polymers and quaternary ammonium ions) had a molecular weight <500, cLogP <5, sum of hydrogen bond donors (as a sum of NH and OH) <5, and sum of hydrogen bond acceptors (as a sum of N and O) <10.[106] Lipinski proposed that poor absorption and permeation are more likely when two or more of these limits are exceeded.[106] In addition to the molecular properties discussed by Lipinski, descriptors such as polar surface area (PSA) have been shown to reliably predict oral bioavailability, particularly for organic anions (*e.g.* carboxylic acid).[107] For instance, the negative impact of a high PSA $>150\text{Å}^2$ and excessive (>10) hydrogen bond donor or acceptor properties on intestinal absorption/permeability has been recognised.[107–110]

An assessment of the physicochemical properties of 111 marketed sulfonamide-based drugs is depicted in Table 5.2. Approximately 90% of the drugs comply with Lipinski's rule of five for successful orally active agents. Examination of the structures of the individual compounds reveals that the sulfonamide motif is present within a vast chemical space with neutral molecules being most common followed by an equal number of acids and bases. The mean range of PSA, hydrogen bond donors and hydrogen bond acceptors for the marketed sulfonamides lies within the confines of the

Table 5.2 Physicochemical properties of the 111 marketed sulfonamide containing drugs.

Property	*Mean*	*Range*
Molecular weight	368	172–606
CLogP	1.8	−2.0–7.8
cLogD @ pH 7.4	0.5	−4.9–4.2
Polar surface area (PSA)	106Å^2	44–200
Hydrogen bond donors (HBD)	3	0–7
Hydrogen bond acceptors (HBA)	4	1–12
Rule of five compliant	90%	–
Acids	20% (22 out of 111)	
Bases	15% (17 out of 111)	
Neutrals	63% (70 out of 111)	
Zwitterions	2% (2 out of 111)	

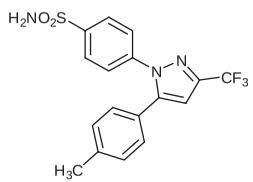
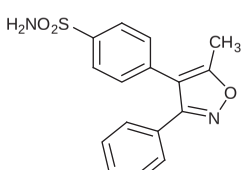
physiochemical space required for good oral absorption of acidic, basic or neutral molecules.

5.2.1.1 Sulfonamide-based Prodrugs–Discovery of Parecoxib

In terms of aqueous solubility, neutral sulfonamides celecoxib and valdecoxib are slightly more soluble than the methylsulfone rofecoxib (Table 5.3).[111,112] However, upon comparison of the solubility parameters with carboxylic acid containing NSAIDs derivatives, neutral sulfonamides possess considerably lower solubility (see Table 5.3). The absorptive apical to basolateral permeability (AB) as measured in the Caco-2 cell line is comparable for the coxibs and the various sub-classes of NSAIDs—a feature consistent with good oral absorption.[111] Lack of asymmetry in the basolateral to apical (BA) direction suggests that efflux transport proteins such as P-glycoprotein or breast cancer resistant protein will not negatively impact oral absorption. The PSA estimates for celecoxib (86.4), valdecoxib (94.6) and rofecoxib (68.8) lie within the range that predicts moderate to good oral absorption.

Consistent with the *in vitro* observations, the coxibs are rapidly absorbed (Tmax $\sim$2.0–3.0 h) in humans following oral administration at their efficacious doses. Differences in physiochemical attributes (*e.g.* solubility and lipophilicity) do appear to play a role in the oral absorption as illustrated with celecoxib and valdecoxib. Systemic exposure as judged from Cmax and $AUC_{0-\infty}$ for valdecoxib (at its efficacious dose of 10–20 mg) is $294 \pm 118\,ng\,mL^{-1}$ and $3306 \pm 1300\,ng\,hr^{-1}\,mL^{-1}$, respectively.[113] The findings from the human mass balance studies on valdecoxib that less than 1% of the administered radioactive dose is recovered in unchanged form in faeces suggests near complete oral absorption of valdecoxib.[114] In the case of celecoxib, C_{max} and $AUC_{0-\infty}$ at the efficacious dose of 200 mg are $797 \pm 498.8\,ng\,mL^{-1}$ and $7600 \pm 5500\,ng\,hr^{-1}\,mL^{-1}$, respectively.[115] The lower oral systemic exposure of celecoxib relative to valdecoxib relates to a greater

Table 5.3 Structure, physiochemical properties, dose and Caco-2 cell permeability of carboxylic acid based NSAIDs and coxibs.

Compound	*MW*	*pKa*	*Dose (mg)*	$P_{Caco\text{-}2} \times 10^6$ *cm/sec* *A to B*	*B to A*	*Equilibrium solubility (pH 7.4) (mg/mL)*
Celecoxib	381	11.1	100–200	17.6	15.1	0.005
						
Valdecoxib	314	11.0	10	20.0	21.1	0.010
						

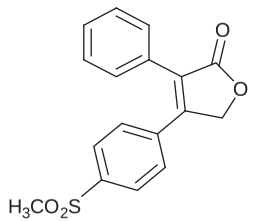
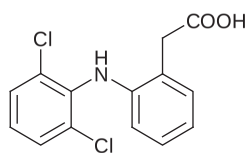
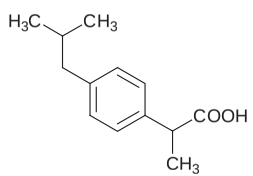
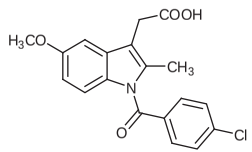
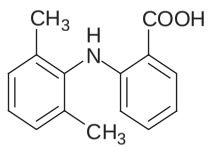
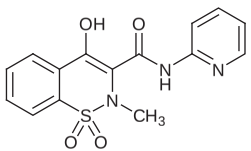
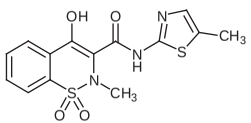
Rofecoxib	314	–	12.5–25	24.1	19.7	0.0009
						
Diclofenac	295	4.2	50	20.2	21.3	15.9
						
Ibuprofen	206	4.4	200–800	10.1	19.8	2.30
						

Table 5.3 (*continued*)

Compound	*MW*	*pKa*	*Dose (mg)*	$P_{Caco\text{-}2} \times 10^6$ cm/sec *A to B*	*B to A*	*Equilibrium solubility (pH 7.4) (mg/mL)*
Indomethacin	357	4.5	25–50	23.8	33.0	1.30
						
Mefenamic acid	241	4.2	250	17.9	22.2	0.10
						

Piroxicam 	331	1.8, 5.1	10–20	24.1	19.7	0.26
Meloxicam 	351	1.1, 4.2	7.5–15	17.6	15.1	0.46

propensity to undergo first pass metabolism, which is apparent upon comparison of the oral clearance values (valdecoxib: $CL/F = 1.7\,mL\,min^{-1}\,kg^{-1}$; celecoxib: $CL/F = 9.5\,mL\,min^{-1}\,kg^{-1}$).[113–116]

The relatively poor aqueous solubility of COX-2 inhibitors like valdecoxib can be considerably improved by conversion of the primary sulfonamide to the corresponding sulfonimide derivative **5.25** (Figure 5.12).[117] Conversion to the sulfonimide derivative imparts acidic character to the sulfonamide motif and consequently provides the options of preparing salts to improve upon the aqueous solubility. Compared with valdecoxib, whose aqueous solubility is $0.010\,mg\,mL^{-1}$, the solubility of **5.25** (as the corresponding sodium salt) is $44\,mg\,mL^{-1}$. Although **5.25** is inactive as a selective COX-2 inhibitor *in vitro*, the compounds displays potent anti-inflammatory activity following oral or intravenous administration in rats due to rapid amidase-mediated hydrolysis of the sulfonimide moiety ($T_{1/2} = 15\,min$) to the active ingredient valdecoxib. However, resistance of **5.25** towards hydrolytic cleavage in dogs, monkeys and human liver preparations precluded further studies on this compound. SAR studies revealed that extension of the alkyl group attached to the sulfonimide nitrogen led to compounds (*e.g.* **5.26** and **5.27**) (Figure 5.12) that retained the aqueous solubility characteristics of **5.25** while undergoing facile hydrolytic cleavage in animals and human liver preparations. Of these compounds, **5.26** (parecoxib sodium) was chosen for clinical development; a randomised, double-blind, placebo-controlled, multiple-dose study in healthy human volunteers demonstrated that parecoxib was rapidly absorbed ($T_{max} \sim 30$ min) and converted to valdecoxib (elimination $T_{1/2} = 0.69$ h).[118] Steady state drug levels were achieved within seven days and linear pharmacokinetics discerned for both the prodrug and the active compound. Parecoxib sodium has been shown to be well tolerated with no clinically significant adverse events observed in this study. Parecoxib sodium is marketed in the European Union as Dynastat for treatment of post-operative pain.[119]

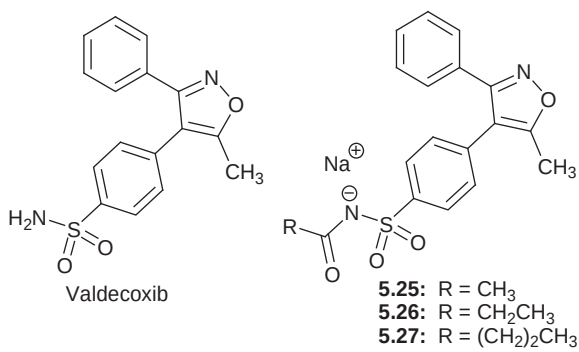


Figure 5.12 Sulfonimide prodrugs of valdecoxib: discovery of parecoxib sodium for parental administration for post-surgical pain management.

In the case of sulfonylurea hypoglycemic drugs, it is interesting to note that the SUR1 receptor is a member of the ABC (adenosine-5′-triphosphate (ATP)-binding cassette). Consequently, some sulfonylureas have been shown to inhibit ABC efflux transporters such P-glycoprotein or multidrug resistance protein (MRP1);[120,121] furthermore, there is evidence that glyburide is a breast cancer resistant protein and MRP3 substrate in transfected cell lines overexpressing these transporters.[122] The impact of these findings on sulfonylurea oral pharmacokinetics and drug–drug interaction potential is unknown.

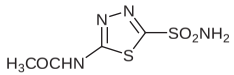
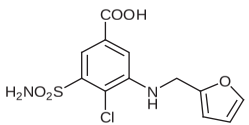
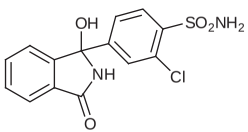
5.2.2 Distribution

Because sulfonamide groups are often used as non-classical carboxylic acid bioisosteres, the affinity of the functional group towards distribution in tissue proteins relative to plasma proteins is often similar to that discerned with carboxylic acid containing drugs. In other words, the distribution volumes (Vd) of drugs containing the two functional groups in a bioisosteric relationship are often comparable. Modulation of Vd for sulfonamide-containing drugs can be influenced by lipophilicity (clogP and/or clogD) and acidity (pKa) in a manner similar for compounds of all physiochemical classes (acids, bases and neutrals).[123] Thus, increases in lipophilicity generally coincide with increases in Vd, and a more acidic character of the sulfonamide group will generally lead to a lower Vd owing to extensive plasma protein (*e.g.* serum albumin) as discerned with carboxylic acid analogs. These principles were outlined in Chapter 2. The pharmacokinetic attributes of structurally diverse sulfonamide derivatives[124] in humans are shown in Table 5.4 and provide a glimpse of the relationship between physiochemical properties and pharmacokinetic parameters such as plasma free fraction (f_u), Vd and clearance.

5.2.2.1 Preferential Red Blood Cell Partitioning of Sulfonamide-based CA Inhibitors

An interesting pharmacokinetic attribute of some sulfonamide derivatives is their extensive binding to red blood cells, which typically results in blood to plasma partitioning ratios >1. Preferential distribution into red blood cells is more commonly discerned with primary sulfonamides due to their affinity for CA isozymes, which are present within the erythrocyte and may act as a site for sequestration of drug. In contrast with primary sulfonamides, hypoglycemic sulfonylureas such as glibenclamide have been shown to preferentially partition to plasma compartment (blood to plasma ratio ~ 0.5).[125] Because of this property, it is possible that sulfonamides could also exhibit non-linear pharmacokinetics as illustrated with the CA inhibitor L-693 612 (Figure 5.13).[126] At blood concentrations achieved in the linear dose range (0.05–0.25 mg kg^{-1}), binding to CA results in extensive red blood cell partitioning of L-693 612, with a constant low free fraction in plasma for elimination (measured *in vitro* blood to plasma ratio of ~ 400). In contrast, higher doses (5–25 mg kg^{-1}) saturate CA

Table 5.4 Physiochemical and pharmacokinetic parameters of representative sulfonamide-containing drugs

Drug	*MW*	*cLogP*	*Log $D_{7.4}$*	f_u	*CL(mL/min/kg)*	*Vd_{ss}(L/kg)*
Loop diuretics						
Acetazolamide	222	−1.13	−0.48	0.04	0.65	0.37
						
Furosemide	330	1.90	−0.13	0.01	1.60	0.12
						
Chlorthalidone	338	0.45	−0.74	0.24	3.90	1.50
						

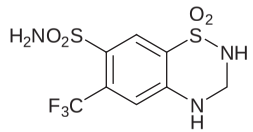
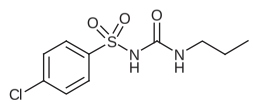
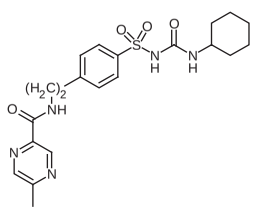
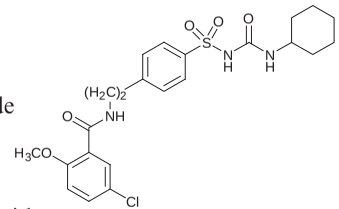
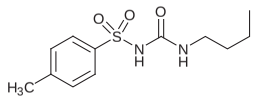
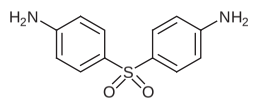
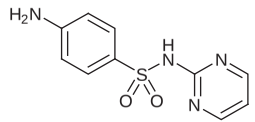
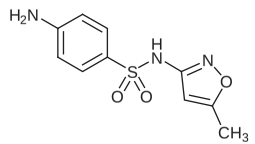
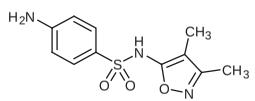
Thiazides						
Hydroflumethiazide	330	−0.21	0.33	N/A	9.70	2.20
						
Sulfonylureas						
Chlorpropamide	276	2.35	0.32	0.03	0.05	0.19
						
Glizipizide	445	2.57	0.14	0.02	0.56	0.16
						

Table 5.4 (*continued*)

Drug	*MW*	*cLogP*	*Log $D_{7.4}$*	*f_u*	*CL(mL/min/kg)*	*Vd_{ss}(L/kg)*
Glyburide 	494	4.24	1.90	0.02	0.82	0.08
Tolbutamide 	270	2.50	0.39	0.05	1.90	0.12
Sulfanilamides						
Dapsone 	248	0.89	0.94	0.25	0.48	0.83

Sulfadiazine 	250	0.10	−0.66	0.44	0.55	0.29
Sulfamethoxazole 	253	0.56	−0.20	0.02	0.36	0.30
Sulfisoxazole 	267	0.22	−0.22	0.08	0.30	0.17

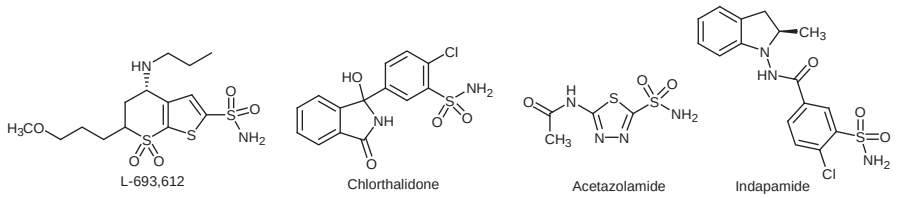


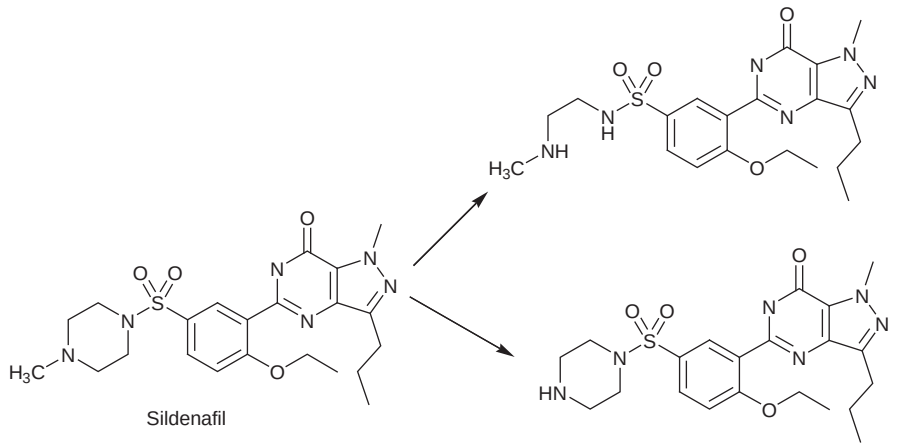
Figure 5.13 Examples of sulfonamides with preferential red blood cell partitioning due to carbonic anhydrase binding.

binding sites in red blood cells; consequently, the free fraction available in plasma for elimination is much greater (blood to plasma ratio reduces to $\sim$6), resulting in significant increases in blood clearance. Likewise, the observed dose-dependent increases in Vd of L-693 612 are consistent with the hypothesis that high-affinity binding to CA confines the compound largely to the blood compartment at low doses, but saturation of CA binding sites at high doses increases availability to peripheral tissues.

Preferential partitioning of sulfonamides into red blood cells renders them susceptible to significant pharmacokinetic interactions with agents that may compete for binding to CA. For instance, a significant drug–drug interaction has been noted in the clinic with the CA inhibitors and anti-hypertensive agents chlorthalidone and acetazolamide (Figure 5.13), wherein acetazolamide was able to displace chlorthalidone from blood cells following administration of the two medications in humans.[127] Thus, intravenous administration of acetazolamide to humans who had previously received [^{14}C]-chlorthalidone resulted in a marked drop in red blood cell radioactivity, whereas that in plasma increased. A likely explanation for the pharmacokinetic interaction is the relatively higher affinity of acetazolamide (*versus* chlorthalidone) towards binding to CA as reflected from the greater than six-fold higher blood to plasma ratio of acetazolamide; the blood-to-plasma ratio of acetazolamide and chlorthalidone has been estimated to be 467 and 70, respectively.[128] Drug–drug interactions due to drug displacement from red blood cells have also been noted with the anti-hypertensive agents and CA inhibitors indapamide (Figure 5.13), chlorthalidone and acetazolamide in the rat. Both chlorthalidone and acetazolamide were able to displace indapamide from rat erythrocytes due to competition for CA binding sites *in vivo*.[129]

5.2.3 Metabolism

Presence of the sulfonamide moiety in a molecule in a secondary or tertiary amide expression usually provides an inert, metabolically robust group that is not typically vulnerable to phase I or phase II metabolising enzymes. Contrary to facile enzyme-catalysed hydrolysis of esters and amides, sulfonamides are generally resistant to cleavage by amidases. An exception to the rule is sulfonimide (acylsulfonamide) derivatives such as valdecoxib, which can undergo



Scheme 5.2 Biotransformation of sildenafil in preclinical species and human.

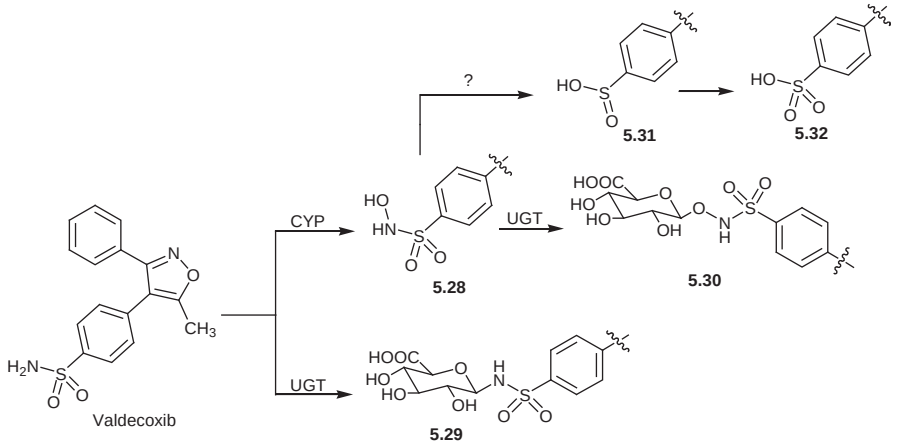
hydrolysis to the parent sulfonamide. A characteristic biotransformation reaction associated with secondary or tertiary sulfonamides include *N*-dealkylation of the substituent attached to the sulfonamide nitrogen as highlighted in the metabolism of the PDE5 inhibitor sildenafil in preclinical species and human (Scheme 5.2).[130]

5.2.3.1 Biotranformation Pathways of Sulfonamides

In contrast with carboxylic acids, which are metabolised by phase II processes such glucuronidation and amino acid conjugation, primary sulfonamide biotransformation can involve *N*-hydroxylation and/or *N*-glucuronidation *via* cytochrome P450 (CYP) and uridine glucuronosyl transferease (UGT) enzymes, respectively.

An example of this phenomenon becomes evident with valdecoxib, wherein a significant component of its metabolic elimination in mice and humans involves hydroxylation and glucuronidation of the sulfonamide moiety to yield metabolites **5.28** and **5.29**, respectively (Scheme 5.3).[114,131] The *N*-hydroxylated metabolite **5.28** of valdecoxib is further metabolised to the corresponding *O*-glucuronide **5.30** (Scheme 5.3). Mass balance studies in humans reveal that ~25% of the administered dose of valdecoxib is eliminated *via* the combined oxidative and glucuronidation pathway depicted in Scheme 5.3.[114] The sulfonamide group in valdecoxib also appears to be susceptible to hydrolytic cleavage, considering that trace amounts (~0.5% of administered dose) of the corresponding sulfinic and sulfonic metabolites **5.31** and **5.32**, respectively, have been detected in human urine.[114]

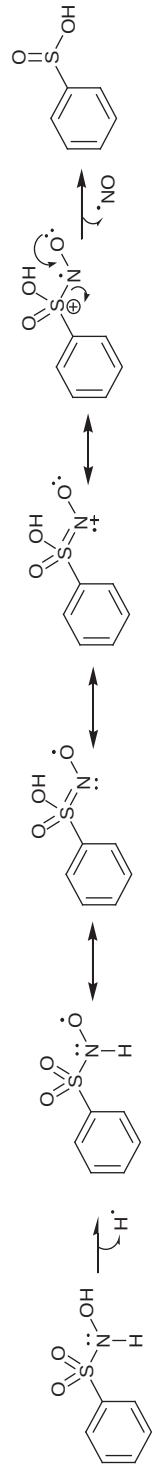
The mechanism of sulfonamide degradation to sulfinic and sulfonic acid derivatives has been examined in some detail and does not appear to be a straightforward enzymatic hydrolysis of the motif in a manner similar to that



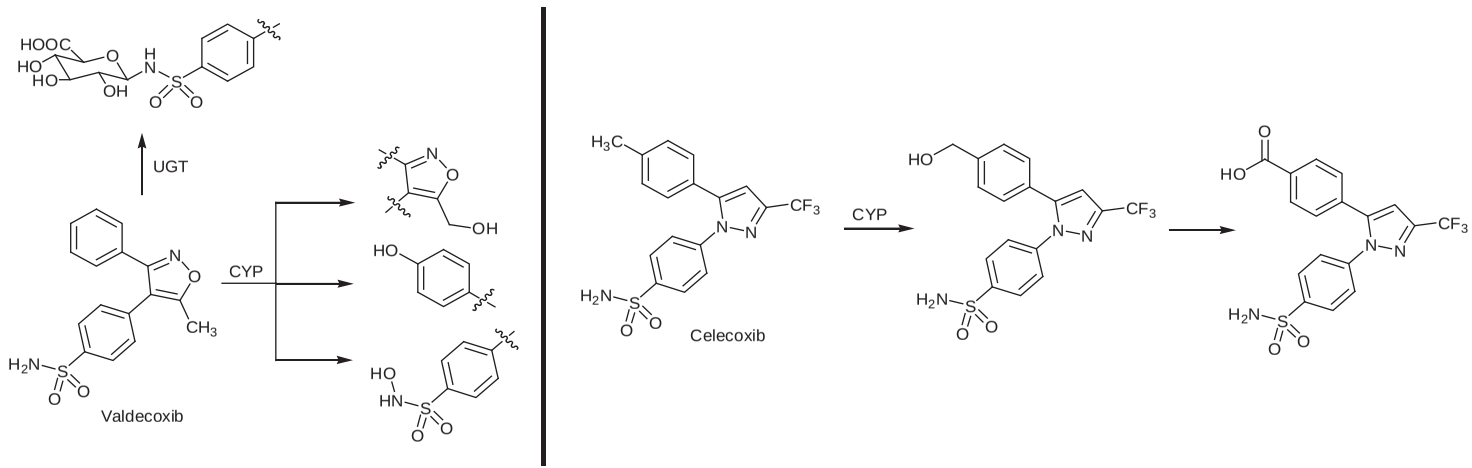
Scheme 5.3 Metabolism of the sulfonamide group as illustrated with the selective COX-2 inhibitor and anti-inflammatory agent valdecoxib.

discerned with esters and amides. Instead, the studies implicate *N*-hydroxylation of the sulfonamide group as the rate-limiting step in the formation of the sulfinic and sulfonic acid metabolites.[132–135] For example, the decomposition of benzenesulfohydroxamic acid (Piloty's acid) (Scheme 5.4) and some of its derivatives under anaerobic and strong alkaline conditions has been reported to yield benzenesulfinic acid and nitrous oxide (N_2O).[132,133] Under physiological conditions (aerobic environment, phosphate buffer, pH 7.4), Piloty's acid decomposes to form benzenesulfinic acid and nitric oxide (NO), which can be intercepted with a nitrone free radical trap.[134] A mechanism, which depicts the formation of NO in the oxidative decomposition of Piloty's acid is shown in Scheme 5.4.[135] Consistent with the general instability of *N*-hydroxysulfonamides under neutral aerobic conditions, attempts to isolate the *N*-hydroxy metabolite of valdecoxib, *i.e.* **5.28**, in pure form by chemical synthesis were met with failure due to facile decomposition at room temperature to afford the corresponding sulfinic acid derivative **5.31**.[136] However, work-up and crystallisation of crude **5.28** using EDTA and ascorbic acid to remove sources of chelating metal ion traces and/or oxidants has afforded a stable monohydrate form of **5.28** whose water content remained constant at room temperatures (under standard humidity conditions) over a period of two years.[136] It is interesting to note that **5.28** is an active metabolite of valdecoxib; however, the COX-2 inhibitory potency and selectivity of **5.28** is significantly lower than that of the parent compound.

Finally, it is noteworthy to point out that the sulfonamide group in celecoxib is resistant to *N*-hydroxylation or *N*-glucuronidation in animals and humans.[116,137] In valdecoxib, CYP mediated hydroxylation occurs on the sulfonamide nitrogen, the 5-isooxazylmethyl group as well on the unsubstituted phenyl ring (Scheme 5.5); in contrast, celecoxib is exclusively metabolised *via* hydroxylation of the *para*-tolyl methyl group (Scheme 5.5). The difference in



Scheme 5.4 Decomposition of *N*-hydroxysulfonamides to sulfinic acid and nitric oxide.



Scheme 5.5 Differences in metabolic profile of the selective COX-2 inhibitors and diarylheterocycle derivatives valdecoxib and celecoxib.

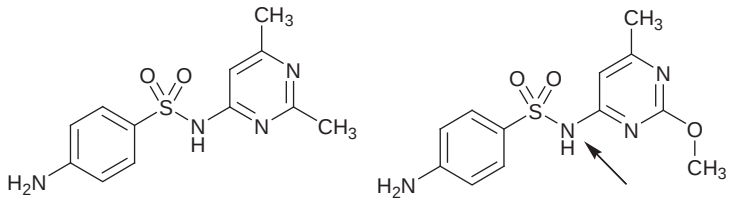


Figure 5.14 An example of structure–activity relationship for sulfonamide *N*-glucuronidation in sulfanilamides. The arrow indicates the site of glucuronidation in sulfamethomidine, which is not discerned with sulfasomidine.

metabolic profile between the two compounds is fairly intriguing and suggests difference(s) in CYP active site binding modes.

Besides valdecoxib, sulfanilamide anti-bacterial drugs are also prone to *N*-glucuronidation on the sulfonamide nitrogen. The degree to which *N*-glucuronidation occurs is highly dependent on the functional group attached to the sulfonamide nitrogen atom as well as the species under consideration. For example, the process of *N*-glucuronidation on the sulfonamide nitrogen in sulfamethomidine is not observed with sulfasomidine (Figure 5.14).[138] The major structural difference is that the pendant 2-methoxy-4-methylpyrimidine substituent in sulfamethomidine is replaced by the 2,4-dimethylpyrimidine motif in sulfasomidine. Furthermore, while sulfonamide glucuronidation constituted a major route of metabolism of sulfamethomidine in humans and primates, the *N*-glucuronide metabolite was not detected in rats and rabbits.[138]

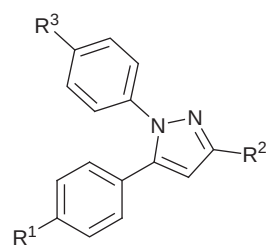
5.2.3.2 Role of CYP2C9 in the Oxidative Metabolism of Sulfonamides

Considering the bioisosteric relationship between sulfonamides and carboxylic acids, it is not surprising that the polymorphic CYP2C9 is involved in the oxidative metabolism of numerous sulfonamide-based compounds in a manner similar to that discerned with carboxylic acid drugs. Structural characteristics of prototypic CYP2C9 substrates include the presence of an anionic group and a hydrophobic zone between the substrate hydroxylation site and the anionic site. Several noteworthy examples of drugs from the antibacterial (*e.g.* sulfamethoxazole and dapsone), antidiabetic (*e.g.* sulfonylureas) and anti-inflammatory (*e.g.* celecoxib and valdecoxib) class of compounds have been reported to undergo CYP2C9-catalysed oxidation.[139–144] Consequently, genetic polymorphisms of CYP2C9 markedly affect the pharmacokinetic (and pharmacodynamic) attributes of the drugs[145–147] Like carboxylate-containing drugs, sulfonamide-based CYP2C9 substrates can be prone to pharmacokinetic interactions with inhibitors or inducers of this isozyme.[148–150]

The structural basis for CYP2C9-mediated oxidation of acidic substrates has been deduced *via* the solution of the crystal structure of this isozyme complexed with the anti-coagulant drug warfarin.[151] For the most part, acidic functionalities (*e.g.* carboxylic acid, sulfonamides, *etc.*) in drugs bind to a active site Arg^{108} residue, which then positions the molecule in proximity of the heme prosthetic group for subsequent oxidation. Using this basic binding mode, molecular docking and/or homology modelling studies have been used to elucidate the structural basis for CYP2C9 mediated metabolism of the sulfonylurea gliclazide.[152] However, oxidative metabolism of all sulfonamide (or for that matter carboxylic acid) derivatives cannot be simply rationalised by the hydrogen bond interaction between the anionic group and the arginine residue as illustrated with celecoxib. While molecular docking studies on celecoxib using the sulfonamide–arginine ion pair as a starting point is consistent with orientation of the *para*-tolyl group towards the heme iron for hydroxylation to occur, experimental studies on the metabolism of neutral derivatives of celecoxib suggest otherwise.[153]

As shown in Table 5.5, in an attempt to disrupt the sulfonamide-arginine interaction, the sulfonamide group of celecoxib was replaced with the corresponding methyl sulfide (**5.33**), methyl sulfoxide (**5.34**) and methyl sulfone (**5.35**), which represent different oxidation states on the sulfur atom. In contrast with the expectation that replacement of the sulfonamide with methylsulfone or methylsulfide would result in a lower propensity towards metabolism by recombinant CYP2C9, *in vitro* studies showed no significant decrease in the extent of metabolism for the sulfide or the sulfone derivative and only a modest decrease (~48% consumption of sulfoxide). Thus, removing apparently important interactions with the enzyme had only little to no influence on the

Table 5.5 SAR studies on sulfonamide replacements in celecoxib as CYP2C9 substrates.



Compound	*R^1*	*R^2*	*R^3*	*% Metabolised by CYP2C9 (1 μM)*
Celecoxib	$-CH_3$	$-CF_3$	$-SO_2NH_2$	94
5.33	$-CH_3$	$-CF_3$	$-SCH_3$	87
5.34	$-CH_3$	$-CF_3$	$-SOCH_3$	48
5.35	$-CH_3$	$-CF_3$	$-SO_2CH_3$	81
5.36	$-CH_3$	$-CF_3$	-COOH	29

metabolism of celecoxib. These results indicated that the hydrophobic core structure of celecoxib has a more significant impact on the total interaction with CYP2C9. What was even more surprising in this SAR exercise is the fact that replacement of the sulfonamide group in celecoxib with a carboxylic acid functionality (*e.g.* compound **5.36**) resulted in a decrease in CYP2C9 catalysed metabolism.

5.2.4 Renal Elimination

The process of secreting organic anions through the proximal tubule cells is achieved by unidirectional transcellular uptake of organic anions into the cells from the blood across the basolateral membrane, followed by extrusion across the brush–border membrane into the proximal tubule fluid. The process is catalysed by the human organic anion transporters (hOATs) 1, 2, 3 and 4.[154] OAT1, OAT2 and OAT3 are localised on the basolateral side of the proximal tubule, while OAT4 is localised on the apical domain of the proximal tubule.[155]

Thiazides and loop diuretics, which are widely used for the clinical management of hypertension and oedema, exhibit their diuretic effect by inhibiting Na^+–Cl^- and Na^+–K^+–$2Cl^-$ co-transporters at the distal tubule and loop of Henle, respectively.[156] Likewise, the CA inhibitor acetazolamide has a strong diuretic effect, although it is principally given for the treatment of glaucoma. It has been hypothesised that all of these compounds are subject to active transport by hOATs to their sites of action primarily based upon evidence from drug-drug interactions studies. Thus, in the case of diuretics, concomitant administration with probenecid, a potent inhibitor of OATs, significantly diminishes their renal clearance in animals and human.[157–159] Likewise, combined use of acetazolamide and the NSAID salicylic acid in humans has been shown to cause severe metabolic acidosis, implying that the agents compete for protein binding and that a common transport pathway mediates their tubular secretion.[160] *In vitro* studies using cell lines overexpressing hOAT isoforms have demonstrated the interaction with representative thiazides, loop diuretics and the CA inhibitor acetazolamide (Figure 5.15); hOAT1 exhibits the highest affinity for thiazides, whereas the affinity for loop diuretics was the greatest with hOAT3.[161] Detailed uptake and efflux studies with bumetanide suggested that the compound is taken up by hOAT1 and hOAT3, and is excreted in the urine by hOAT4.[162] Overall, the findings on the active OAT mediated transport of thiazides, loop diuretics and acetazolamide is consistent with their weakly acidic nature.

Most first and second generation sulfonylurea hypoglycemic agents are eliminated *via* metabolism.[163,164] Chlorpropamide and glisoxepide are the exceptions; while metabolism represents a significant component of elimination, $\sim$22–40 % of the administered doses of the compounds is renally excreted in the unchanged form.[163] However, the values for percentage of dose excreted in the unchanged form need to be interpreted with caution. For example, while the mean amount of chlorpropamide excreted unchanged in the urine is $\sim$22%,

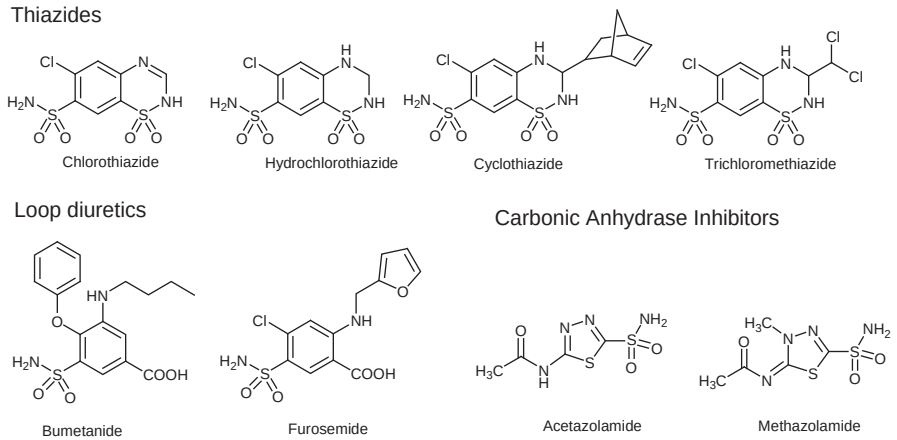


Figure 5.15 Sulfonamide-based substrates for the human renal OAT transporters.

a variation from 10–60% has been discerned in human volunteers.[162] From an SAR perspective, it is interesting that chlorpropamide but not tolbutamide is subject to renal excretion as parent. Noteworthy structural differences are the replacement of the chlorine atom in chlorpropamide with a methyl group in tolbutamide and extension of the terminal alkyl chain in chlorpropamide by one methylene in tolbutamide (see Table 5.4).

It is tempting to speculate that the difference in renal excretion pattern occurs because of the difference in plasma free fraction (range of chlorpropamide and tolbutamide bound to albumin is 57–87% and 86–99%, respectively) of the two drugs which, in turn, governs the process of passive filtration and tubular secretion.[165,166] The involvement of active renal transport of chlorpropamide by human OAT isoforms remains uncertain; although hOAT1 can be potentially ruled out on the basis of the findings that most sulfonylureas (including chlorpropamide and tolbutamide) are not substrates for the rat OAT1 transporter.[167]

Sulfonamide antibacterials are known to be eliminated by hepatic metabolism (*N*-acetylation and CYP oxidation of the N4 amine) as well as renal processes. Renal excretion (mostly likely transport mediated) appears to be dependent on the acidity of the sulfonamide group, which in turn is influenced by the heterocyclic substituent attached to the sulfonamide nitrogen.[168–171] This is illustrated with three sulfanilamide derivatives, sulfisomidine, sulfametomidine and sulfadimethoxine, respectively (Figure 5.16).[172] The rank order for renal clearance of the three compounds in humans is: sulfisomidine $>>>$ sulfametomidine $>$ sulfadimethoxine. Thus, replacement of the 2,6-dimethylpyrimidine group in sulfisomidine with 2-methyl-6-methoxy- or 2,6-dimethoxypyrimidine groups dramatically lowers renal clearance. Of interest is the observation that these functional group changes also significantly increase the half-life of sulfisomidine from 8 h to 28–35 h. It has been speculated that the difference in half-life may be

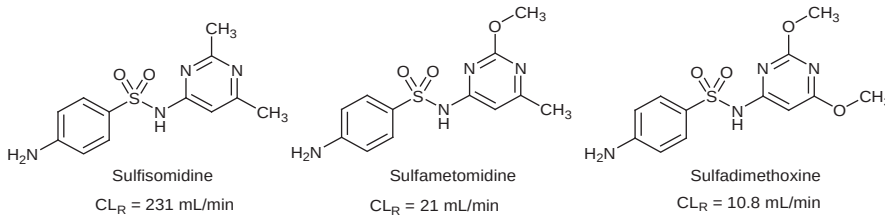


Figure 5.16 SAR for renal excretion of sulfanilamides.

due to a reduction in phase I/II metabolism, active renal secretion or by increased tubular reabsorption. The individual renal transporters that mediate renal transport of sulfanilamide remain largely unexplored.

Finally, human mass balance studies on sulfonamide-based coxibs (*e.g.* celecoxib and valdecoxib) indicate little to no renal excretion of the parent compounds.[114,116]

5.3 Adverse Drug Reactions (ADRs) with Sulfonamide Drugs

Sulfanilamide antibacterials have been implicated in causing hypersensitivity reactions. The most common manifestation of a hypersensitivity reaction is rash and hives. However, there are several life-threatening manifestations of hypersensitivity to sulfa drugs, including Stevens-Johnson syndrome (also referred to as toxic epidermal necrolysis), agranulocytosis, haemolytic anaemia, thrombocytopenia and fulminant hepatic necrosis, among others. Approximately 3% of the general population has ADRs when treated with sulfonamide antibacterials. Of note is the observation that patients with HIV have a much higher prevalence, at about 60%.[173]

5.3.1 Types of Hypersensitivity Reactions with Sulfanilamide Antibacterials

At least two types of ADRs have been described that are related to the sulfanilamide-type structure.[174,175]

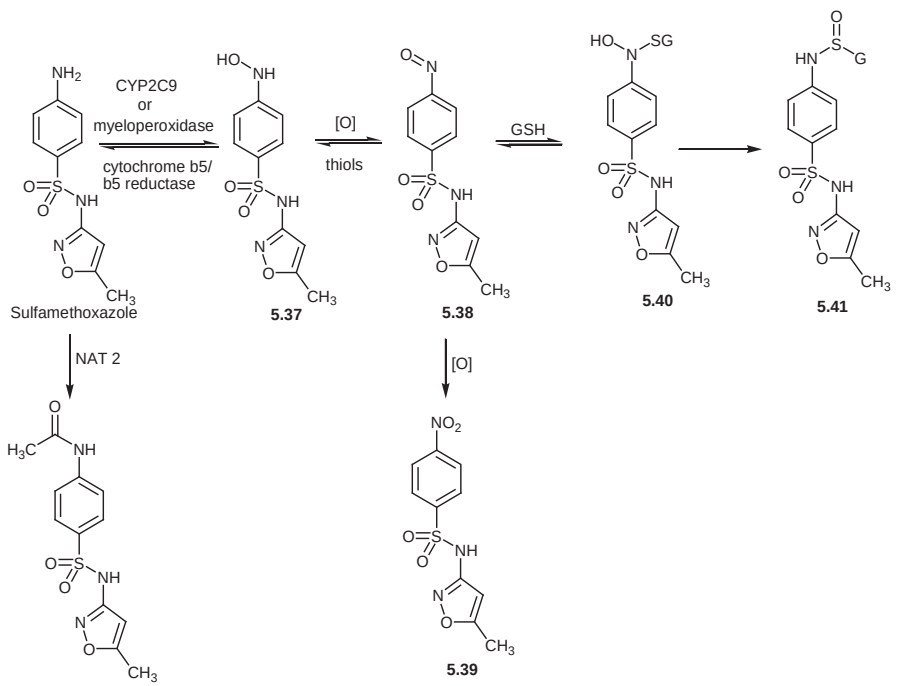
The first is a type 1 hypersensitivity reaction (immediate allergy) which is immunoglobulin (Ig) E mediated and presents most commonly as either a maculopapular eruption or an urticarial rash that develops within 1–3 days of initial medication and resolves spontaneously upon discontinuation of the drug. Anaphylaxis may develop upon repeated exposure. The heterocyclic ring system that is attached to the sulfonamide nitrogen is believed to be the immunological determinant of type 1 hypersensitivity response.[174,175]

The second is a type 2 hypersensitivity reaction that is cytotoxic and immune-mediated and initially presents as a fever and a non-urticarial rash that

may progress to erythema multiforme and multi-organ toxicity. The onset is delayed (usually 7–14 days) and resolves upon withdrawal of the medication. Type 2 hypersensitivity reactions are typically mediated by IgM and IgG antibodies.

5.3.2 Mechanism of Type 2 Hypersensitivity by Sulfonamide Antibacterials

Although the biochemical mechanisms involved in type 2 hypersensitivity associated sulfonamide antibacterials have not been fully elucidated, reactive metabolites, as opposed to the parent compounds, appear to play a pivotal role. The obligatory step in the bioactivation of aniline derivatives involves *N*-hydroxylation on the primary amine nitrogen leading to the formation of the hydroxylamine metabolite (Scheme 5.6).[176] Sulfamethoxazole hydroxylamine (**5.37**), a metabolite generated from the CYP2C9 and myeloperoxidase catalysed oxidation of sulfamethoxazole in humans[177] (and putative analogs thereof) are toxic to cells of the immune system *in vitro*.[178,179] Sulfamethoxazole metabolites are thought to play an additional role in the pathogenesis of hypersensitivity reactions because they have been shown to activate dendritic cells and to be immunogenic.[180,181] Once formed, hydroxylamine derivatives



Scheme 5.6 Bioactivation of the aniline motif in sulfonamide antibacterials—sulfamethoxazole as an example.

such as **5.37** can autoxidise to the corresponding nitroso intermediate (**5.38**). The nitroso metabolite of sulfamethoxazole and related anilines are unstable in solution and undergoes further oxidation to the stable nitro metabolite **5.39**.[182] The nitroso metabolite of sulfamethoxazole has been showed to react with glutathione *in vitro* to produce a semimercaptal intermediate **5.40**, which can rearrange to a stable sulfinamide **5.41** (Scheme 5.6).[183,184] Covalent adduction of the sulfamethoxazole nitroso metabolite **5.38** with cysteinyl residues in peptides and proteins has recently been demonstrated, which then implicates the potential for intracellular protein haptenisation by **5.38**.[185] Consistent with these overall findings, glutathione and related thiols have been shown to reduce toxicity and protein binding associated with sulfamethoxazole *in vitro*.[186,187] Furthermore, the finding that sulfamethoxazole and related compounds such as dapsone can also be oxidised in keratinocytes suggests that bioactivation and haptenisation may occur directly in the skin.[188] Support for this hypothesis is provided by the findings that covalent adduct formation by sulfamethoxazole hydroxylamine has been detected in human epidermal keratinocytes with protein complexes observed in the region of 160, 125, 95 and 57 kDa.[189]

5.3.2.1 Risk Factors Associated with Type 2 Hypersensitivity of Sulfonamides

Slow acetylation by the polymorphic *N*-acetyltransferase (NAT) 2 has been considered as a risk factor for hypersensitivity to sulfonamide antibacterials because the principal route of elimination of these drugs in humans involves *N*-acetylation of the aniline moiety by NAT2 resulting in the neutral amide metabolites (see Scheme 5.6).[190] In a NAT2-deficient population the aniline motif has the potential to undergo bioactivation *via* the *N*-hydroxylation route.

However, given the low incidence of type 2 hypersensitivity reactions to sulfonamide antibacterials (1 in 1000), slow acetylation cannot be the sole mechanism of predisposition in the population. Interindividual variability in the formation and detoxication of reactive metabolites of the sulfonamide antibacterials could be another possibility with regards to individual risks to type 2 hypersensitivity reactions, especially since the formation of the reactive intermediate precursor is mediated by the polymorphic CYP2C9 isozyme. However, the reason why certain individuals are susceptible to these adverse effects is at present still largely unknown, as the nature of the process from formation of the reactive metabolite and downstream consequences leading to toxicity remains largely undetermined.[191,192]

5.3.2.2 Are All Sulfonamides Created Alike?

It is commonly believed that people who have a hypersensitivity reaction to one member of the sulfonamide class of antibacterials are likely to have a similar reaction to other non-antibacterial sulfonamide-containing drugs. The clinical significance of cross-reactivity of medications in a patient with a 'sulfa allergy'

continues to perplex clinicians and complicates decisions regarding patient safety. Historically, the term 'sulfa' refers to a derivative of the antibacterial agent sulfanilamide. More recently, the term has been broadly applied to include numerous and chemically diverse set of drugs that contain the sulfonamide motif including NSAIDs such as celecoxib, valdecoxib and meloxicam. For instance, the package insert for the COX-2 inhibitor celecoxib states that '*celebrex should not be given to patients who have demonstrated allergic-type reactions to sulfonamides*'.

The potential of sulfonamide (*e.g.* celecoxib, valdecoxib and meloxicam) and methylsulfone (*e.g.* rofecoxib) derived NSAIDs to cause Steven-Johnson syndrome has been examined using the US Food and Drug Administration (FDA) adverse event reporting system (AERS). No instance of severe epidermal necrolysis has been reported for meloxicam, and incidences for the other three agents were depicted to be in the order valdecoxib > celecoxib > rofecoxib.[193]

There is no doubt that Steven-Johnson syndrome is a low-incidence ADR of most, if not all, NSAIDs, but the studies conducted to date have suggested that there is no association with the sulfonamide moiety. For instance, a meta-analysis has explored the incidence of allergic reactions associated with celecoxib in: [194]

- patients in North America;
- patients taking part in international arthritis trials;
- patients with a history of hypersensitivity reactions to sulfonamide antibacterials;
- patients receiving medications containing sulfonamide antibacterials.

Data from the 11 008 patients studied demonstrated that the incidence of hypersensitivity with celecoxib was statistically indifferent from that observed with placebo or active comparators (NSAIDs) when the entire cohort was examined. Although skin reactions occurred with greater frequency in patients with a history of sulfonamide functionality, the trend was consistent across all three treatment groups (celecoxib, NSAIDs and placebo), indicating a general patient susceptibility rather than a functional group-specific effect.

Furthermore, a prospective study assessed the incidence of cross-reactivity in 28 patients with a self-reported history of sulfonamide antibacterial allergy who then received celecoxib.[195] Sulfonamide allergy was assessed by either skin prick, intradermal testing, and/or lymphocyte toxicity assay (LTA) using sulfamethoxazole. Positive reactions occurred in four out of 28 patients administered a skin test and two out of 10 patients evaluated by LTA. Patients with a negative skin test received an oral challenge with sulfamethoxazole. All patients underwent a low- and high-dose oral challenge test with celecoxib without any adverse events. Follow-up in 25 patients showed that 15 (60%) continued to tolerate therapy with celecoxib, five did not take celecoxib after the oral challenge, and five discontinued celecoxib therapy secondary to adverse effects. In the six patients with a positive skin test, four continued to take celecoxib, one discontinued therapy secondary to gastrointestinal adverse

effects, and one did not take the drug after the oral challenge upon the advice of the primary care physician. The authors concluded that the risk for cross-reactivity of celecoxib in patients with a sulfonamide antibacterial allergy is low.

5.4 Bioactivation Pathways Involving the Sulfonamide Motif

There are several examples in bioorganic chemistry where the sulfonamide group has been shown to participate in reactive metabolite formation. In addition, there are instances of 2-sulfonamidoheterocycles, which owing to their electrophilic nature, undergo nucleophilic displacement reactions with endogenous nucleophiles such as glutathione either under chemical or enzymatic conditions.

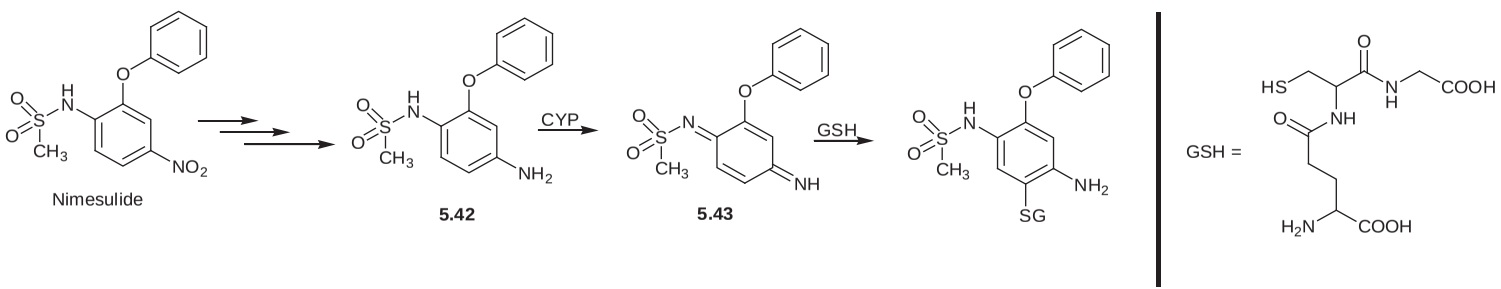
5.4.1 Bioactivation of Sulfonanilides

Reactive metabolite formation can occur *via* a two-electron oxidation process on aryl sulfonamides, which contain electron rich functionalities in an *ortho* and/or *para* framework relative to the sulfonamido group. Reactive intermediates derived from such bioactivation reactions can be generally categorised as quinone (quinone-imine, quinone-methide and diiminoquinones) derivatives that react with glutathione and/or protein nucleophiles in a 1,4-Michael fashion. The overall two-electron oxidation process with aryl sulfonamides resembles the one observed with the acetanilide derivative acetaminophen, which yields the electrophilic quinone-imine NAPQI.[196] The aryl sulfonamide-based selective COX-2 inhibitor nimesulide illustrates the concept.

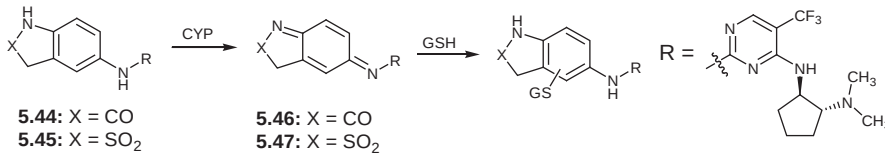
After widespread clinical use of nimesulide, hepatotoxicity—including both acute and hepatitis and the more severe fulminant hepatic failure—were reported.[197,198] The relatively high occurrence of these adverse events in Finland and Spain caused the drug to be withdrawn from the market in these countries. Circumstantial evidence linking reactive metabolite formation with idiosyncratic hepatotoxicity has been presented by Li *et al.*[199]

As shown in Scheme 5.7, the rate-limiting step in nimesulide bioactivation involves reduction of the nitrobenzene group to the corresponding electron-rich aniline metabolite **5.42**, which has been detected in human urine. Two-electron oxidation of **5.42** by CYP and/or peroxidase enzymes generates the reactive diiminoquinone species **5.43**, which adducts with sulfydryl nucleophiles or to human serum albumin.

Recent studies from our laboratory have shown a similar bioactivation pathway on 5-aminooxindole-and 5-aminobenzosultam-based inhibitors of proline rich tyrosine kinase (PYK2) exemplified by analogs **5.44** and **5.45**, respectively, which are metabolised by CYP3A4 to form electrophilic diiminoquinone species **5.46** and **5.47**, respectively, amenable to trapping with glutathione (Scheme 5.8).[200] Interestingly, 5-aminobenzosultam **5.45** was less



Scheme 5.7 Bioactivation of the aryl sulfonamide derivative nimesulide to reactive metabolites.



Scheme 5.8 Bioactivation of 5-aminooxindole and 5-aminobenzosultam derivatives to reactive diiminoquinones

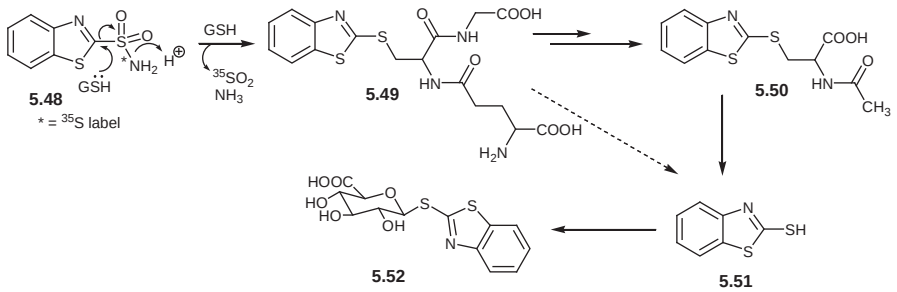
susceptible towards bioactivation than the 5-aminooxindole derivative **5.44** as judged from the dramatically lower peak area ratio of the glutathione conjugate of **5.45** (relative to the glutathione conjugate of **5.44**). Estimates of the relative rates of oxidation of the 5-aminooxindole and 5-aminobenzosultam fragments using theoretical quantum chemical calculations suggest that 5-aminobenzosultam fragment is more difficult to oxidise, with approximately $+4.5$ kcal mol^{-1} more energy required for oxidation of this component.[201]

5.4.2 Intrinsic Electrophilicity of 'Activated' Sulfonamides

The presence of the sulfonamido group in the α position of certain heterocyclic rings generates electrophiles that can react with glutathione. The phenomenon was first demonstrated with the carbonic anhydrase inhibitor benzothiazole-2-sulfonamide **5.48** (Scheme 5.9). Although **5.48** inhibits carbonic anhydrase enzyme(s) *in vitro* with potency greater than the traditional inhibitors of this enzymes (*e.g.* acetazolamide and methazolamide, see Figure 5.5), **5.48** is devoid of *in vivo* pharmacological activity in preclinical species even after administration of very high doses.

The complete absence of **5.48** in circulation and/or urine after administration of ^{35}S-radiolabeled **5.48** to rats provides a rational explanation for the *in vitro–in vivo* pharmacological disconnect. While $>80\%$ of the total radioactivity was eliminated in urine, none of the characterised metabolites (benzothiazole-2-mercapturic acid **5.50**, benzothiazole-2-mercaptan **5.51** and benzothiazole-2-mercaptoglucuronide **5.52**) were radioactive implicating the loss of the ^{35}S label (see Scheme 5.9).[202,203] The proposed mechanism, which collectively accounted for these observations, involves a *quantitative* nucleophilic displacement of the 2-sulfonamide group in **5.48** by the endogenous pool of glutathione in the mammal to first afford the sulfydryl conjugate **5.49**, $^{35}SO_2$ and ammonia. The demonstration that all the urinary radioactivity was due to inorganic sulfate confirms the nucleophilic displacement of the sulfonamide group by glutathione. Subsequent breakdown of **5.49** affords the mercapturic acid conjugate **5.50**. A carbon–sulfur bond cleavage in **5.49** (or **5.50**) can generate the mercaptan metabolite **5.51**, which can undergo *S*-glucuronidation to afford **5.52**.

A side-by-side comparison on the *in vitro* reactivity of acetazolamide, methazolamide and some 6-hydroxybenzothiazole-2-sulfonamide derivatives with glutathione at 37 °C (pH 7.4 buffer) revealed that acetazolamide and



Scheme 5.9 *In vivo* nucleophilic displacement of the sulfonamide group in benzothiazole-2-sulfonamide by glutathione.

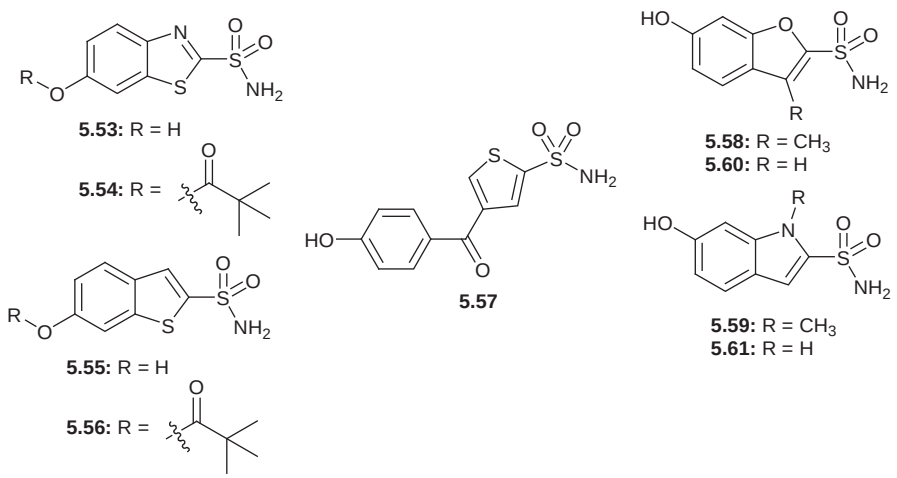


Figure 5.17 SAR studies on the *in vitro* reactivity of glutathione with 2-sulfonamido-heterocycles.

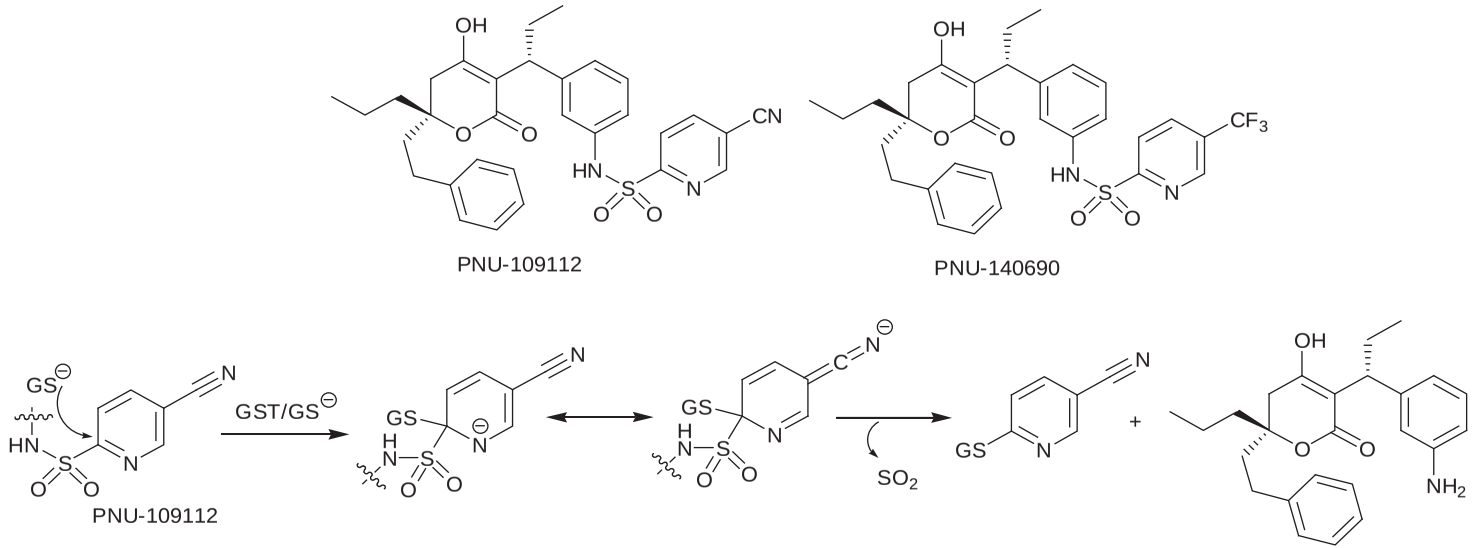
methazolamide possessed significantly diminished capability to undergo nucleophilic displacement with glutathione.[204] Likewise, the parent 6-hydroxybenzothiazole-2-sulfonamide **5.53** was the slowest to react with glutathione; conversion of the hydroxy group to lipophilic ester derivatives increased the rate of nucleophilic displacement by glutathione (Figure 5.17).[204] Interestingly, the lipophilic pivalic acid ester derivative of 6-hydroxybenzothiazole-2-sulfonamide **5.54** was shown to be a potent allergen in guinea pig model of dermal-sensitisation potential.[204] While the molecular basis for this adverse effect remains unclear, it is likely a nucleophilic displacement of the sulfonamide by protein nucleophiles in the skin can trigger the process of haptenisation leading to an immune response.

The finding that de-aza versions of **5.53** and **5.54**, *i.e.* 2-benzo[*b*]thiophene-sulfonamide analogs (*e.g.* compounds **5.55** and **5.56**, Figure 5.17), are

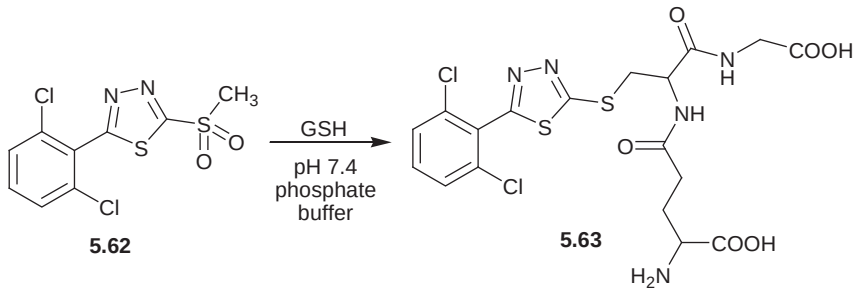
practically inert towards reaction with nucleophiles such as glutathione suggests that the electrophilic nature of benzothiazole-2-sulfonamide is derived from the presence of the *imino* group in the thiazole ring system.[205] Consistent with this general argument, thiophene-2-sulfonamide analogs (*e.g.* compound **5.57**, Figure 5.17) have also been shown to be latent towards reaction with glutathione.[206] Although benzofuran- and indole-2-sulfonamide derivatives (*e.g.* **5.58** and **5.59**), which do not contain the heterocyclic imine group, showed much lower levels of reactivity toward glutathione than benzothiazole-2-sulfonamides, they were measurably more electrophilic than the corresponding benzothiophene-2-sulfonamides.[207] Concurrent with these findings, several benzofuran- and indole-2-sulfonamide were noted to cause skin sensitisation.[207] In addition, the 3-methyl- and *N*-methyl-substituted derivatives of the benzofuran- and indolesulfonamides (*e.g.* compounds **5.58** and **5.59**) were appreciably more reactive towards glutathione than the desmethyl compounds **5.60** and **5.61**, respectively. Likewise, electron-donating substituents in benzofuran- and indolesulfonamides did not affect or accelerate the rate of displacement reaction. This is in contrast to the benzothiazole series, where a 6-hydroxy substituent markedly reduced the electrophilicity of the system.[204]

A final example of the *in vitro* and *in vivo* reactivity of glutathione with activated sulfonamides is evident with the HIV-1 protease inhibitor PNU-10912.[208] From an SAR perspective, PNU-140690 that is obtained by substituting the cyanopyridinyl group in PNU-10912 with a trifluoromethylpyridinyl moiety (Scheme 5.10), was shown to be stable towards reaction with glutathione *in vitro* and *in vivo* studies in animals.[208] Furthermore, these studies were the first to demonstrate the catalytic role of glutathione-*S*-transferase (GST) enzymes in the sulfonamide nucleophilic displacement reaction. Additional SAR studies on pyridine-2-sulfonamides further confirmed the requirement of an electrophilic centre (pyridinyl nitrogen) α to the sulfonyl group for reaction with glutathione.[209] Sulfonamide reaction with glutathione was not dependent on the substituents attached to the nitrogen, however, pyridine ring substituents markedly influenced the process. Thus, *ortho*- and *para*-pyridine ring substituents capable of withdrawing sufficient electron density from the carbon atom α to the sulfonyl group were an absolute requirement for the GST-mediated sulfonamide displacement. A proposed mechanism in which the role of electron-withdrawing groups in facilitating sulfonamide displacement by glutathione is shown in Scheme 5.10 for PNU-10912.

It is of great importance to note that the nucleophilic displacement reaction is not limited to sulfonamides; 2-alkylsulfonylheterocyclic compounds also succumb to this pathway as exemplified with the 2-methylsulfonylthiadiazole derivative **5.62**. The compound is a leptin receptor agonist *in vitro* but does demonstrate any pharmacology (spontaneous reduction of food intake) *in vivo* in rats or mice, a phenomenon which appears to be related to the lack of systemic drug levels following intravenous and oral administration of **5.62** to rodents. In phosphate buffer in the presence of glutathione, **5.62** was shown to be unstable and underwent quantitative conversion to the glutathione



Scheme 5.10 SAR and mechanistic studies on the glutathione mediated nucleophilic displacement of pyridinyl-2-sulfonamides.



Scheme 5.11 Nucleophilic displacement of a 2-methylsulfonylthiadiazole derivative by glutathione.

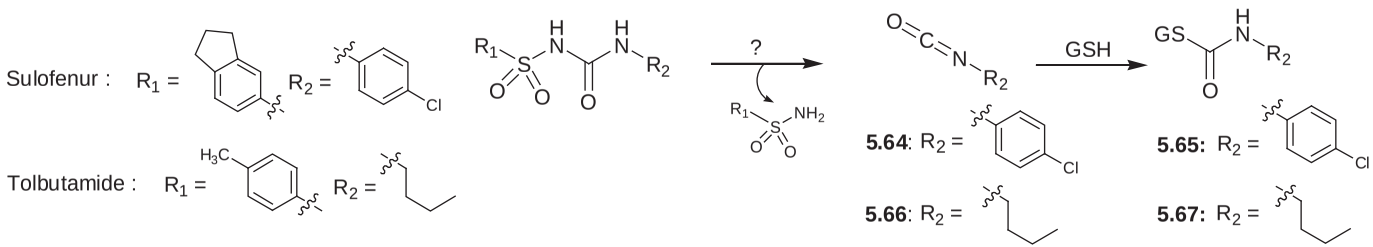
conjugate **5.63**, which is obtained *via* nucleophilic displacement of the methylsulfone group in **5.62** (A. S. Kalgutkar, unpublished findings) (Scheme 5.11). Consistent with the *in vitro* observations, rat plasma sample analysis after intravenous or oral administration of **5.62** indicated the presence of **5.63** as the exclusive component in the biological matrix.

5.4.3 Bioactivation of Sulfonylureas

Although diarylsulfonylureas such as sulofenur have demonstrated utility as antitumor agents against a wide range of cancers, dose-limiting toxicities in humans (including methemoglobinemia and haemolytic anaemia), have hampered wide usage.[210] In paediatric patients with refractory malignant solid tumors, methemoglobinemia was observed at all doses during a phase I clinical trial.[211] Likewise, the antidiabetes attributes of tolbutamide has been offset by its teratogenic properties in several animal species and humans, and its use is contraindicated in pregnant diabetic patients.[212] In both cases, circumstantial evidence has been presented which links toxicity with bioactivation of the sulfonylurea motif to protein-reactive metabolites. Following *in vivo* administration of sulofenur to rats, Jochheim *et al.*[213] characterised a novel glutathione conjugate **5.65** derived from conjugation of the thiol nucleophile with *p*-chlorophenylisocyanate (**5.64**) (Scheme 5.12). Similarly, analysis of rat bile following administration of tolbutamide led to the identification of *S*-(n-butylcarbamoyl)glutathione (**5.67**), a glutathione conjugate derived from the reaction with *n*-butylisocyanate (**5.66**) (see Scheme 5.12).[214]

The biochemical mechanism(s) for sulfonylurea decomposition to the corresponding isocyanates remains unclear since sulfonylureas have been found to be stable towards decomposition at physiological pH and increasing temperature. Likewise, no glutathione–isocyanate conjugate formation is discernible in incubations of the parent sulfonylureas and glutathione under chemical or biochemical (GST catalysis) conditions.

Finally, it is interesting to note that the isocyanate and corresponding glutathione conjugate, derived from tolbutamide metabolism, are time-dependent



Scheme 5.12 Bioactivation of sulfonylureas to reactive isocyanate intermediates.

inactivators of glutathione reductase (GR) enzyme that presumably cause inactivation *via* protein carbamoylation.[213,214] It has been speculated that the teratogenic effects of tolbutamide in rats could be linked to tolbutamide-mediated depletion of glutathione through inhibition of GR in rat embryos.[214] GR catalyses the reduction of the oxidised disulfide form of glutathione to glutathione and thus maintains intracellular levels of the thiol antioxidant in the embryo, which is critical for cell viability and normal growth. Glutathione depletion by exogenous compounds has been reported not only to enhance the embryo-lethal, teratogenic and growth-retarding properties of these chemicals, but also to cause an increase in the number of dead and malformed rat embryos *in vivo*.[215]

5.5 Conclusions

The juxtaposition of terminologies around the sulfonamide-containing drugs and sulfonamide drugs can cause confusion around the issue of hypersensitivity reactions (sulfa allergy). Historically, the term 'sulfa' refers to a derivative of the antibacterial agent sulfanilamide. More recently, the term has been applied generously to a diverse group of drugs, all of which contain the sulfonamide motif. This is largely for historic reasons, as many of the drugs were approved prior to any toxicity mechanism investigations. In addition, some non-antibacterial sulfonamides (*e.g.* sulfonylureas and loop diuretics) are classified as derivatives of the antibacterials because their discovery has been influenced in large part by the polypharmacology of the original sulfa drugs.

The sulfa allergy association raises concerns for medicinal chemistry and the use of the sulfonamide group in drug discovery. However, to date, a thorough examination of the literature does not support the dogma of sulfonamide antibacterial cross reactivity with non-antibacterial sulfonamides. In fact, rigorous studies examining cross reactivity between sulfonamide antibiotics and sulfonamide non-antibiotics suggest that predisposition to allergic reactions is the primary risk factor for the association, not a true cross reactivity.[95,194]

In retrospect, the sulfonamide group should remain an essential part of the medicinal chemist's arsenal in the search of new chemical entities with pharmacological action. There are numerous examples of blockbuster drugs which contain this motif and yet are not associated with any significant incidence of sulfa allergy type adverse drug reactions. In the current climate, which is focused on a heightened awareness of toxicophores (structural alerts) and toxicity, it is recommended that the sulfonamide motif should not be treated as a toxicophore, although appropriate caution needs to be exercised in terms of novel bioactivation pathways involving this functional group.

Finally, the finding that metabolic elimination of many acidic sulfonamide derivatives is principally mediated by CYP2C9 needs to be taken into consideration from a drug design perspective, given the polymorphic expression of this isozyme in humans and its contribution towards variability in pharmacokinetic and pharmacodynamic response.

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CHAPTER 6

Influence of Aromatic Rings on ADME Properties of Drugs

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6.1 Introduction

Aromatic rings are commonly found in most drugs. Historically, these rings have been a classic structural feature of drugs ever since the discovery of aspirin in the late 1800s. Since then several drug molecules that span all therapeutic areas have been designed and contain a combination of these rings along with other side chains and functional groups. The best selling drugs shown in Figure 6.1, as well as other marketed drugs, contain at least one aromatic ring system.

Carbocyclic six-membered aromatic rings are widely present in both natural products and endogenous ligands, and are therefore commonly incorporated by medicinal chemists into new chemical entities. Aromatic rings are also preferred replacements of linear and branched alkyl/cycloalkyl groups. Such a replacement not only imparts greater rigidity to the molecule but can potentially increase the non-covalent interactions between the macromolecule and the small molecules. These interactions generally involve aromatic amino acid side chains of the receptor and/or aromatic and heteroaromatic rings of the ligand. Analysis of the ligand–protein complexes reveals that aromatic rings are

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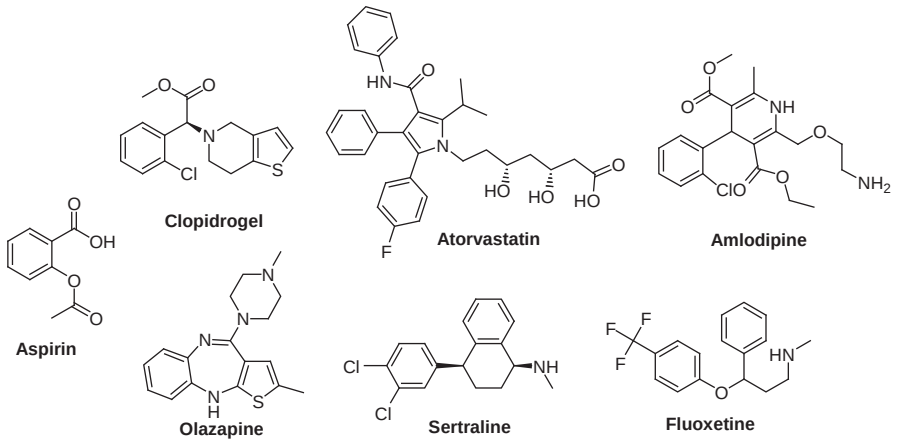


Figure 6.1 Some drugs containing aromatic rings.

primary involved in π–π stacking, O–H/π, and cation–π interactions.[1] From a drug design perspective, aromatic rings and their functionalised counterparts can be considered as versatile pharmacophores that increase these individual non-bonding and electrostatic interactions between the ligand and macromolecules. Hence, incorporation of these rings into a molecule can improve the binding affinity and therefore the potency of newly synthesised analogs relative to their aliphatic or alicyclic ring congeners.

The presence of an aromatic ring in a molecule also provides a platform for functionalisation of a compound. It serves as a core that helps to orient other groups in a structure in the right direction and therefore enhance the interactions with functionalities in the receptor. This chapter reviews the influence of phenyl and substituted phenyl rings on the adsorption, distribution, metabolism and excretion (ADME) properties of the molecules. Wherever possible, calculated physicochemical properties (clogP or clogD) are used to illustrate the effect of the aromatic substituent on ADME properties.

6.2 Physicochemical Properties of Aromatic and Substituted Aromatic Rings

The most basic structure in aromatic rings is the benzene ring (or a phenyl group). It is essentially a fully unsaturated analog of a cyclohexyl ring with a hydrogen to carbon ratio equal to one (lower than most other rings and functionalities). In spite of its low H : C ratio, these rings confer exceptional chemical stability compared with simple unsaturated congeners (*e.g.* the cycloalkene or the open chain hexatriene). The high thermodynamic and chemical stability of this system is attributed to the delocalisation of p-orbitals containing 6π-electrons (resonance stabilisation of a conjugated cyclic triene).

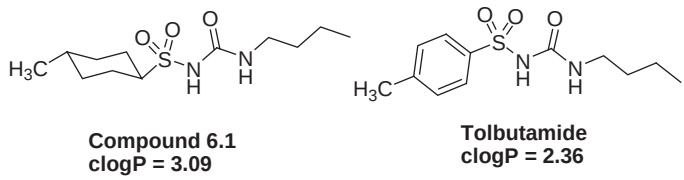


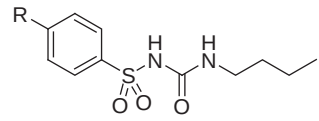
Figure 6.2 Comparison of cLogP of alicylic and aromatic analogs.

The phenyl ring is a neutral moiety and therefore its presence in a molecule does not affect its pKa. Further, in the absence of any hydrogen bonding capabilities of the phenyl ring, the inclusion of this group in a molecule does not have an effect on its polar surface area (PSA). However, addition of a phenyl ring into a molecule addresses another important physicochemical property such as lipophilicity and therefore influences the absorption, distribution and clearance (excretion and metabolism) properties of a compound. Although less lipophilic relative to cyclohexane ring (clogP of cyclohexane is 3.16 and clogP of benzene is 2.18), the clogP of a phenyl ring is still sufficiently high to change the lipophilicity of the whole molecule. For example, a hypothetical methylcyclohexyl analog of tolbutamide (compound **6.1**) has a clogP of 3.09 while tolbutamide has a clogP of 2.36 (Figure 6.2).

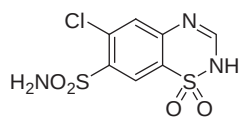
Addition of a substituent or a functional group in an aromatic ring in place of hydrogen atom can significantly perturb the electronic, steric, hydrophobic, hydrophilic and hydrogen-bonding parameters of the ring depending on the characteristics of that group. These changes can affect the interactions of the compound with the receptors and therefore alter its biological activity. For example, the replacement of an aniline group in carbutamide with a tolyl group in tolbutamide results in separation of the antibacterial effect from the antidiabetic effect (Figure 6.3).[2] Similarly chlorothiazide is an antihypertensive agent with a strong diuretic effect. Replacement of the sulphonamide moiety in chlorothiazide with hydrogen atom results in an antihypertensive without the diuretic activity (Figure 6.3).[2]

An isosteric change of a functional group or a substituent on a phenyl ring has a major effect on the lipophilicity of the molecule and can therefore influence the disposition (absorption, distribution or excretion) of compounds. In addition to lipophilicity, the change in polarity brought about by perturbation of electron distribution of the aromatic ring due to inclusion of functional groups can also influence the metabolism of the compound in the body.

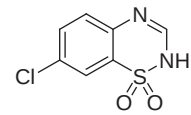
Hammett was the first to explore the effect of substituents on reactivity of the phenyl ring.[3,4] He demonstrated that both the inductive and resonance effect of a substituent could affect the electron density of the ring. Based on these studies, an electronic parameter, (σ), also called as the Hammett constant, was assigned to each substituent. The σ value for each substituent is dependent on the electronic property of that substituent (electron withdrawing or electron donating) and the position of the substituent on the ring (*para* or *meta* relative to other group). The more electron withdrawing a substituent, the more positive is its



R = NH_2; **Carbutamide**
R = CH_3; **Tolbutamide**



Chlorothiazide



Diazoxide

Figure 6.3 Effect of functional group modification on the biological activity.

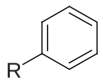
σ value (relative to H, which is set at 0); conversely, the more electron donating, the more negative is its σ value. Table 6.1 shows the Hammett constants (σ_{meta} and σ_{para}) for some commonly used groups. A list of Hammett constants for several groups is given in the review by Hansch and co-workers.[5]

The Hammett relationship does hold true for the substituents at the *ortho* position where it is complicated due to steric interactions and polar effects. As the substituent becomes more electron withdrawing, it potentially reduces the electron density of the phenyl ring, making the ring less reactive. In contrast, the electron donating substituents enhance the reactivity of the ring. Thus, substitution of the phenyl ring with a cyano or the nitro group deactivates the phenyl ring and makes it less susceptible to oxidative metabolism, while replacement of hydrogen atom with substituents such as the methyl group can make the phenyl ring more prone to P450 catalysed oxidation.

Like the substituent constants derived by Hammett, Hansch and co-workers derived constants for the contribution of individual functional groups to the partition coefficient of the phenyl ring (and eventually to the logP of the overall molecule).[6,7] The lipophilicity substitution constant (π), dictates the influence of a particular substituent on the partition coefficient (clogP) of the compound. The positive value indicates that the substituent makes the phenyl ring more lipophilic and therefore increases the logP of the molecule. Likewise, the negative value indicates that the substituent makes the molecule more polar by decreasing its lipophilicity. Table 6.1 also shows the π values for various substituents that are commonly incorporated in molecules. A comprehensive listing of all π values for various substituents is depicted in the review by Hansch and co-workers.[5]

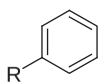
The third important parameter which addresses the interaction of a drug with the receptor or an enzyme is the steric effect. The most widely used parameter for steric substituent effects is the steric parameter, E_s. This was defined by Taft in much the same way as the Hammett's electronic effects, but was standardised to the methyl group ($E_{s\ methyl} = 0.0$) as opposed to the hydrogen by Hammett.[8,9] While the Hammett equation accounts for how field,

Table 6.1 Aromatic substituent constants of some commonly used functional groups



R	*Formula*	π	σ_p	σ_m	*Molar refraction (MR)*	*No. of hydrogen bonddonors*	*No. of hydrogen bond acceptors*
H	Hydrogen	0.00	0.00	0.00	1.03	0	0
F	Fluoro	0.14	0.06	0.34	0.92	0	0
Cl	Chloro	0.71	0.23	0.37	6.03	0	0
OH	Hydroxy	−0.67	−0.37	0.12	2.85	1	1
OCH_3	Methoxy	−0.02	−0.27	0.12	7.87	0	1
SH	Mercapto	0.39	0.15	0.25	9.22	1	0
SCH_3	Methylmercapto	0.61	0.00	0.15	13.82	0	0
$SOCH_3$	Sulfoxide	−1.58	0.49	0.52	13.70	0	1
SO_2CH_3	Methylsulfone	−1.63	0.72	0.60	13.49	0	1
NO_2	Nitro	−0.28	0.78	0.71	7.36	0	1
NH_2	Amino	−1.23	−0.66	−0.16	5.42	1	1
$NHCH_3$	Methylamino	−0.47	−0.84	−0.30	10.33	1	1
$N(CH_3)_2$	Dimethylamino	0.18	−0.83	−0.15	15.55	0	1
$NHSO_2CH_3$	Methylsulfonamido	−1.18	0.03	0.20	18.18	1	1
COOH	Carboxy	−0.32	0.45	0.37	6.93	0	1
$COOCH_3$	Methylcarboxylate	−0.01	0.45	0.37	12.87	0	1
$OCOCH_3$	Acetate	−0.64	0.31	0.39	12.47	0	1
$CONH_2$	Carboxamido	−1.49	0.36	0.28	9.81	1	1
CN	Cyano	−0.57	0.66	0.56	6.33	0	1
$COCH_3$	Methylketone	−0.55	0.50	0.38	11.18	0	1

Table 6.1 (*continued*)



R	*Formula*	π	σ_p	σ_m	*Molar refraction (MR)*	*No. of hydrogen bonddonors*	*No. of hydrogen bond acceptors*
	Phenyl	1.96	−0.01	0.06	25.36	0	0
C≡CH	Ethynyl	0.40	0.23	0.21	9.55	0	0
$HC{=}CH_2$	Ethenyl	0.82	−0.02	0.05	10.99	0	0
CH_2CH_3	Ethyl	1.02	−0.15	0.07	10.30	0	0
CH_3	Methyl	0.56	−0.17	−0.07	5.65	0	0
	$CH_2CH_2CH_3$	n-Propyl	1.55	−0.13	−0.07	14.96	0
0							
$CH(CH_3)_2$	Isopropyl	1.53	−0.15	−0.07	14.96	0	0

$(CH_2)_3CH_3$	n-Butyl	−0.02	−0.27	0.12	7.87	0	0
	$CH_2CH(CH_3)_2$	iso-Butyl	2.05	−0.12		19.59	0
0							
	$CH(CH_3)C_2H_5$	sec-Butyl	2.04	−0.12		19.59	0
0							
$C(CH_3)_3$	tert-Butyl	1.98	−0.20	−0.10	19.62	0	0
SO_2NH_2	Sulfonamido	−1.82	0.57	0.46	12.28	1	1
CF_3	Trifluoromethyl	0.88	0.54	0.43	5.02	0	0
OCF_3	Trifluoromethoxy	1.04	0.35	0.38	7.86	0	1
SO_2CF_3	Trifluoromethyl-sulfone	0.55	0.93	0.79	12.86	0	1
CF_2CF_3	Pentafluoroethyl	1.68	0.52	0.47	9.23	0	0
CH_2CN	Cyanomethyl	−0.57	0.01	0.16	10.11	0	1
CH_2OH	Hydroxymethyl	−1.03	0.00	0.00	7.19	1	1
$NHCONH_2$	Urea	−1.30	−0.24	−0.03	13.72	1	1
$NHCSNH_2$	Thiourea	−1.40	0.22	0.16	22.19	1	1
$NHCOCH_3$	Acetamido	−0.97	0.0	0.21	14.93	1	1
$NHCO_2CH_3$	Methylcarbamate	−0.37	−0.15	0.07	16.53	1	1
CH_2OCH_3	Methoxymethyl	−0.78	0.03	0.02	12.07	0	1
	Cyclopropyl	1.14	−0.21	−0.07	13.53	0	0

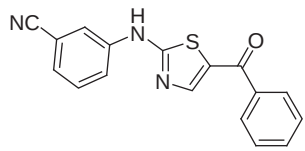
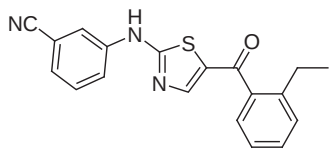
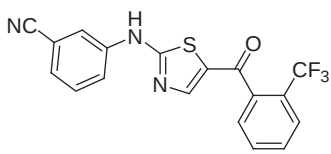
inductive and resonance effects influence reaction rates, the Taft equation also describes the steric effects of a substituent. This parameter is useful for studying intramolecular steric effects.

However, since E_s constants have not been determined for the majority of substituents and since biochemical–biomedical 'steric' requirements are often of the 'bulk' type, two parameters readily available for each substituent; molar refraction (MR) and molecular weight (MW) are commonly employed to understand the biological quantitative structure–activity relationship (QSAR). The molar refraction values not only address the steric bulk of substituent but also measure the electronic effects and therefore the dipole–dipole interactions at the active site.[10] The greater the MR value of a substituent, the larger is its steric or bulk effect. Table 6.1 also gives MR values for some commonly used substituents; a comprehensive listing of values for all substituents is presented by Hansch and co-workers.[10]

Several other parameters have been described to assess the QSAR of compounds. However, σ, π and molecular weight are the three parameters commonly used in practice by most medicinal chemists to modulate the overall biological and ADME properties of a molecule.

The following example illustrates the concept of the influence of substitution of a phenyl ring or interchange of a functional group on lipophilicity and solubility. Replacement of a hydrogen atom in synthesis of NPY5 receptor antagonists[11] **6.2** with an ethyl in compound **6.3** or a CF_3 group in compound **6.4** results in the change of clogP and calculated solubility (Table 6.2). This in

Table 6.2 Effect of substitution on the lipophilicity and solubility.

No.	*Structure*	*cLogP*	*cSolubility (mg/mL)*
6.2		2.43	0.01
6.3		3.51	0.002
6.4		4.90	0.0001

turn can affect the absorption and permeability of the molecule, and also affect the distribution characteristics of the compound. The introduction of a CF_3 group on the phenyl ring can possibly make the phenyl ring less reactive and therefore less susceptible to P450 mediated oxidative metabolism.

6.2.1 Importance of Fluorine Substitution on Phenyl Rings

Fluorine group is the most commonly used functional group in the recent years.[12–15] Approximately 5–15% of the launched drugs contain a fluoro group as a part of their structure. Some of the top-selling fluorinated pharmaceuticals include the antidepressant fluoxetine (which contains fluorines in the form of a CF_3 group), the cholesterol-lowering drug atorvastatin (Figure 6.1).

Inclusion of a fluorine atom in a drug molecule can, in theory, influence both the disposition of the drug and the interaction of the drug with its pharmacological target; hence it deserves a special mention with respect to impact and influence on the physicochemical properties of a molecule.

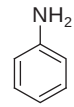
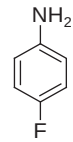
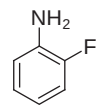
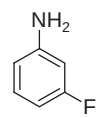
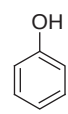
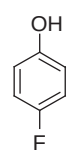
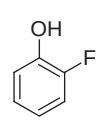
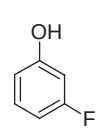
The electronic properties of fluorine make it a unique atom. It is both comparable to hydrogen and the most highly electronegative element in the periodic table(3.98 Pauling scale). Even though it is considered as an isostere of hydrogen, the size and stereoelectronic influences of the two atoms are quite different. The van der Waal radius of fluorine is larger than hydrogen and (1.47 *versus* 1.20Å) and much closer to that of oxygen (1.52Å), as is its electronegativity (3.98 for F *versus* 3.44 for O).[16] The C–F bond length (1.39Å) is also in the same range as the C–O bond length (1.40Å), but much bigger than C–H (1.09Å).[17,18]

When fluorine is substituted for hydrogen in aryl ring, the resulting change in the electron distribution affects the pKa, dipole moments, and overall reactivity and stability of neighbouring functional groups within the molecule.[14,15] For instance, replacement of a hydrogen atom with one fluorine group can affect the basicity of amines including aromatic amines (aniline) and the acidity of the phenol.[18] The magnitude of the change in the pKa depends on the distance between the fluorine atom and the functional group (Table 6.3). Thus, the presence of a fluorine atom *ortho* to a phenolic group or the amine is associated with a reduced pKa, whereas *meta* fluoro substitution has much less effect in both rings.

In terms of lipophilicity, H/F exchange leads to a more lipophilic molecule. Whereas fluorination of alkanes will actually decrease lipophilicity, the addition of fluorine group on an aryl ring increases lipophilicity.[14] Although the value of clogP may differ depending upon the substitution of fluorine at the *ortho*, *para* or *meta* position, there is an overall increase in the clogD as depicted in Table 6.3.

Due to effect of fluorine on the lipophilicity and pKa, this group can influence a number of different parameters in lead optimisation including solubility and binding affinities (potency, selectivity), and therefore the absorption and distribution properties of a drug. Enhanced lipophilicity due to the replacement of

Table 6.3 Effect of fluorine on aniline and phenol.

Substrate	*Structure*	*pKa*	*cLogD$_{7.4}$*
Aniline		4.61	1.14
p-Fluoroaniline		4.66	1.2
o-Fluoroaniline		3.2	1.47
m-fluoroaniline		3.6	1.47
Phenol		9.86	1.54
p-Fluorophenol		9.92	1.84
o-Fluorophenol		8.71	1.82
m-Fluorophenol		8.97	2.06

hydrogen with a fluoro group can potentially favour the membrane penetration and therefore an increased absorption and improved blood–brain barrier penetration of a compound.

Fluorine also forms a strong bond with carbon. The C–F bond energy is higher than the C–H or C–O bond (116, 99 and 85 $kcal^{-1}$, respectively).[18] This in turn increases the thermal and oxidative stability of the ring and makes the C–F bond less sensitive to metabolic degradation.[18] The electronic change also makes the overall ring less reactive to P450 mediated oxidation.

6.3 Influence of Aromatic and Substituted Aromatic Rings on ADME Properties of Compounds

As discussed earlier, incorporation of phenyl and substituted phenyl rings into drugs can affect their physical properties and hence influence their pattern on the disposition and toxicity. The following sections illustrate the influence of these aromatic rings on the absorption, distribution and clearance (metabolism and excretion) of drugs.

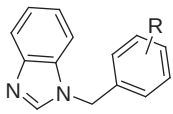
6.3.1 Absorption

A majority of drugs are administered orally. Two main factors influencing intestinal absorption are the solubility of a compound in the gastrointestinal fluid and its permeability through the gastrointestinal wall.[19,20] Both these parameters are dependent on several physicochemical properties of a molecule such the molecular size, lipophilicity, polar surface area (PSA) and hydrogen bond donating and accepting capacity of the polar atoms.[21] Although molecular size impacts the transport of molecules through biological membranes, lipophilicity has been proven to be the most important parameter affecting both the permeability and aqueous solubility of compounds.

The logP value of a molecule primarily governs its ability to partition into biological membranes. Since addition of a phenyl ring to a molecule increases its lipophilicity, it can have a positive influence on the membrane permeability of the compound. Modulation of the log P/log D value of a compound by adding a substituent on the ring will further enhance or reduce its absorption. Substituents such as alkyl and trifluoromethyl groups or the halogen atoms (Cl or F), which commonly increase the logP value of a molecule, can increase its membrane permeability. Alternatively, inclusion of the polar groups such as carboxy, carboxamido, cyano groups (which have a negative π value) can negatively influence the permeation of the compound through the gastrointestinal membrane.

Fichert and co-workers have explored structure–permeability relationships, particularly the effect on cell permeability of some commonly used functional groups attached to the drug-like core structures in lead optimisation efforts

Table 6.4 Effect of commonly used functional groups on cell permeability.[22]



	$P_{Caco-2} \times 10^{-6}$ cm/sec	Log D
H	71.1	2.91
*m*CN	65.8	2.47
PCO_2H	3.19	−0.61
PSO_2NH_2	23.4	1.04
pSO_3H	0.08	−1.78
mNH_2	57.4	1.92
m$NHSO_2CH_3$	23.4	2.08
m$CONH_2$	25.1	1.70
mCO_2H	1.56	−0.72
mCH_2NH_2	27.6	0.21

(Table 6.4).[22] For this set of compounds, those with log D values within the range >0 to <3 showed high permeability, while compounds with log D values <0 display variable permeability.

For simple drugs like β-adrenoceptor antagonists, the log $D_{7.4}$ values are remarkably predictive of the absorption potential. All these compounds have a molecular weight that ranges from 250 to 310; however, their log D values differ depending on the substituents on the phenyl ring. As seen in Table 6.5, incorporation of the cyclohexane diol or the methyl carboxamido group in the molecule results in a negative clog$D_{7.4}$ value, which in turn affects the absorption of these two drugs through the gastrointestinal tract. On the other hand, introduction of the alkyl or phenyl group in betaxolol and propranolol increases the lipophilicity of these compounds, which results in their 100% absorption following oral administration[23](Table 6.5)

Aqueous solubility dictates the amount of drug in solution and hence the amount of drug available for absorption from the gastrointestinal tract. Compounds with low solubility may suffer from dissolution-limited absorption. Although an increase in lipophilicity enhances the permeability, a higher logP value has a deleterious effect on the aqueous solubility of compounds. In general, aqueous solubility is inversely proportional to lipophilicity, and compounds with a high log$D_{7.4}$ value (>3) may show poor dissolution and resultant poor bioavailability. For instance, promazine (Figure 6.4) has been classified as a high permeability high solubility drug in the biopharmaceutics classifications system while chlorpromazine belongs to high permeability-low solubility class of compounds.[24] The only difference in the two compounds is the replacement of one of the hydrogen atoms in promazine with a chloro group in chlorpromazine (Figure 6.4). This subtle change in chlorpromazine results high permeability but low aqueous solubility (Figure 6.4). The effect on solubility is probably due to the increase in the clogP of chlorpromazine relative to promazine.

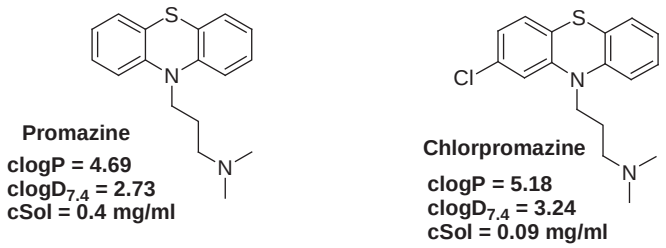


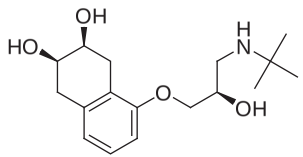
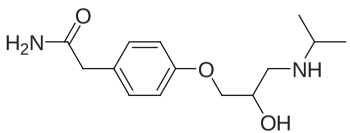
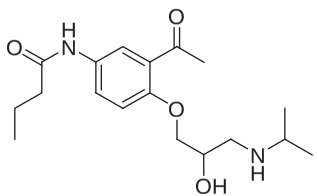
Figure 6.4 Structures and physicochemical properties of promazine and chlorpromazine.

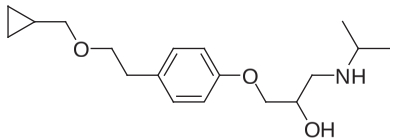
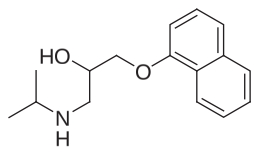
Even though incorporation of polar groups increases the aqueous solubility of compounds, the increased hydrogen bonding capabilities and therefore the increased polar surface area of these groups can affect the transfer of compounds across the gastrointestinal membrane.[21] Desolvation and the breaking of hydrogen bonds becomes the rate-limiting step to transfer across the membrane. Thus, in order optimise the absorption of a compound, it is important to strike a balance between the hydrophilic and lipophilic parameters of a compound.

The impact of hydrophilic substituents on absorption can be observed by comparing the pharmacokinetics of two D_2-type receptor antagonists, sulpiride and remoxipride (Table 6.6). Sulpiride is a sulfonamide derivative which is incompletely absorbed through the gastrointestinal tract and has a bioavailability of only 27%.[25] Remoxipride on the other hand is well absorbed (bioavailability of 90%) and is comparable to other standard neuroleptics.[26] Comparison of the physicochemical properties of these two drugs indicates that sulpiride has a negative value ($logD_{7.4}$ -1.07) and high PSA (110.1 $Å^2$) which is due to its increased number of hydrogen bond donors and acceptors. On the other hand, remoxipride has a $logD_{7.4}$ of 0.38 and a PSA of 50.8 $Å^2$ (Table 6.6). Hence the low absorption of sulpiride is attributed to its high polarity in addition to low lipophilicity.

The drug efflux transporter P-glycoprotein (P-gp), which is known to confer multidrug resistance in cancer chemotherapy, is also expressed in the apical surface of intestinal epithelial cells.[27] Hence, it can play an important role in oral absorption of a drug. Although numerous drugs have been identified as P-gp transporter substrates, no clear structure–activity relationships with respect to the influence of phenyl and substituted phenyl rings can be drawn. Based on clinical and animal studies with P-gp modulators, it has become apparent that the effect of P-gp on drug absorption is not as important as generally believed. It is important to note that, regardless of whether a compound is a substrate for P-gp or not, a drug molecule has to passively gain intracellular access prior to being acted upon by this transporter. Overall, this makes permeability and solubility the most important factors for intestinal absorption, as described above.

Table 6.5 Physicochemical properties of representative beta-blockers and its influence on absorption.

Name	*Structure*	*MW*	*cLogP*	*clogD$_{7.4}$*	*PSA*	*% Absorbed*[23]
Nadolol		309	0.56	−2.1	81.95	30
Atenolol		266	0.33	−1.76	84.5	50
Acebutolol		336	1.77	−0.3	87.6	90

Betaxolol		307	2.53	0.43	50.72	100
Propranolol		259	2.9	0.79	41.49	100

6.3.2 Distribution

Distribution of drugs entails passage of a compound from circulation into the tissues. Like absorption, there is a trend for increasing tissue permeability with increasing lipophilicity.[21] Hence the incorporation of the phenyl rings in a molecule is likely to increase its affinity for the tissues. As in the case of absorption, the substituents that increase the logP value of the compounds will enhance distribution into the tissues more than the polar groups. For example, propranolol and alprenolol with a clogP value of 1.2 and 1.0, respectively, show good penetration into the cerebrospinal fluid (CSF) from the plasma as shown by the CSF–plasma ratio (Table 6.7), while nadolol and atenolol are not permeable through the blood–brain barrier.[28] Clearly, incorporation of cyclohexanediol in the ring system makes the compound quite polar for it to cross the blood–brain barrier, whereas very hydrophilic nature of atenolol hinders its permeability into the brain as is the case in the absorption of these two drugs.

A similar relationship between lipophilicity and transfer across the blood–brain barrier has been observed among the benzodiazepines midazolam, alprazolam and triazolam.[29] As seen in Table 6.8, the increase in lipophilicity increases the brain to serum ratio.

Plasma protein binding is also an important parameter that influences tissue distribution. Only free drug in plasma can cross the membrane and equilibrate with free drug in the tissues. Incorporation of neutral lipophilic substituents on the phenyl ring tends to increase the binding of the drug to plasma proteins and therefore reduce the free fraction that is available to transfer to the tissues. Comparison of the brain to unbound serum concentration ratio of lorazepam, in which one of the hydrogen atom on the phenyl ring is replaced by a chloro group (Table 6.9), *versus* oxazepam suggested that extraction of the latter into the brain was slightly enhanced.[29] Since the change in lipophilicity is minimal between the two compounds, the increase in brain exposure is attributed to more unbound fraction of oxazepam.

It has been demonstrated that other factors such as the hydrogen bonding capabilities of a compound can also affect its penetration across the blood–brain barrier.[30,31] Hence, the established relationship between permeability across the blood–brain barrier, protein binding and lipophilicity may not necessarily hold in all cases of lipophilic drugs. For example, in spite of similar clogD and protein binding values of flunitrazepam compared to diazepam (Table 6.10), its brain to serum ratio is much lower than diazepam. This disconnect is probably attributed to the increase in PSA of flunitrazepam (78.94 $Å^2$) due to presence of a nitro group in one of its aromatic rings.

The effect of hydrogen bond donor and polar functionality in the phenyl ring on brain exposures was also demonstrated in the following example (Table 6.11). Compound **6.5** was synthesised as one of the analogs of mGluR2 potentiators but exhibited poor brain exposure following administration to rats.[32] Replacement of the phenolic group with an ethoxy group (compound **6.6**)

Table 6.6 Comparison of physicochemical properties of sulpiride and remoxipride.[25,26]

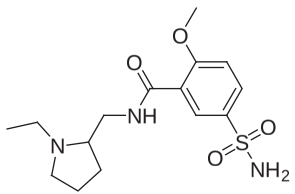
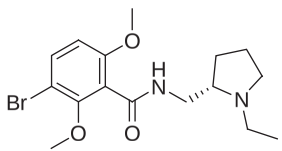
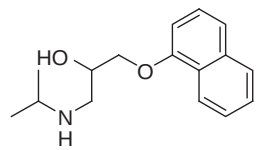
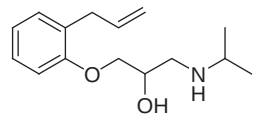
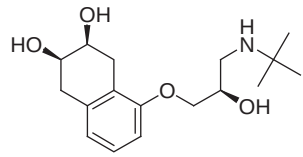
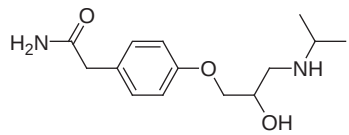
Name	*Structure*	*MW*	*clog$D_{7.4}$*	*PSA Å^2*	*CSolubility mg/mL*	*% F*
Sulpiride		341	−1.07	110.1	126	27
Remoxipride		371	0.38	50.8	84	90

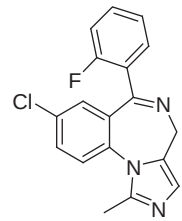
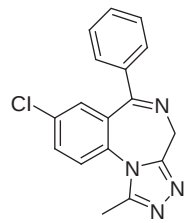
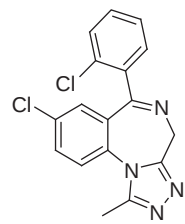
Table 6.7 Brain penetration of β-blockers and its relationship to lipophilicity.[28]

Compound	*Structure*	*cLogP*	*CSF/Plasma*
Propranolol		1.2	1
Alprenolol		1.0	1
Nadolol		−1.5	ND
Atenolol		−1.1	ND

gave better brain levels and higher brain-to-plasma ratio (1.2:1). Detectable levels for **6.6** were attributed to the drop in the polar surface area by 16 $Å^2$ compared with compound **6.5**, in addition to the increase in the log D of the molecule.

Another factor that affects brain penetration is the efflux of the compound from blood–brain barrier by P-gp transporter.[27] In contrast to absorption, the P-gp transporter plays an important role in brain uptake as it limits cellular uptake of drugs from the blood circulation into the brain. As mentioned above, the structure–activity relationships in terms of the role of the substituted phenyl ring systems are not fully understood. However, an increase in brain exposure *via* modulation of PSA, which in turn affects the P-gp efflux properties, has been demonstrated by Roberts and co-workers.[33] This group focused on the reduction of polar surface to influence the efflux properties of compounds. 2-Imidazole $\alpha 1_A$ partial agonist (**6.7**) was synthesised for its potential utility in treating stress urinary incontinence (Table 6.12). However, **6.7** showed poor brain penetration (rat free plasma/CSF 1:0.08), which was attributed to high P-gp-mediated efflux when assessed in MDCK MDR-1 cells (AB/BA ratio of 7:29). The medicinal chemistry strategy focused on reducing the PSA of new

Table 6.8 Comparison of brain to serum (B/S) ratio of midazolam, alprazolam and triazolam and its physicochemical properties.[29]

Name	*Structure*	*cLogD$_{7.4}$*	*B/S ratio*[a]
Midazolam		3.78	33.91
Alprazolam		1.92	2.62
Triazolam		2.08	19.52

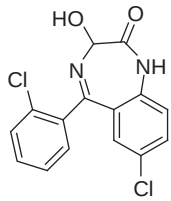
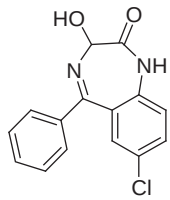
[a]B/S ratio = brain to unbound serum ratio.

analogs in order to reduce the potential for P-gp recognition and therefore improve blood–brain barrier penetration.

As shown in Table 6.12, the replacement of the sulfonamide with dialkylethers such as the methoxymethyl ether **6.8** resulted in retention of the $\alpha 1_A$ partial agonist potency with no evidence of P-gp efflux. Since the log D values of all the analogs made were similar, the modulation of the P-gp mediated efflux of **6.7** by addition of the ether functionalities was attributed to the decrease in PSA of the molecule. The brain penetration was further enhanced by increasing the lipophilicity of the compounds *via* incorporation of the chloro and fluoro groups (Table 6.12).

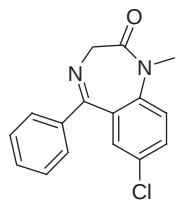
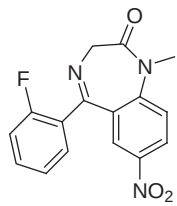
Although incorporation of polar groups in phenyl rings has a negative influence on the permeability across the blood–brain barrier, the strategy of substituting the aromatic hydrogen with polar moieties can be used in preventing serious central nervous system (CNS) side effects that may be caused by some lipophilic compounds. This has been demonstrated in the following two

Table 6.9 Comparison of the brain to unbound serum concentration and plasma protein binding of lorazepam and oxazepam.[29]

Compound	*Structure*	*clogD$_{7.4}$*	*Unbound fraction*	*B/S ratio*[a]
Lorazepam		2.38	0.157	16.01
Oxezapam		2.22	0.171	20.30

[a]B/S ratio = brain to unbound serum ratio.

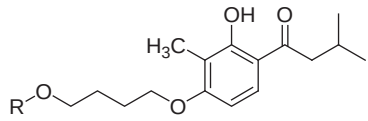
Table 6.10 Relationship between unbound fraction, physicochemical properties and brain to serum ratios of diazepam and flunitrazepam.[29]

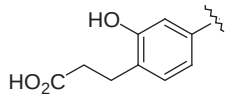
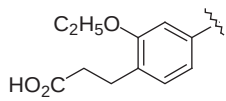
Name	*Structure*	*Total B/S*[a]	*B/unbound S*[b]	*clogD$_{7.4}$*	*PSA (Å^2)*
Diazepam		3.34	26.05	2.8	32.67
Flunitrazepam		1.15	5.97	2.2	78.94

[a]Total B/S = total brain to serum ratio,
[b]B/unbound S = brain to unbound serum concentration.

examples. Compound **6.11** (Figure 6.5), which was designed as a cardiotonic agent, resulted in 'bright visions' upon administration to patients. The side effect was probably due to CNS activity and was a result of permeability of the drug across the blood–brain barrier. Kutter and Austel optimised the structure

Table 6.11 Effect of replacing a phenolic group with an ethoxy group on brain to plasma ratio.[32]



No.	*R*	*MW*	*PSA (Å²)*	*clogD*	*HBD*[a]	*B/P*[b]
6.5		473	102	3.8	2	BQL[c]
6.6		424	86	5.6	1	1.2

[a]HBD = hydrogen bond donor,
[b]B/P = brain to plasma ratio,
[c]BQL = below quantitation limits.

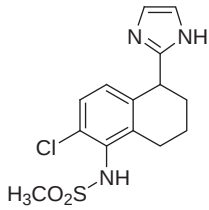
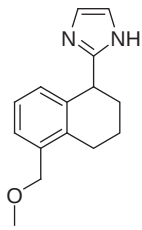
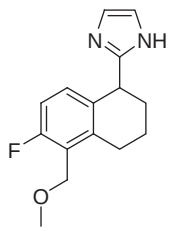
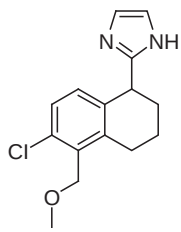
by substituting the methoxy group in **6.11** with more hydrophilic substituents that could potentially prevent blood–brain penetration.[34] Replacement of a methoxy group with a bioisosteric methylsulfoxide moiety in sulmazole (**6.12**) sufficiently modified the lipophilicity of **6.11** from a clogP of 2.59 to 1.17 (Figure 6.5). Furthermore, the increase in the PSA of **6.12** from 60.03 to 87.08 prevented it from crossing the blood–brain barrier, thus eliminating the unwanted CNS related side effects but retaining the desired activity.

Rubenstein and co-workers have also used a similar approach for limiting the brain exposure of a class of antimitotic agents.[35] Compounds **6.13** and **6.14** were synthesised as irreversible inhibitors of tubulin polymerisation and were effective against a variety of tumors, including those that express the multidrug resistant (MDR) phenotype. However, CNS toxicity was observed for both compounds possibly due to the high brain levels of **6.13** and **6.14** (Table 6.13). Synthesis of less lipophilic analogues of **6.13** was therefore undertaken in order reduce brain exposure of these agents and therefore increase the therapeutic window. Acyl derivatives of aniline **6.14** were designed that could potentially increase the polar surface area and decrease log D of both **6.13** and **6.14** (Table 6.13). Introduction of the polar acyl groups (compounds **6.15**–**6.17**) successfully prevented the compounds from crossing the blood–brain barrier. Unfortunately, analogs **6.15**–**6.17** were less potent and displayed weak concentration-dependent inhibition of tubulin polymerisation *in vitro* than **6.13** and **6.14**.

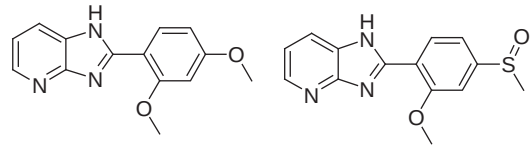
6.3.3 Clearance

Metabolism and excretion are the two primary routes of elimination of a drug from the body. Like absorption and distribution processes, the physicochemical

Table 6.12 Modulation of P-gp efflux by reducing total polar surface area.[33]

No.	*Structure*	*MDCK-MDR1 ratio*	*PSA (Å^2)*	*log$D_{7.4}$*
6.7		7/29	83	1.5
6.8		40/36	38	1.8
6.9		39/37	38	2.0
6.10		40/36	38	2.6

properties of a drug and its structure can primarily dictate its route of elimination.[21] Among all the physicochemical properties, lipophilicity is the key factor determining the ultimate route by which the drug will be eliminated.[21] It governs the accessibility of the drug to many of the drug metabolising enzymes as well as the ability of a molecule to cross the renal tubular membrane. The latter governs the urinary excretion of a compound. Since substituted aromatic rings play a major role in modulating a drug's lipophilicity, incorporation of a

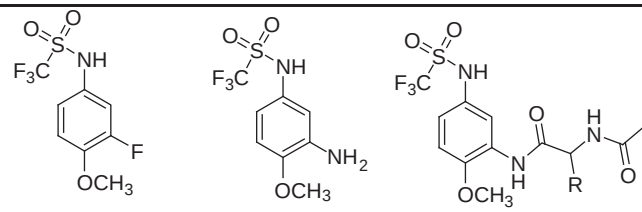


Compound 6.11
cLogP = 2.59
PSA = 60.03

Sulmazole 6.12
logP = 1.17
PSA = 87.08

Figure 6.5 Structures and physicochemical properties of cardiotonic agents **1**, and sulmazole **2**.

Table 6.13 Prevention of brain permeability by addition of polar acyl groups.[35]



1 **2**

3 R = CH_3
4 R = H
5 R = $CHCH_3OH$

No.	*MW*	*PSA (Å^2)*	*clogD*	*Brain levels*
6.13	371	55	2.0	860
6.14	368	81	1.2	1200
6.15	481	114	0.36	BQL[a]
6.16	467	134	0.020	BQL
6.17	497	134	0.040	BQL

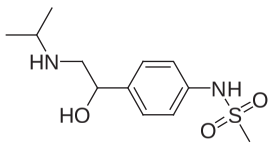
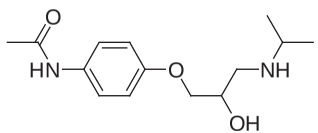
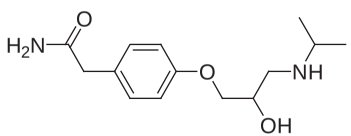
[a]BQL = not detected

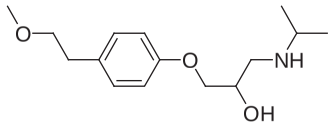
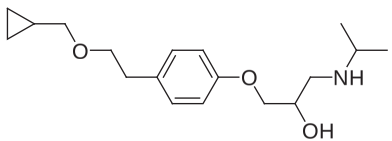
substituted aromatic ring in a molecule influences the route of elimination of a drug.

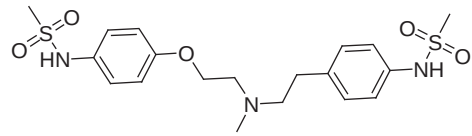
6.3.3.1 Excretion

Molecules that have a relatively small size (approximate molecular weight of 200–300) and are polar are generally excreted unchanged in the urine. Since incorporation of substituted phenyl rings adds a certain degree of lipophilicity to the molecule, most molecules with one or more phenyl rings generally undergo metabolism. However, replacement of a hydrogen atom in an aromatic ring with polar functionalities can decrease the $\log D_{7.4}$ and can result in excretion of the drug renally. For example, comparison of structures of various beta blockers and their physicochemical properties (Table 6.14) indicates that analogs with polar functionalities which increase the PSA and decrease the

Table 6.14 Influence of physicochemical properties on renal clearance of β-blockers.[36]

Name	*Structure*	*MW*	*clog$D_{7.4}$*	*PSA ($Å^2$)*	*% Excreted unchanged in urine*
Sotalol		272	−1.7	86.81	80
Practolol		266	−1.5	70.59	95
Atenolol		266	−1.7	84.5	85

Metoprolol		267	−0.47	50.72	10
Betaxolol		307	0.43	50.72	15



Dofetilide
clogD$_{7.4}$ = 0.52 (clogP = 1.38)
PSA = 121.57 Å^2

Figure 6.6 Structure and physicochemical properties of dofetilide.

clogD value (*e.g.* atenolol, sotalol and practolol) are more prone to undergo renal excretion than those with lipophilic groups such as betaxolol and metoprolol.[36]

The effect of polar functionality has also been demonstrated in the pharmacokinetics of the neuroleptic sulpiride. Assessment of unchanged sulpiride in the urine indicates that metabolism is a minor route of clearance for this compound and about 70% of the dose is excreted unchanged in the urine.[25] In contrast, the analog of sulpiride, remoxipride is extensively metabolised and only 25% of the dose is excreted in the urine.[26] This can be correlated to the physicochemical properties of these two drugs (Table 6.6). The highly polar nature of sulpiride (clogD$_{7.4} = -1.07$) is linked to the high renal clearance of the drug relative to remoxipride.

Even though it is commonly believed that hydrophilic compounds are excreted unchanged renally and that the log D value of renally excreted drugs is generally <0, there are some discrepancies. For example, the class III antiarrhythmic agent, dofetilide, is an amine with a log D$_{7.4}$ value of 0.52 (Figure 6.6). The moderate lipophilicity of this compound suggests that it has the potential to undergo oxidative metabolism by P450. However, assessment of the renal clearance of dofetilide in humans indicates that 80% of the compound is excreted unchanged in the urine.[37] This is probably due to the presence of the two phenyl sulfonamide groups in the molecule, which increase the hydrogen bonding potential and therefore the PSA of the molecule (121.57 Å^2). Taken together, dofetilide more closely resembles atenolol or sotalol as reflected by the renal clearance of the compound.

6.3.3.2 Biliary Excretion

Several uptake and efflux transporters are present in the hepatobiliary system.[38] These are quite broad in their specificity and have a high capacity for eliminating metabolites of drugs *via* the bile. In some instances, these transporters are responsible for the excretion of unchanged drug in the bile. Although the structure–activity relationship of these transporters is unknown, a rough correlation between the physicochemical parameters of a drug and its substrate property for the biliary transporters is exemplified in the following example. Compound **6.18** is a prostaglandin D$_2$ receptor antagonist which is primarily

excreted unchanged in the bile after administration to bile-duct cannulated rats (Table 6.15).[39] This result suggested a role for active transport of parent from plasma circulation into the bile. Modification of **6.18**, with substituents that decreased its PSA (Table 6.15) reduced the biliary clearance significantly, as observed for **6.19** and **6.20**. Similarly, there was a trend towards increasing log $D_{7.4}$ value and decreasing biliary concentration of the parent.

6.3.3.3 Metabolism

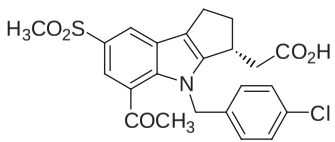
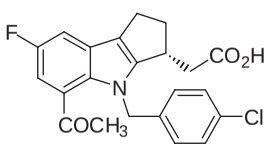
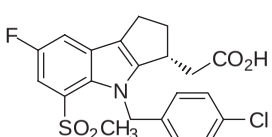
As mentioned earlier, metabolism is a primary route of clearance for most compounds containing one or more aromatic rings. Lipophilic compounds are susceptible to oxidation by liver enzymes (namely cytochrome P450) as well as by other conjugative enzymes such as uridinylglucuronyltransferase (UGT).[21]

Incorporation of phenyl rings into a molecule affects its metabolism in two ways. Firstly, the presence of unsubstituted or substituted aromatic rings in the molecule increases its lipophilicity; this facilitates an easy transfer of the molecule across the membrane and brings it into close proximity to the xenobiotic metabolising enzymes. Secondly, the aromatic rings themselves become a site of metabolism. The latter, however, is dependent upon the reactivity of the ring. As discussed in Section 6.2, an aromatic ring is a delocalised system with 6π electrons. Although aromatic molecules display enhanced stability due to the delocalisation of these electrons, these rings can undergo electrophilic aromatic substitution reactions that involve replacement of hydrogen atom in the ring by an electrophilic substituent. In the case of CYP450 catalysed oxidations, the hypervalent iron–oxene complex is widely accepted as 'the oxidant species' that is ultimately responsible for the oxidation of compounds.[40] This high-energy oxo-species can add into the aromatic ring to produce a tetrahedral intermediate that subsequently rearranges *via* an epoxide (an arene oxide) or a ketone intermediate and ultimately results in an arenol (Figure 6.7).

Like electrophilic aromatic substitution reactions, the substituents on the ring influence the oxidation of the phenyl ring.[41] Hence, aromatic rings that are activated (contain electron rich substituents) are more susceptible to aromatic hydroxylation.[42] For example, aniline containing analogs are hydroxylated extensively. The final location of the oxygen is further explained by simple organic principles. Ring opening of the arene oxide of compounds containing electron rich rings is expected to occur in the direction that results in a more stable carbocation. Thus, aniline derivatives are hydroxylated in the *ortho* and *para* positions of the amino group as observed in atorvastatin (Figure 6.8).[43,44]

On the other hand, deactivated rings (rings with electron withdrawing groups) are hydroxylated at a slower rate or not at all. For example, the uricosuric agent, probenecid, undergoes no detectable aromatic hydroxylation (Figure 6.9).[45,46] The presence of two deactivating groups on the ring—the carboxy group and the sulfonamide moiety—results in a significant decrease in the electron density of the phenyl ring. The same principle applies to drugs that contain two phenyl rings. In case of chlorpromazine or fluphenazine, the

Table 6.15 Correlation of biliary clearance of D_2 prostaglandin receptor antagonists with their physicochemical properties.[39]

No.		*Concentration in rat bile (μM)*	*clogD$_{7.4}$*	*cLogP*	*PSA*	*MW*	*pKa*
6.18		1100	0.16	2.96	101.82	459.9	4.56
6.19		81	1.19	3.98	59.3	399.8	4.56
6.20		103	0.85	3.66	84.7	435	4.54

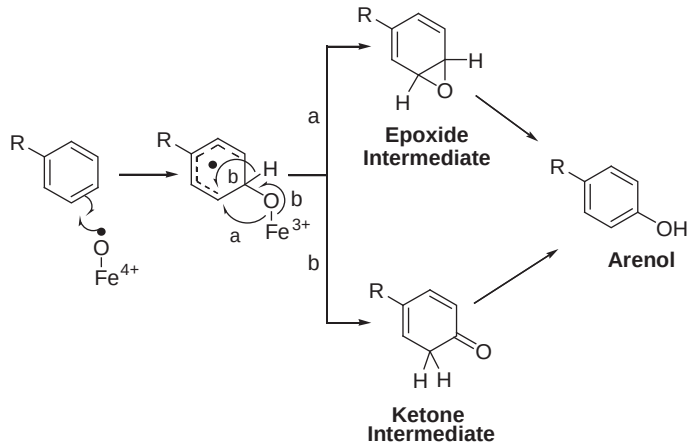


Figure 6.7 Oxidation of aromatic rings by iron-oxo species.

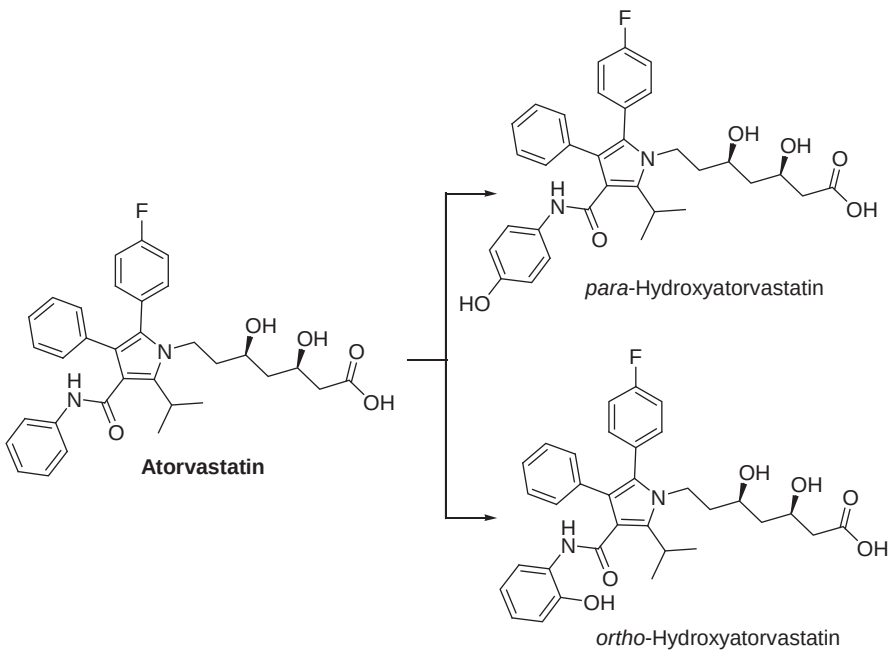


Figure 6.8 Structure of atorvastatin and its CYP3A4 mediated metabolites.

unsubstituted phenyl ring is hydroxylated while no oxidation is observed on the less reactive substituted ring (Figure 6.9).[47,48]

In light of these observations, several strategies to reduce the metabolic clearance of compounds have been employed. One strategy involves

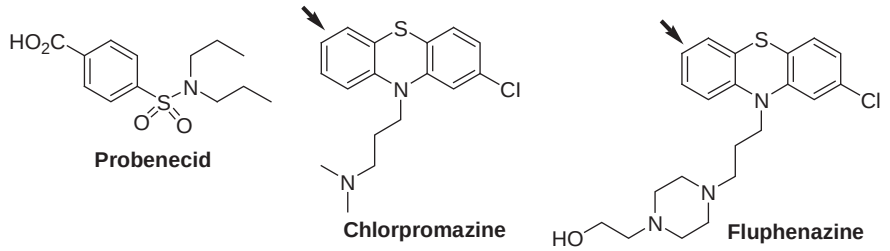


Figure 6.9 Structures of probenecid, chlorpromazine and fluphenazine. The arrow indicates the site of metabolism

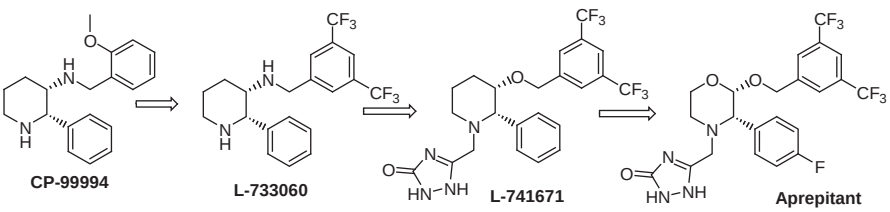


Figure 6.10 Structures of NK1 receptor antagonists.

inactivation of aromatic rings towards oxidation by substituting them with strongly electron withdrawing groups (*e.g.* CN, SO_2NH_2, SO_3H). Since such groups reduce the lipophilicity of the molecule, it becomes necessary to compensate by adding a lipophilic group at another site in the molecule to retain activity. Alternatively, a lipophilic trifluoromethyl group (CF_3) is frequently incorporated into the aromatic ring in the compound. Not only does the CF_3 group add lipophilicity to the molecule but it also leads to the deactivation of the ring.

The following example illustrates the use of trifluoromethyl groups to improve metabolism. CP-99994 (Figure 6.10) was one of the lead NK1 receptor antagonists but was discontinued from phase II clinical trials due to poor bioavailability,[49] which was attributed to the compound's high metabolic liability. To improve bioavailability, the molecule was modified by replacing the methoxyphenyl moiety with a 3,5-bistrifluoromethylphenyl moiety. This modification resulted in enhanced activity and improved metabolism. Further improvement in metabolic liability and basicity finally resulted in aprepitant (Figure 6.10).[49]

A more frequently employed strategy to circumvent the problem of metabolic instability involves blocking the reactive site by the introduction of a halogen atom (Cl or F). The design of fenclofenac (an analog of diclofenac) illustrates the role of a halogen atom in potentially blocking the site of metabolism. Diclofenac is metabolised relatively rapidly *via* hydroxylation of the 4-position of the dichlorophenyl ring (Figure 6.11), yielding 4-hydroxydiclofenac

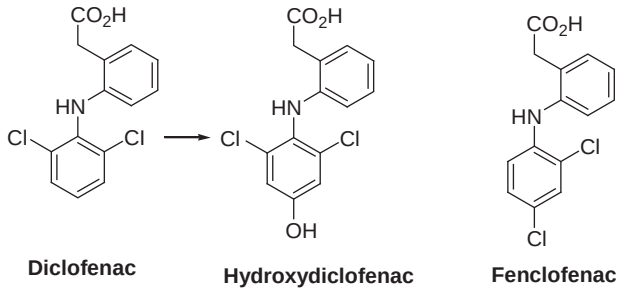


Figure 6.11 Metabolism of diclofenac and structure of fenclofenac

and resulting in a short half-life in humans. A change in the position of the chloro group from the 6-position to the 4-position in fenclofenac not only improves its metabolic stability but also increases its half-life to 20 h.[50]

Given the unique properties of fluorine (see Section 6.2) and the convenient methods of introducing it, this group is more popular and is commonly used to alter the rate and route of metabolism, and therefore improve the bioavailability and half-lives of compounds of interest.[14,17,18] As described earlier, the carbon–fluorine bond is much more resistant to direct chemical attack by cytochrome P450 in comparison to the carbon–hydrogen bond. The introduction of a fluorine on a phenyl ring is also said to decrease the rate of interaction between the π system and the iron-oxo intermediate.[17] Thus even though the aromatic rings with a fluorine group are metabolised, the rate of metabolism is slower than the unsubstituted aromatic ring.

Some examples of where introduction of the fluoro group in the molecule alters the metabolic rate are demonstrated in the discovery of ezetimibe, a cholesterol absorption inhibitor, and a calcium entry blocker, flunarizine, used in the treatment of cerebral and vascular insufficiency.[51,52] Both cases involve the blocking of unwanted metabolic oxidation of the aromatic ring by fluorine (Figure 6.12). This altered the metabolic route of the compounds and increased their stability and half-lives.

In another example 3-piperidinylindole, which was synthesised as an antipsychotic agent, had poor bioavailability due to high metabolic instability and poor permeability.[53] Although γ-fluorination of the piperidine group decreased the pKa of 3-piperidinylindole and increased its permeability, it suffered from high metabolic instability due to hydroxylation of the 6-position on the indole ring. Structural modification *via* incorporation of fluorine at this position led to a greater improvement in bioavailability and increased binding affinity by an order of magnitude (Table 6.16).

Discovery of celecoxib presents an interesting case where substituents were introduced on the aromatic ring of the molecule to incorporate metabolic soft spots and decrease the half-life of the compound.[54] The fluoro- and chlorophenyl- analogs (**6.21** and **6.22**) were discovered as an early lead in the synthesis of cyclooxygenase-2 (COX-2) inhibitors. Although both compounds

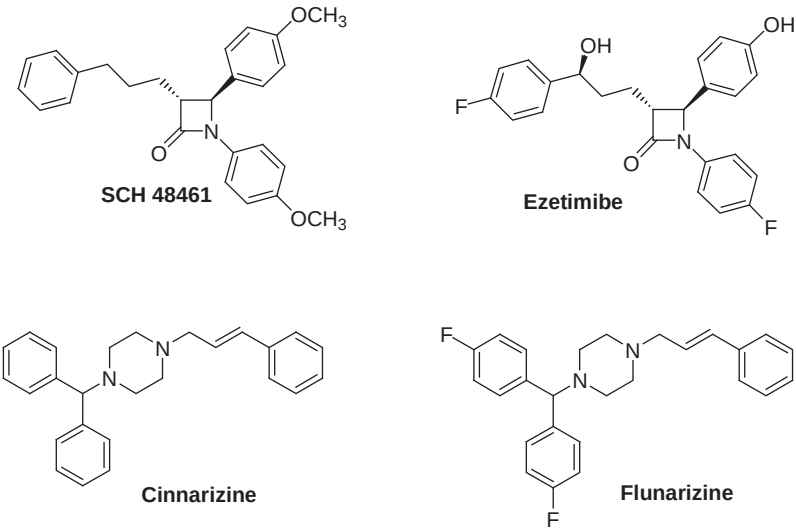
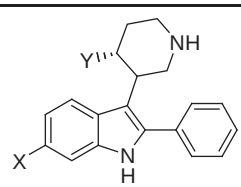


Figure 6.12 Examples of fluorine substitution in rational drug design results in alteration of metabolic routes.,

Table 6.16 Modification of 3-piperidinylindole, an antipsychotic, to improve bioavailability.[53]



X	*Y*	*F (%)*	*cLogP*	*pKa*
H	H	Poor		10.4
H	F	18		8.5
F	F	80		

showed good efficacy in models of inflammation, they had an unacceptably long plasma half-life in rats (221 h and 117 h, respectively). Replacement of chloro group in **6.22** with a methyl group (celecoxib) resulted in a minor change in its physicochemical properties but decreased its half-life to 3.5 h in rats. The rapid elimination of celecoxib was attributed to oxidation of the tolyl group to the corresponding carboxylic acid (Figure 6.13).

Appropriate incorporation of substituents on aromatic rings of a molecule can also alter the rate of the primary metabolic routes and therefore influence their duration of action. For example, the electron donating capability of the two methyl groups at the 2- and 6-positions in lidocaine is speculated to reduce

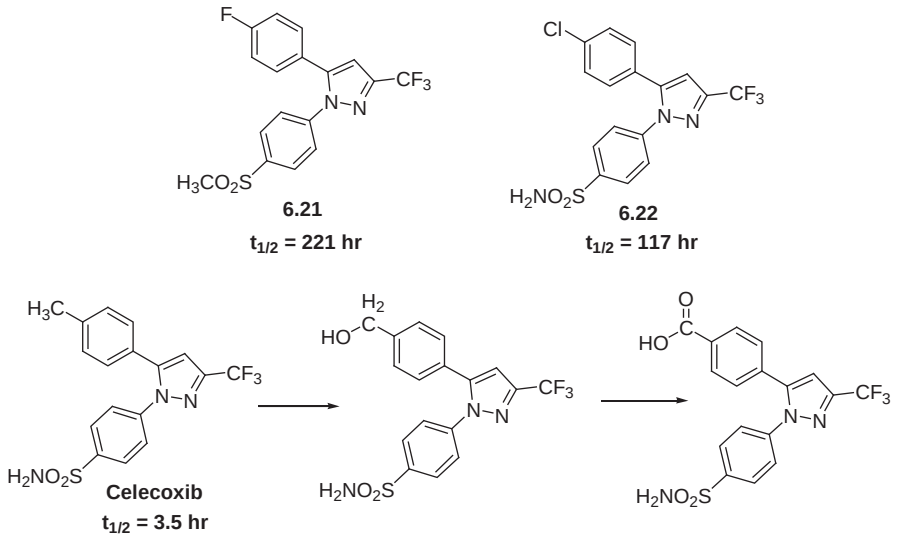


Figure 6.13 Introduction of metabolically labile substituents to decrease half-life of cylcooxygenase-2 (COX-2) inhibitors and discovery of celecoxib.

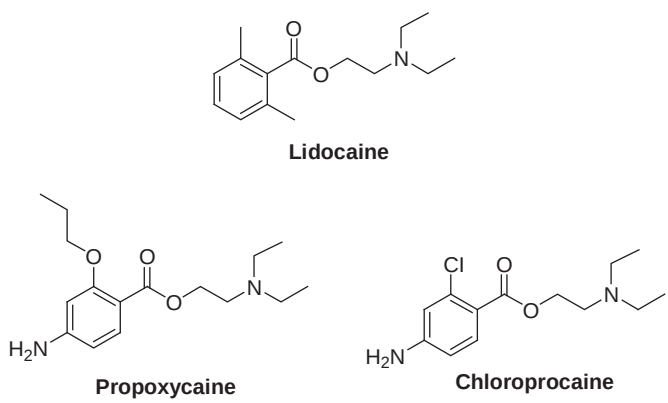


Figure 6.14 Structures of lidocaine, propoxycaine and chloroprocaine.

its rate of hydrolysis by esterases by chemically reducing the electrophilicity of the carbonyl carbon to hydrolytic attack relative to its desmethyl analog, and therefore the enhance its anaesthetic potency (Figure 6.14).[55] A similar rationalisation has been put forth to explain the different duration of actions of procaine analogs. Prolonged action of propoxycaine (Figure 6.14) is due to reduced rate of hydrolysis of the ester linkage as a result of inductive effect of the *o*-propoxy group as well as the steric hindrance due to its location on the ring. In contrast, the short of duration of action of chloroprocaine is attributed

to the electron withdrawing effect of the *o*-chloro group, which increases the electrophilicity of the carbonyl group and makes the ester group more susceptible to hydrolysis by plasma and tissue esterases.[55]

6.4 Toxicity

Aromatic rings can play a major role in influencing the toxicity of drugs. As discussed above, hydrogen atoms in the aromatic rings of compounds are generally replaced with lipophilic functional groups to improve their ADME properties and potency. These changes in the physicochemical properties of compounds also enhance their interactions with targets and proteins, and result in side effects. For example, amiodarone (Figure 6.15) is a lipophilic compound belonging to this category. This antiarrhythmic agent is quite efficacious but causes a number of side effects including pulmonary toxicity and phospholipidosis.[56,57] The toxicity is attributed to the presence of a diiodophenyl moiety in the molecule which increases the lipophilicity of the compound (clogP = 7.81).[58] This enhances its permeation and subsequent accumulation into several tissues and results in a very long half-life in humans (~9 to 77 days).[59] In another example, the alkyl and cyclohexyl groups present in proxicromil, an anti-allergy agent, allow this compound absorb readily through the gastrointestinal tract (Figure 6.15).[60] However, this highly lipophilic, detergent-like drug tends to accumulate in the biliary canaliculus, which may be a reason for its hepatotoxicity.[61]

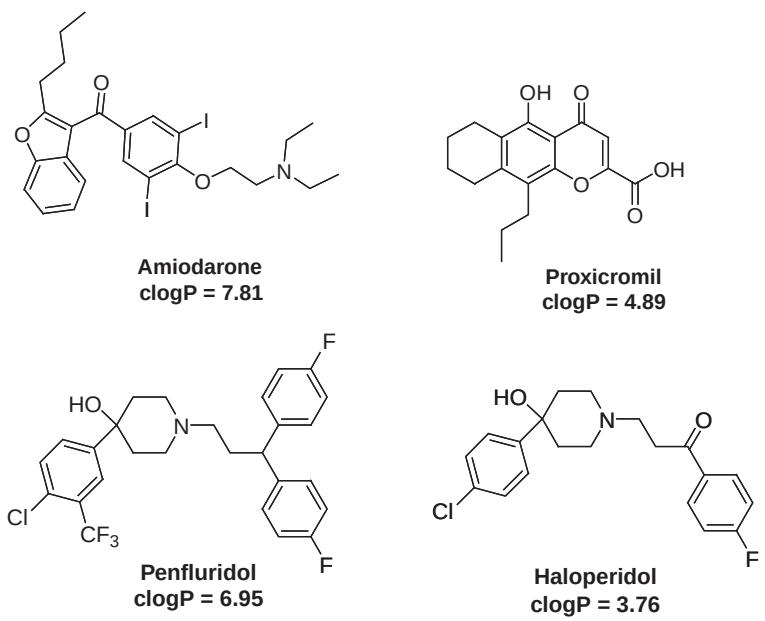


Figure 6.15 Structures of amiodarone, proxicromil, penfluridol and haloperidol.

Other examples exist where the introduction of lipophilic groups has resulted in increased volume of distribution or accumulation into tissues, which ultimately result in long half-lives. A classic example is haloperidol *versus* penfluridol (Figure 6.15). Introduction of various blocking groups to improve the metabolism of haloperidol lead to the discovery of penfluridol. The compound is more potent than haloperidol and penetrates the blood–brain barrier very readily, probably due to its high lipophilicity (clogP = 6.95). Although toxicities have not been observed with penfluridol, its potential for accumulating in the tissues and the resulting long half-life (199 h) raises concern, especially in case of an overdose of the drug.[62,63]

Metabolic activation or bioactivation is another way in which the aromatic rings can influence toxicity of a compound. Most xenobiotic metabolising enzymes are not only responsible for detoxication of drugs but can induce the formation of reactive intermediates. The electrophilic intermediates thus formed are capable of covalently reacting with nucleophiles found in proteins and nucleic acids, which in turn may be responsible for the drug's toxic manifestations including mutagenesis, carcinogenesis and hepatic necrosis.[64,65]

As described above, most lipophilic aromatic compounds undergo P450 mediated metabolic oxidation *via* epoxide formation. The reactivity and the importance of epoxides was first realised with polyaromatic hydrocarbons. These compounds underwent oxidative metabolism to reactive epoxide that covalently bound to DNA.[66,67] Furthermore, there was a good correlation between the DNA adduct formation and carcinogenesis.[68] Drugs possessing structural features prone to metabolic epoxidations are abundant.[69] Aromatic anticonvulsants such as phenytoin, phensuximide and phenobarbital are known to exert side effects such as hepatic necrosis and aplastic anaemia *via* the chemically reactive epoxide metabolites formed by P450 oxidation (Figure 6.16).

It is important to realise, however, that not all epoxides exert toxic effects. The reactivity of the epoxide intermediate varies greatly with its molecular geometry, stability, electrophilic reactivity and relative activity as substrates of epoxide-transforming enzymes (epoxide hydrolase, glutathione transferase and others). In most cases, epoxides are quite unstable and rearrange rapidly to the arenols (see above). Only the ones that are stable enough and capable of traversing membranes are exposed to macromolecules and form adducts.

Aromatic compounds containing phenolic moieties and aromatic amines are susceptible to bioactivation by several oxidative enzymes such as the peroxidases and cytochrome (CYP) enzymes. Phenolic compounds generally undergo a two electron oxidation to quinoid intermediates namely, *o* and *p* quinone imines or *o* and *p* quinone methides (Figure 6.17).[70] Quinone imine and quinone methides are reactive electrophiles and may adduct to cysteine and lysine residues of proteins, or they may act as haptens and initiate immunological responses.[71] These intermediates are congeners of α,β-unsaturated carbonyl compounds and are capable of undergoing 1,4-addition of a nucleophile (Michael addition) to form conjugates as shown in Figure 6.17. *In vivo* these intermediates react with macromolecules and form covalent adducts,

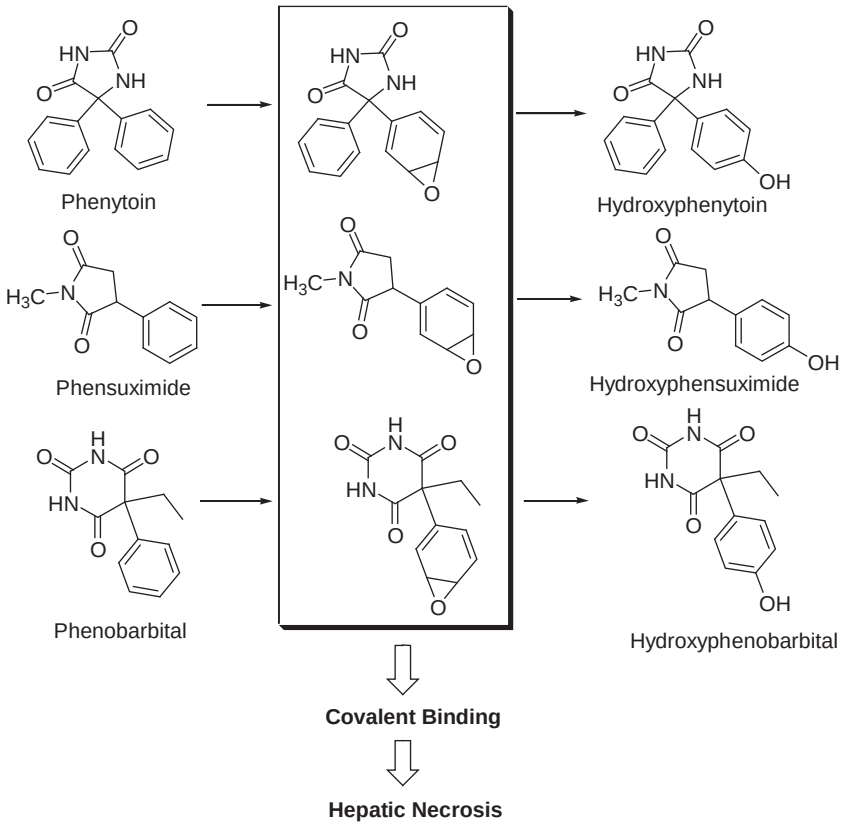


Figure 6.16 Bioactivation of compounds containing aromatic rings to epoxides.

which may trigger mechanisms that finally lead to cell necrosis, immune responses or blood toxicities.

Metabolic activation of acetaminophen represents a classic example in this category.[72–74] Although safe at therapeutic doses, this widely used analgesic causes fulminant hepatic necrosis when taken in overdose. The cause of hepatotoxicity is ascribed to P450 catalysed 2-electron oxidation of acetaminophen to a quinone imine intermediate (NAPQI) and subsequent covalent binding of this electrophilic intermediate with various proteins in the liver (Figure 6.18). Some additional examples include bioactivation of amodiaquine (Figure 6.18) and troglitazone (Figure 6.19).[75–78] The hepatotoxicity and agranulocytosis observed following administration of antimalarial amodiaquine is triggered by formation of reactive quinone imine intermediate (similar to that observed for acetaminophen) which is produced in the liver as well as other sites in the body (Figure 6.18).[75]

Troglitazone was developed for the treatment of type 2 diabetes but was withdrawn from the clinic due to rare cases of liver failure and deaths. The

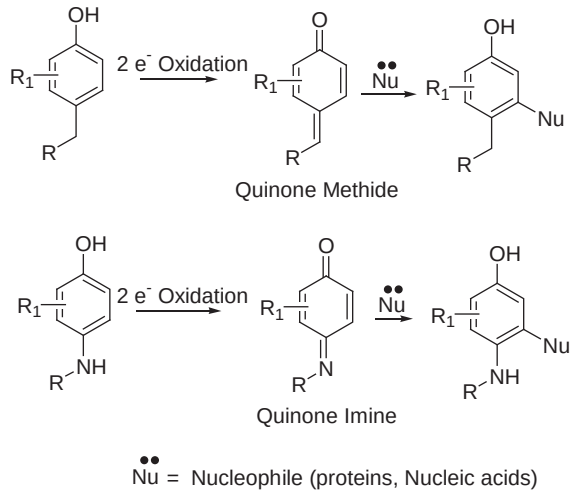


Figure 6.17 Two electron oxidation of phenolic compounds to quinone methides and quinone imines and their reaction with nucleophiles.

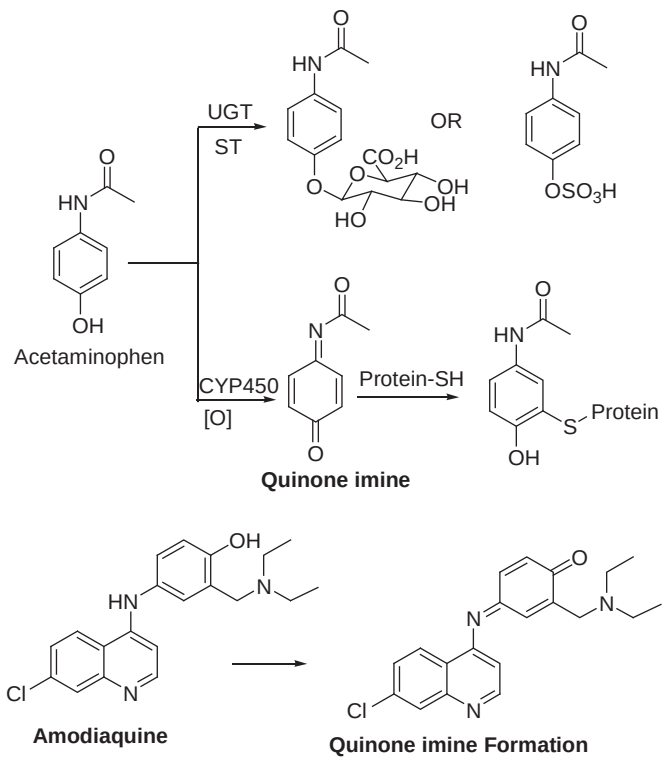


Figure 6.18 Metabolic activation of acetaminophen and amodiaquine to a quinone imine.

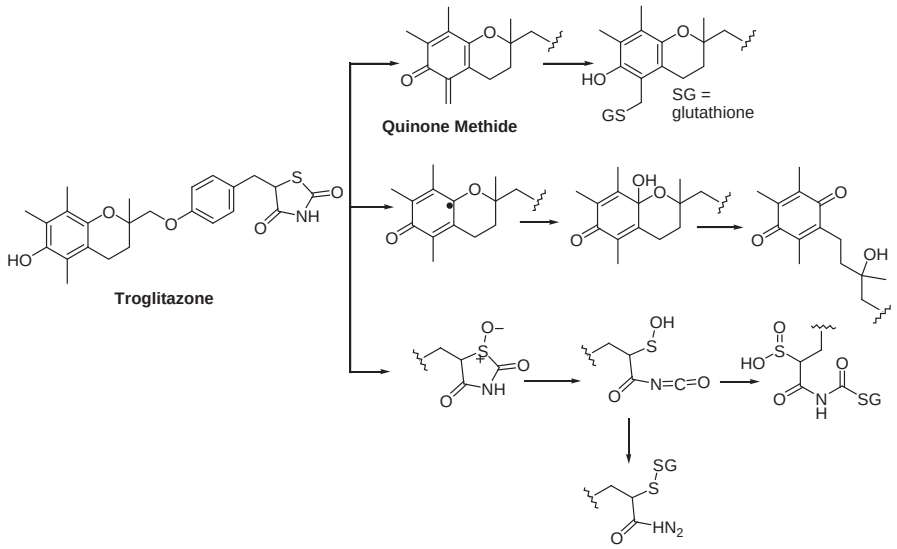


Figure 6.19 Metabolic activation of troglitazone.

observed hepatotoxicity has been associated with the formation of reactive intermediates. Three different mechanisms for troglitazone bioactivation that results in covalent binding to proteins have been proposed. While two mechanisms involve metabolic activation of the thiazolidinedione moiety, one of the proposed mechanisms involves the oxidation of the chromane moiety of troglitazone to a reactive *o*-quinone methide (Figure 6.19).[79] The formation of the quinone methide has been confirmed by trapping the intermediate with glutathione or *N*-acetylcysteine, and the structure of the adduct has been determined unequivocally by NMR.

Phenolic compounds can also form reactive intermediates *via* catechols. Most often phenolic compounds are cleared *via* glucuronidation or sulfation of the hydroxyl group (as shown for acetaminophen, Figure 6.18). However, given the electronic distribution of the hydroxyphenyl ring, these compounds can be oxidised to catechol or hydroquinone derivatives. These hydroxylated derivatives of phenols are readily oxidised to chemically reactive quinones and semiquinones, which form covalent bonds with proteins and DNA.[80,81] Alternatively, these can generate toxic reactive oxygen species that could damage the cellular mechanisms. The natural estrogens such as estrone and estradiol and drugs such as 17α-ethynylestradiol undergo extensive CYP-mediated oxidation in the 2- or 4-positions.[82] One pathway implicated in the tumorgenic process involves the metabolism of estrogens to catechols mediated by P450s and further oxidation of these catechols to estrogen *o*-quinones (Figure 6.20). The estrogen quinones react with DNA and form N3-adenine or N7-guanine DNA adducts, resulting in mutagenic apurinic sites.[83] A similar bioactivation pathway is also demonstrated for equilin and equilenin, which are used for estrogen replacement therapy.[84]

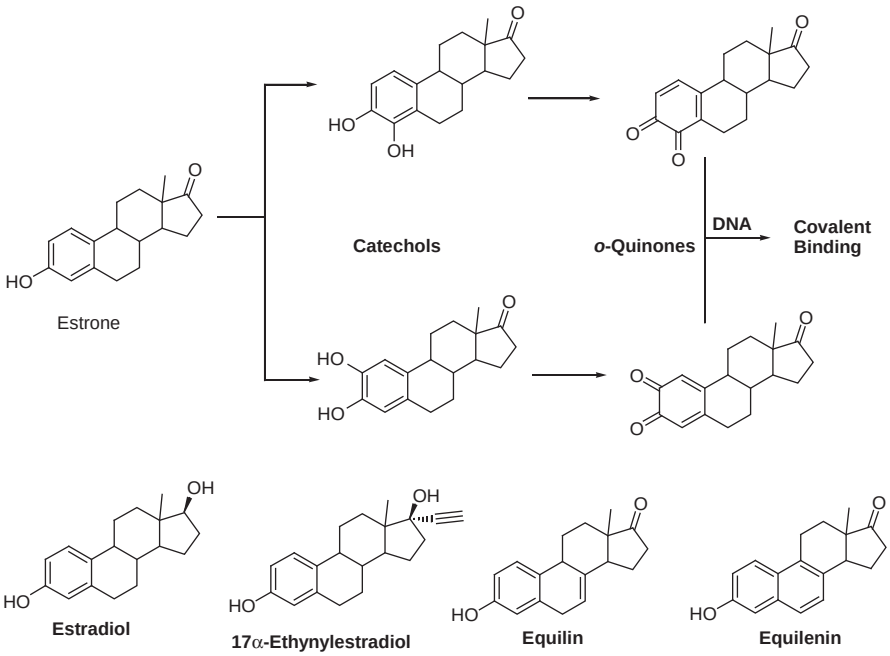


Figure 6.20 Metabolic activation of estrogens via the catechols.

Hydroxylated metabolites of substituted or unsubstituted aromatic compounds are also susceptible to formation of quinone methide or quinone imine intermediates that are capable of binding to proteins *in vivo* and induce heptatotoxicity. For instance, diclofenac is a non-steroidal anti-inflammatory agent (NSAID) that undergoes metabolism *via* glucuronidation of the carboxylic acid.[85] However, a minor pathway of diclofenac metabolism involves CYP2C9 or 3A4 mediated hydroxylation of the two aromatic rings (Figure 6.21). The hydroxy group is perfectly positioned to undergo a two electron oxidation to the corresponding quinone imine metabolite (Figure 6.21). The reactivity of these metabolites is implicated in the hepatotoxicity of this compound.[86,87] Similarly, the toxicity of high incidence of tacrine hepatotoxicity has been correlated with the formation of 7-hydroxytacrine.[88] The position of the hydroxy group on the aromatic ring in the hydroxytacrine metabolite is suggestive of the formation of a quinone imine moiety as depicted in Figure 6.21.[89]

Likewise, a causative role of the quinone imine intermediate in nefazodone hepatotoxicity has also been speculated.[90] Following incubation of nefazodone with microsomes or recombinant P4503A4 in the presence of sulfydryl nucleophiles, conjugates derived from the addition of thiol to a monohydroxylated nefazodone metabolite have been identified (Figure 6.22). Tamoxifen is an effective drug in the prevention and treatment of breast cancer, but long-term usage of this anti-estrogenic drug has been linked to an increased risk of uterine cancer. A potential pathway leading to toxicity of tamoxifen

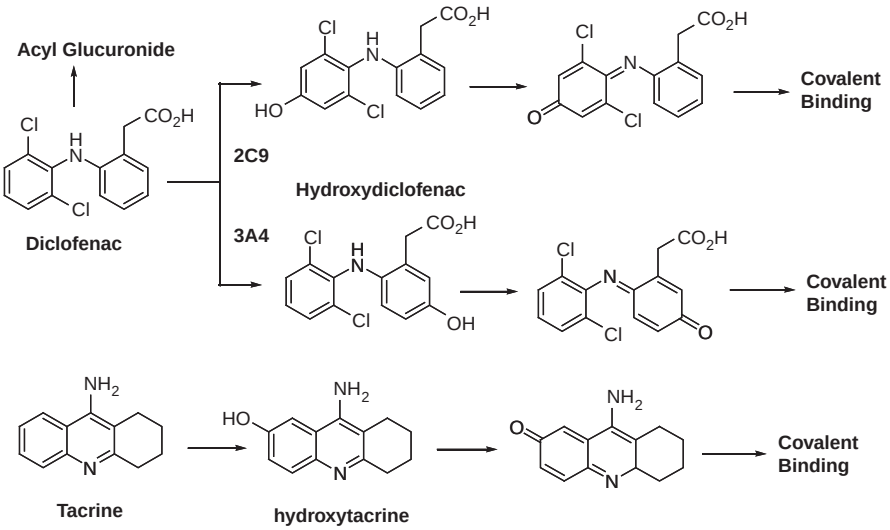


Figure 6.21 P450 catalyzed bioactivation of diclofenac and tacrine to quinone imines via their hydroxylated metabolites.

involves bioactivation of 4-hydroxytamoxifen *via* several pathways as shown in Figure 6.22.[91] These involve formation of the *o*-quinone *via* the catechol or extended quinone methides (Figure 6.22). All these electrophilic species react with glutathione (GSH) and DNA, and may contribute to the genotoxicity of tamoxifen. However, the one that is actually responsible for genotoxicity has not been explicitly identified.

Like epoxides and even though phenolic derivatives are prone to oxidation and generation of reactive metabolites, not all compounds that contain a phenolic moiety are toxic. Hence factors that affect the amount of reactive metabolite formed *in vivo* can influence the toxicity elicited by these compounds *via* metabolic activation. Some factors that can play a major role in controlling the amount of reactive metabolite formed include the dose of the compound and the efficiency of the pathways that detoxify the reactive metabolites or the parent compound.

The quinoid intermediates formed by metabolic activation of phenolic compounds or the phenolic metabolites can also lead to inactivation of CYP enzymes that produce them. For example, nefazodone is an inactivator of CYP3A4.[90] The mechanism of inactivation possibly involves alkylation of the nucleophiles in the active site of the molecule. Raloxifene, a very popular selective estrogen receptor modulator (SERM) used in treatment of osteoporosis and breast cancer, also inactivates CYP3A4 *via* bioactivation ($Ki = 9.9\ \mu M$; $kinact = 0.19\ min^{-1}$).[92] The inactivation of the enzyme is ascribed to the formation of extended quinoid species that is formed by CYP3A4 (Figure 6.23).[92,93] The reactive species has been shown to alkylate the thiol moiety in the active site of 3A4.[94]

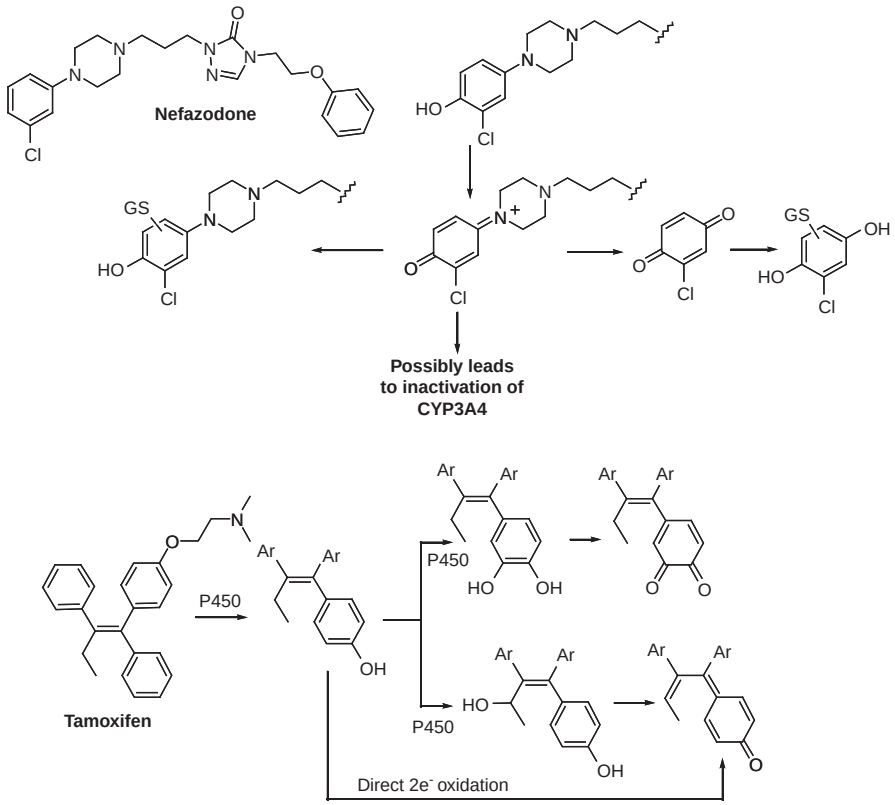


Figure 6.22 Metabolic activation of nefazodone and tamoxifen.

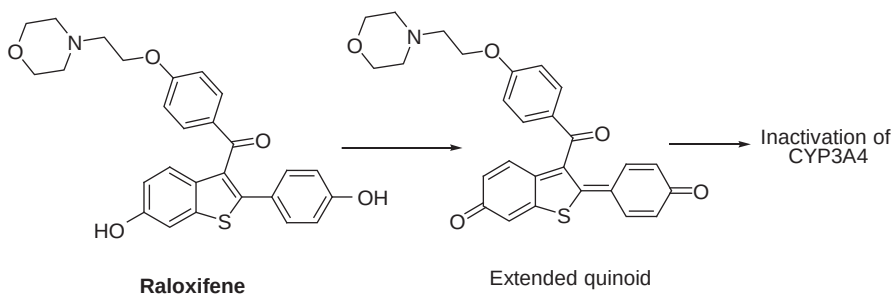


Figure 6.23 Metabolic activation of raloxifene to an extended quinoid intermediate.

Fluorine substitution has been commonly used as a tool to block metabolic activation of compounds and in the development of safer drugs (Figure 6.24). Liehr found that 2-fluoro-estradiol was as estrogenic as estradiol but non-carcinogenic in the Syrian hamster.[95] *In vivo* studies indicate that the presence

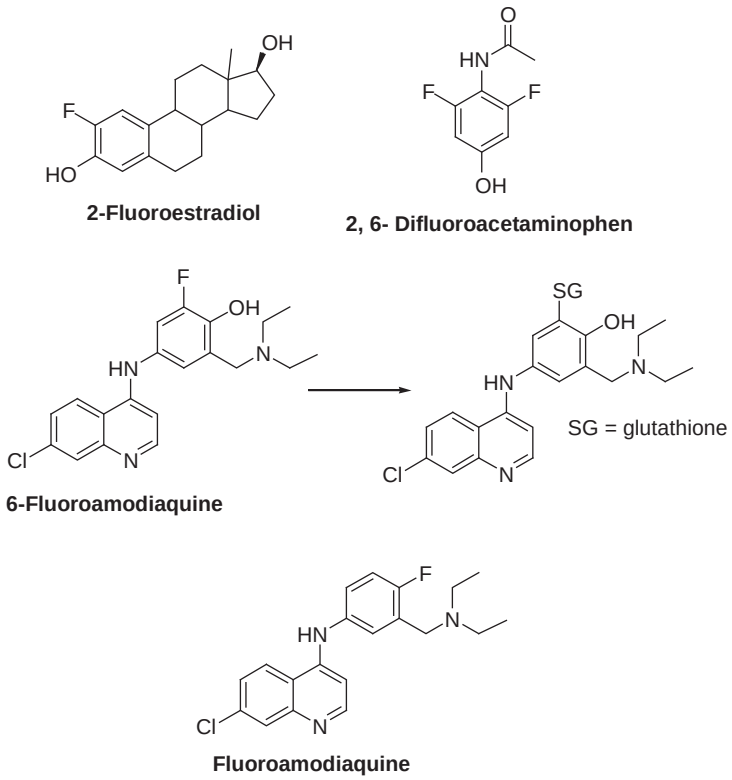


Figure 6.24 Introduction of fluorine into the molecule prevents bioactivation.

of a 2-fluoro substituent diverts the metabolism of the steroid from 2-hydroxylation to glucuronidation and therefore prevents its bioactivation to the quinone intermediates. Introduction of fluorine into acetaminophen also affects its NAPQI formation by altering the oxidation potential.[18] This influence is dependent on the number of hydrogen atoms that are replaced in the molecule and their positions. The presence of fluorine at the 2- and 6-positions (Figure 6.24) increased the oxidation potential of acetaminophen from 1.14 to 1.52, and reduced the propensity of the molecule to undergo oxidative bioactivation thereby reducing hepatotoxicity.[18] However, the glucuronidation and sulfation pathways are not affected by this change and therefore do not affect the elimination of the compound from the body. As is the case for acetaminophen, 6-fluorination of the aminophenol ring of amodiaquine (Figure 6.24) produced an analogue with significantly raised oxidation potential and reduced bioactivation *in vivo*.[18] However, some bioactivation was observed due to defluorination followed by formation of the quinone imine, as observed in amodiaquine. Similarly, isosteric replacement of the hydroxyl function with fluorine also provided an analogue, fluoroamodiaquine

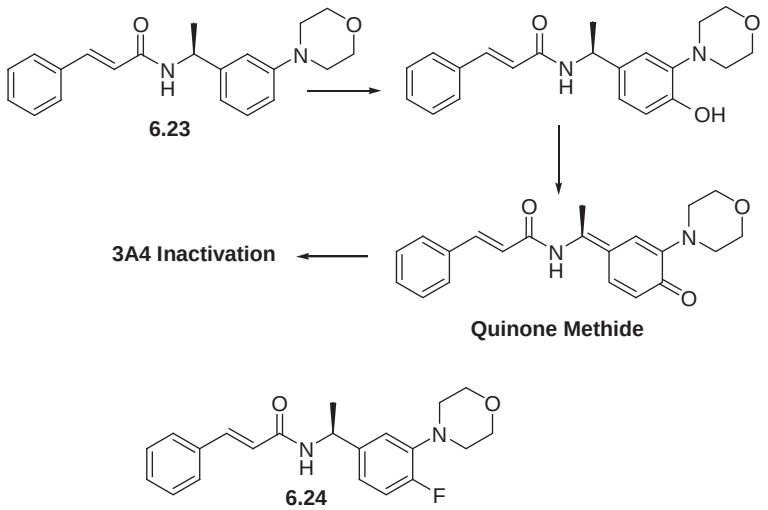


Figure 6.25 Inactivation of CYP3A4 by KCNQ2 potassium channel opener **6.23**.

(Figure 6.24), which had similar antimalarial potency to the parent drug but was not bioactivated to toxic metabolites *in vivo*.[18]

The following example demonstrates modification of the lead compound to prevent mechanism based inhibition by introducing fluorine into the molecule. The lead compound (**6.23**) was designed to be a KCNQ2 potassium channel opener and had excellent oral bioavailability and pharmacological activity (Figure 6.25).[96] However, **6.23** was an inactivator of CYP3A4. P450 inactivation was consistent with a bioactivation pathway involving the initial aromatic hydroxylation *ortho* to the morpholine ring (or *para* to the benzylamine methine). Further two electron oxidation of this initial metabolite by P450 can result in the formation of the reactive quinone methide intermediates. The potential formation of either reactive intermediate was avoided by the introduction of the fluorine atom on the position ortho to the morpholine ring. Compound **6.24** is not only devoid of P450 inactivation liability, but also retains the pharmacological and pharmacokinetic properties of the prototype compound **6.23**.

Although fluorine atoms have been commonly used in blocking metabolism and bioactivation (as described earlier), it is important to note that fluorination of the aromatic ring does not necessarily prevent the compound from undergoing bioactivation or from inactivating the enzyme.[18] More recently, dasatinib, a tyrosine kinase inhibitor, has been shown to inactivate CYP3A4 and this inactivation proceeds through a reactive intermediate.[97] The major mechanism of inactivation proceeds through hydroxylation at the 4-position of the 2-chloro-6-methylphenyl ring in the molecule followed by further oxidation forming a reactive quinone-imine (Figure 6.26). Interestingly, blocking the para

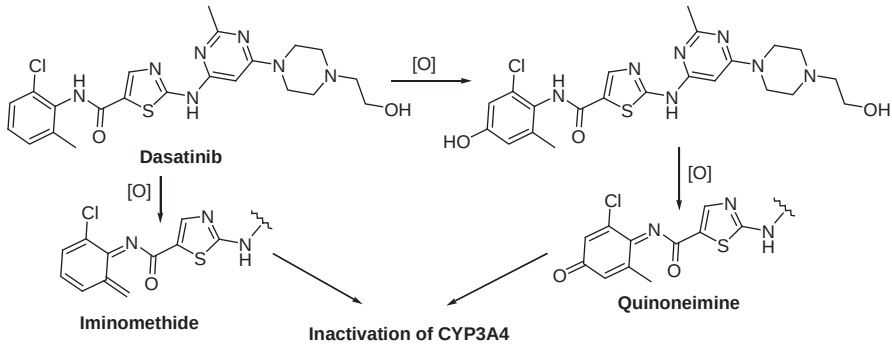
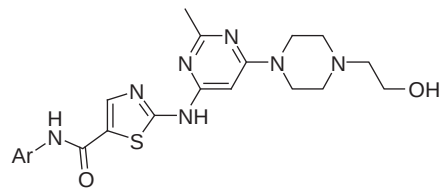
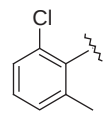
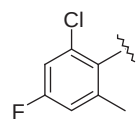
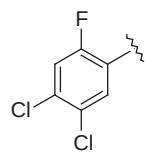


Figure 6.26 Bioactivation of Dasatinib.

Table 6.17 Effect of fluorine on inactivation of CYP3A4 by dasatinib and its analogs.[97]



Compound	*Ar*	K_I *(μM)*	K_{inact} *(min^{-1})*
Dasatinib		6.3	0.034
6.25		5.4	0.039
6.26		No inactivation	

position with fluorine did not prevent inactivation of CYP3A4 (Table 6.17). The rates of inactivation were virtually identical and the K_I for the fluorinated analog was lower, implying that it is more efficiently bioactivated possibly by virtue of increase in the clogP value due to the incorporation of fluorine.

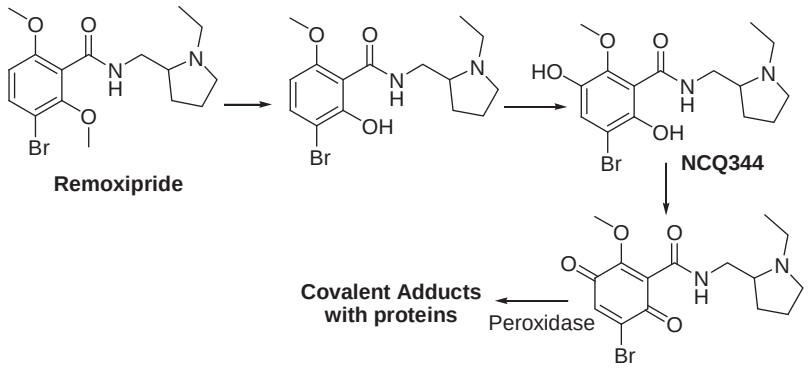


Figure 6.27 Metabolic activation of remoxipride via *O*-dealkylation.

However, introduction of three halogens on the molecule prevented the bioactivation and therefore the inactivation of the compound.[97]

Other functional groups present on the aromatic rings that generate phenolic metabolites also play a role in mediating toxicity *via* metabolism. For instance, an *O*-dealkylated metabolite resulting in a phenolic species has been implicated in aplastic anaemia observed for the neuroleptic remoxipride.[98,99] Elegant mechanistic studies conducted by Erve and co-workers have demonstrated that NCQ344, a hydroquinone metabolite formed *via* O-dealkylation and subsequent oxidation, is capable forming a reactive *para*-quinone (Figure 6.27) by stimulated human neutrophils or myeloperoxidase.[100]

A similar metabolic activation pathway has been observed in metabolism studies of an investigational maxi-K channel opener, MaxiPost, being developed to treat ischemic stroke.[101] The metabolite M1 of MaxiPost is bioactivated *via* M1 to form a quinone methide, which has been shown to covalently bind to proteins (Figure 6.28). Although major toxic events have not been associated with this compound, covalent binding of the compound *in vivo* in humans poses a major risk of possible idiosyncratic reactions.

Compounds containing aromatic amines (anilines) induce a variety of toxicological responses including carcinogenicity and hepatotoxicity. Several drugs containing an aniline moiety, which have been withdrawn from the market, have a black box warning on their labels. Therefore, anilines have been put on the black list of functional groups that the medicinal chemists generally avoid. Anilines can also undergo a two electron oxidation to dimines or quinone imines depending on the functionality at the 4-position of the amino group (some of the drugs discussed above are derivatives of aniline).

However, the primary pathway of bioactivation of an aromatic amine is hydroxylation of the amino group.[102,103] *N*-oxidation leads to generation of the reactive species *via* conjugation (sulfation or acetylation) of the corresponding hydroxylamine. The electrophilic intermediate thus formed by elimination of the sulfate moiety or the acetoxy moiety can covalently bind to cellular macromolecules (Figure 6.29). Alternatively, the hydroxylamine is

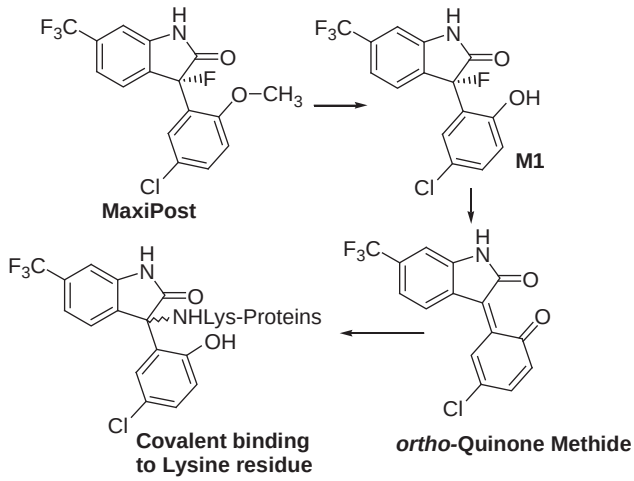


Figure 6.28 P450-catalyzed metabolic activation of MaxiPost.

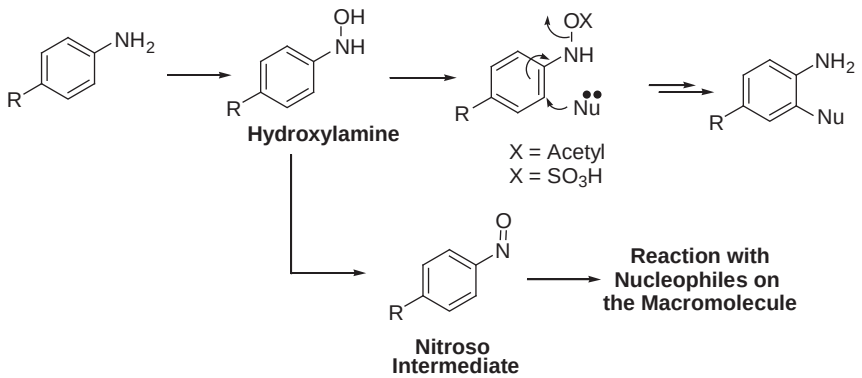


Figure 6.29 Activation of aromatic amines.

oxidised to a nitroso derivative, which reacts with the nucleophile on the macromolecule.[104]

Some drugs that contain an aniline group and have been withdrawn from the clinic include nomifensine (Figure 6.30) and carbutamide (Figure 6.3). Nomefensine was withdrawn from the market due to acute immune haemolytic anaemia. The aniline moiety in this drug has not been implicated in its toxicity, but its role in inducing toxic events *via* reactive metabolites cannot be ruled out.[105] Carbutamide, an oral antidiabetic, was withdrawn from the market due to life-threatening bone marrow toxicity in man.[106] The toxicity was attributed to the activation of the aniline moiety since tolbutamide, in which the amino group was replaced with a methyl group (Figure 6.3), was devoid of toxicity.

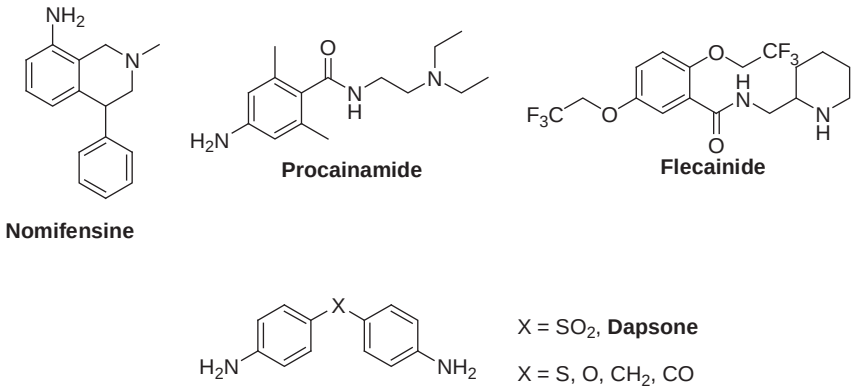


Figure 6.30 Structures of aniline containing compounds and its analogs.

Procainamide (Figure 6.30) is another drug where removal of the aniline moiety translates into a markedly improved safety profile. Bone marrow aplasis and lupus syndrome observed upon administration of procainamide is not observed upon administration of flecainide, which is devoid of aniline moiety.[107]

Dapsone (Figure 6.30), an antibacterial, is also shown to induce agranulocytosis, aplastic anaemia and cutaneous adverse drug reactions; all these toxicities are associated with *N*-oxidation of aniline nitrogen.[108–110] The influence of electronics on bioactivation potential is evident in structure–toxicity assessment on dapsone analogues. Replacement of the sulfone group in dapsone with sulfur, oxygen, methylene or carbonyl substituents significantly reduce methemoglobinaemia in human erythrocytes.[111] Although the reason for the influence of these substituents is unclear, a good correlation between haemotoxicity and the Hammett constant (σ_p) has been demonstrated in this analysis indicating that the substituent in the 4-position can potentially affect the rate of oxidation of the aniline nitrogen.

Compounds containing nitroaromatic rings, which can be metabolically reduced to anilines, can also elicit toxicity. Tolcapone, a catechol-*O*-methyltransferase inhibitor (Figure 6.31), has been associated with a number of problems including abnormalities in liver function tests and three cases of fatal hepatotoxicity.[112] These problems have led to withdrawal of the drug from the market in some countries and the introduction of a black box warning and intensive monitoring requirements in the United States. A significant portion of tolcapone biotransformation proceeds *via* reduction of the nitrobenzene group to the aniline derivative, which is transformed to the corresponding anilide by *N*-acetyltransferase. Both the aniline and the anilide metabolites of tolcapone undergo facile two electron oxidation to the corresponding quinone-imine metabolites that are trapped with GSH (Figure 6.31).[113] Interestingly, the structural analog of tolcapone, entacapone, does not undergo similar bioactivation in spite of the presence of the same structural motif. This is attributed to minimal (or lack of) reduction of the nitro group to the aromatic amine in

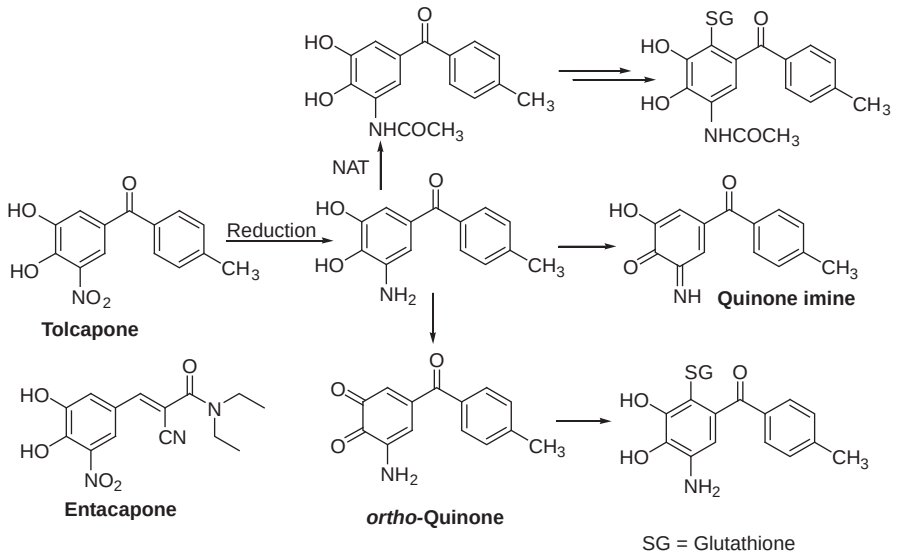


Figure 6.31 Metabolic activation of tolcapone via reduction of the nitro group.

humans. Instead, its principal clearance pathway involves *N*-de-ethylation of the tertiary amide substituent and isomerisation of the active *E*-isomer to the inactive *Z*-isomer, followed by glucuronidation of the catechol moiety.[114]

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CHAPTER 7

Influence of Heteroaromatic Rings on ADME Properties of Drugs

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7.1 Introduction

Aromatic heterocycles play a critical role in medicinal chemistry and drug design.[1–3] More than half of known drugs contain at least one heterocyclic component in their structure. New chemical entities are generally designed as analogues of endogenous ligands that are vital to biochemical processes or, in some cases, as simplified congeners of complex natural products. Since most of these substrates contain heterocyclic rings, these rings by default become core structures of the newly designed compounds. These drugs compete with endogenous ligands and mimic (agonist) the ligands in their action. Alternatively, they block (antagonist) the active site in the receptor and therefore its normal function. Some common endogenous ligands that have heteroaromatic rings as their core structure include histamine, 5-hydroxytryptamine (serotonin), adenosine 5′-triphosphate (ATP) and nucleic acid bases such as the

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uracil, thymine, cytosine, guanine and adenine (Figure 7.1). Figure 7.2 represents some drugs that are structurally similar to these ligands.

Cimetidine, an antiulcer drug (Figure 7.2), was one of the first compounds developed through rational drug design.[4] This is a classic example of where the endogenous ligand, histamine, was used as a lead to design a H_2 receptor

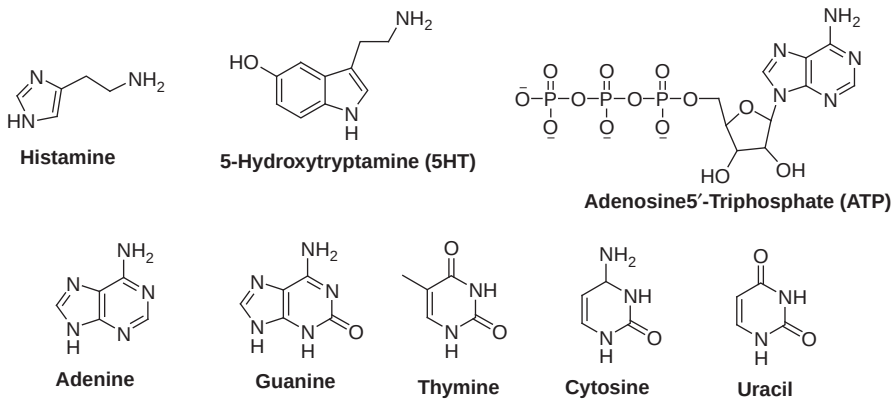


Figure 7.1 Structures of endogenous ligands that contain heterocyclic rings.

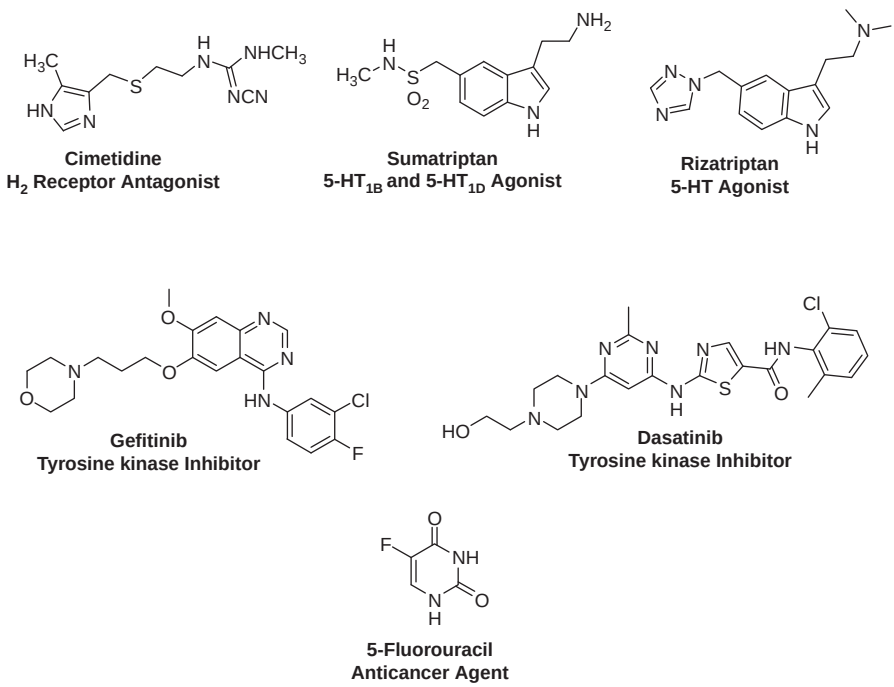


Figure 7.2 Drugs that structurally mimic endogenous ligands.

antagonist that could block the undesired effects of this endogenous ligand, which is a mediator of gastric hypersecretory disorders and allergic responses. Like histamine, cimetidine also has an imidazole ring and can bind to the active site of the H_2 receptor. It competitively inhibits histamine at the H_2 receptors of parietal cells and therefore reduces acid secretion.

Since then, several endogenous transmitters have been used to design drugs that can either mimic or antagonise the effects of the natural ligand. For instance, triptans mimicked serotonin and were designed as specific and selective agonists for the 5HT receptors for the treatment of migraine and cluster headaches.[5] Since the discovery of sumatriptan, several analogues with improved potency/selectivity and/or oral bioavailability have been made. All triptans contain an indole nucleus and basic nitrogen at a similar distance from the nucleus like serotonin but differ in their side chain (Figure 7.2).

Another example involves designing small molecules that inhibit various protein kinases.[6,7] These compounds compete with ATP and prevent the phosphorylation of important physiological substrates. Although not structurally similar to ATP, these compounds possess heterocyclic cores that interact with various residues in the ATP binding site of the enzymes.[8] Figure 7.2 represents two such receptor tyrosine kinase inhibitors that have been recently approved in treatment of cancer.

Several natural products containing heteroaromatic rings have been used as leads to design new molecules that can mimic their pharmacological activity.[9] One example includes quinine, an antimalarial, which was used as a lead for two drugs—primaquine and chloroquine. The latter two drugs remain therapeutic standards for the treatment of malaria (Figure 7.3). Other historical examples of analog design from natural products include the design of local anaesthetic procaine from the alkaloid cocaine, or the synthesis of potent 3-hydroxyl-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor ('statins') such as rosuvastatin, inspired from lovastatin.

Even where the natural substrate or ligand for a biological target does not contain a heterocycle, compounds designed to act on that target frequently contain heterocyclic groups. These compounds can function as agonists or antagonists of the receptor depending upon the presence of the heterocyclic motifs incorporated in them. Figure 7.4 shows some examples of this class.

In some cases, compounds that resemble essential metabolites can provide false synthons in biosynthetic processes. Some examples include anti-metabolites such as methotrexate (an analog of folic acid) or gemcitabine, cladribine or 5-fluorouracil, which resemble deoxycytidine, deoxyadenosine or uracil, respectively (Figure 7.5).

Heteroaromatic rings have been used as bioisosteres by medicinal chemists to design new candidates. Several functional groups including phenyl rings or carboxylic acid and its ester analogues have been replaced by heterocyclic rings and have resulted in therapeutically attractive compounds.[10,11] These replacements often confer structural integrity to the molecules and hold functionalities in a particular geometry. Alternatively, the presence of a heteroatom in the ring has been shown to increase interactions with the receptor or the enzyme, and thus render greater pharmacological activity or specificity to resulting

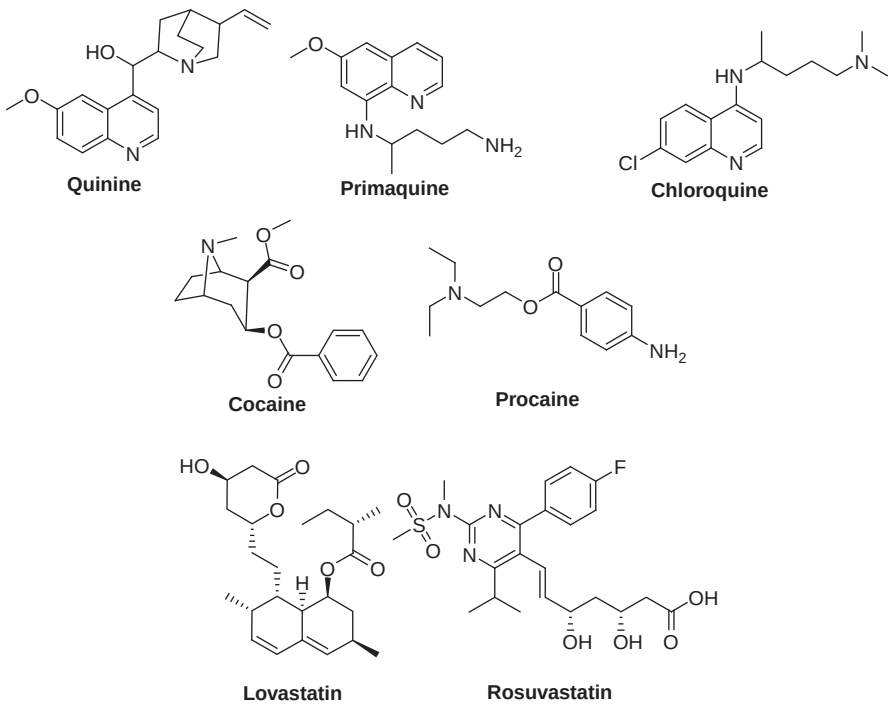


Figure 7.3 Structures of some naturally occurring ligands and their synthetic analogs.

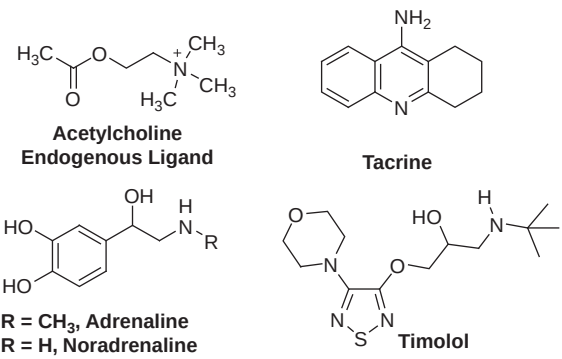


Figure 7.4 Examples endogenous ligands that lack heteroaromatic rings and their structural mimics.

compounds. For example, the increased potency of pioglitazone (Figure 7.6) compared with its predecessor ciglitazone is primarily attributed to fortuitous incorporation of an additional receptor binding group (pyridine moiety).[12]

Introduction of heteroaromatic rings into drug molecules also affects their physicochemical properties, which in turn can alter their absorption, distribution,

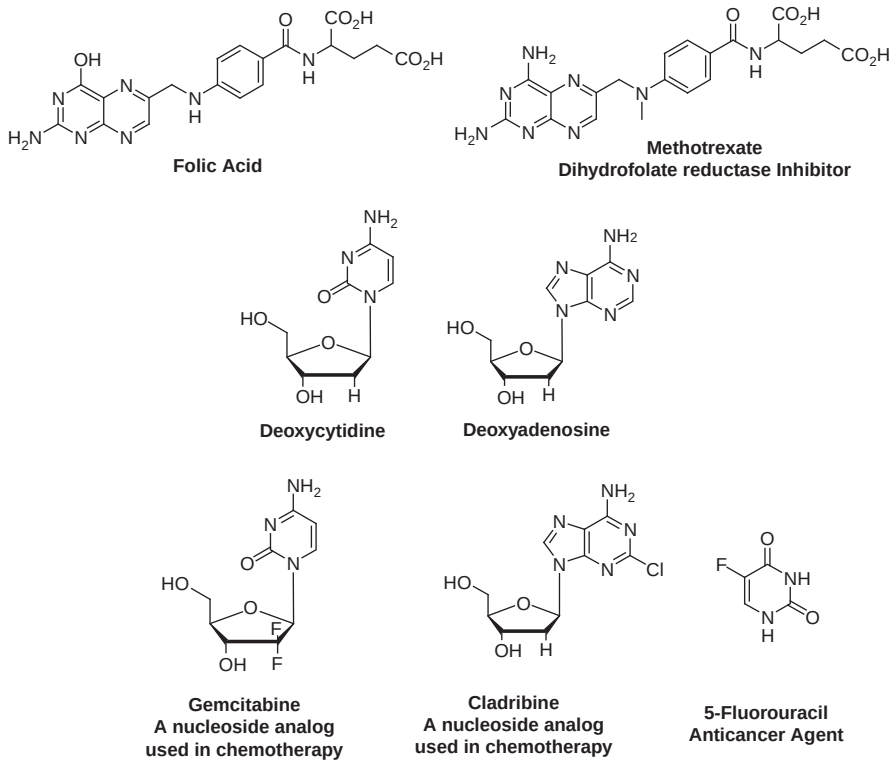


Figure 7.5 Structures of antimetabolites.

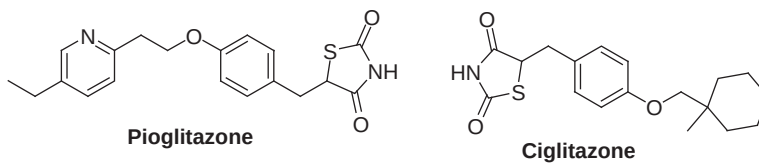


Figure 7.6 Structure of pioglitazone and ciglitazone.

metabolism and excretion (ADME) profiles. In the following sections, the influence of the heteroaromatic rings on the physicochemical and ADME properties of a molecule are reviewed. Attempts are made to correlate the effect of the change in the physicochemical properties such as log P/log D, acidity/basicity (pKa), hydrogen bonding capability of molecules, and polar surface area (PSA) on their ADME properties. Calculated physicochemical parameter values using ACD software are used to describe the effect of a structural change (incorporation, removal or replacement of an aromatic heterocycle) on ADME characteristics.

7.2 Types of Heteroaromatic Rings and their Physicochemical Properties

Two types of heteroaromatic rings that are commonly found in drugs are the six-membered heteroaromatic ring and five-membered heteroaromatic ring systems.[13–15]

All six-membered heterocycles and their benz-fused derivatives are analogs of carbocyclic rings (phenyl or the napthyl ring), where one or more methine (CH) groups in the rings are replaced by a sp^2-hybridised nitrogen atom. These rings maintain the same electronic structure as the carbocyclic rings (they have a complete cycle of p-orbitals containing 6π-electrons). The most commonly used six-membered rings and their benz-fused derivatives that constitute sub-structures of drugs are shown in Table 7.1.

Table 7.1 Physicochemical properties of commonly used six-membered rings.[a]

Heterocycle	*Name*	*MW*	*LogP*	*pKa*	*PSA*
	Benzene	78.1	2.18		
	Pyridine	79.1	0.84	5.23	12.89
	Pyrimidine	80.1	0.26	1.78	25.78
	Pyridazine	80.1	–0.51	2.82	25.78
	Pyrazine	80.1	0.002	0.92	25.78
	1,2,3-Triazine	81.1	−0.73	1.33	38.67
	1,2,4-Triazine	81.1	−0.92	1.32	38.67
	1,3,5-Triazine	81.1	−0.73	1.59	38.67
	Tetrazine	82.1	−1.47	1.6	51.56
	Quinoline	129.2	2.13	4.97	12.89
	Quinazoline	130.2	1	3.44	25.78
	Cinnoline	130.2	1.14	2.79	25.78
	Pthalazine	130.2	1.42	0.29	25.78

[a]All values were calculated using ACD software (version 11.0).

Although the presence of an electronegative nitrogen atom in the ring does not alter the character of the π-orbitals of these rings, it distorts the distribution of the π electrons and therefore lowers the energies of π-orbitals relative to the phenyl ring. This coupled with the inductive effect of the electronegative nitrogen atom makes it less reactive and renders properties that corroborate with conjugated imines or conjugated carbonyls.[15] As the number of sp^2 nitrogen atoms in the ring increase, there is a further decrease in the reactivity of the rings. This makes it less susceptible to P450 mediated oxidation. However, the lone pair of electrons on the nitrogen atom that are planar to the ring provide a site for protonation and other reactions such as *N*-oxidation (equivalent to a tertiary amine), which is not analogous to the carbocyclic ring system.

The presence of sp^2 nitrogen atoms in the ring also affects the physicochemical properties of these rings. These rings are weak bases in comparison with their alicyclic counterparts, piperidine or piperazines. As shown in Table 7.1, pyridine and quinoline are the most basic of them all, with a pKa of ~ 5. As the number of nitrogen atoms in the ring increase, the pKa decreases. The pKa is also affected by the position of the second nitrogen atom in the ring (Table 7.1). The replacement of a methine group of the phenyl ring with nitrogen atoms also affects the lipophilicity (log P value) and the polarity of heteroaromatic rings compared with the benzenenoid rings. As the number of ring nitrogens increase, the log P of the fragment decreases and the polarity increases (Table 7.1). Thus, introduction of one nitrogen atom lowers the log P of a phenyl ring from 2.13 to 0.65, while the log P value of pyrimidine and triazine ring is -0.3 and -0.7, respectively.

Five-membered heterocyclic rings form the second popular class of heteroaromatic ring systems that are commonly used as substructures in compound synthesis. The more commonly used five-membered heterocyclic rings and their benz-fused analogs are shown in Table 7.2 and Table 7.3, respectively. Like six-membered rings, all five-membered rings and their benz-fused derivatives share the same electronic structure of the phenyl ring, though the presence of only five atoms in the ring instead of six makes these rings smaller in size relative to the carbocyclic and six-membered heteroaromatic rings.

Five-membered rings with a single heteroatom are the simplest ring systems that belong to this class. The most common rings in this class are the pyrrole, furan and the thiophene and their respective benz-fused analogs (indole, benzofuran and benzothiophene); they contain a nitrogen, an oxygen and a sulfur atom, respectively, in the ring system. Given the electron rich nature of these rings, the five-membered rings are more reactive than the six-membered rings and can undergo oxidative metabolism like the benzenoid rings (see Section 7.3.3). However, the degree of reactivity and the physicochemical properties of these rings are dependent on the nature and the electronegativity of the heteroatom present in the ring (Table 7.2). Thus, thiophene and furan is the most lipophilic of all with a log P value that is quite similar to benzene (1.74 and 1.95 *versus* 2.13) while the log P value of pyrrole is 0.85. The pyrrole moiety possesses an NH group that is typical of a secondary amine; however, the basicity of pyrrole, pKa = -3.8 for a conjugated acid, is much less than that of a secondary amine (pKa = 10.87). This large difference is primarily due to the

Table 7.2 Physicochemical properties of commonly used five-membered rings.

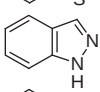
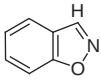
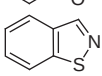
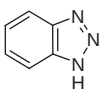
Heterocycle	*Name*	*MW*	*LogP*	*pKa*[a]	*PSA*	*pKa*[b]
	Benzene	78.1	2.18			
	Pyrrole	67.1	0.85	−0.27	15.79	17
	Furan	68.1	1.74		13.14	
	Thiophene	84.1	1.95		28.24	
	Imidazole	68.1	−0.1	7.1	28.68	14
	Oxazole	69.1	0.12	0.98	26.03	
	Thiazole	85.1	0.44	2.72	41.13	
	Pyrazole	68.1	0.36	2.83	28.68	14
	Isoxazole	69.1	0.51	−2.03	26.03	
	Isothiazole	85.1	1.14	4	41.13	
	1,2,3-Triazole	69.1	−0.63	1.2	41.57	8.7
	1,2,4-Triazole	69.1	−0.58	2.7	41.57	10.5
	4*H*-1,2,4-Triazole	69.1	−0.89	2.7	41.57	10.2
	2*H*-1,2,4-Triazole	69.1	0.009	1.2	41.57	8.7
	1,2,5-Oxadiazole	70.1	0.16	−1.47	38.92	
	1,2,4-Oxadiazole	70.1	−0.69	−0.28	38.92	
	1,2,3-Oxadiazole	70.1	−0.68	−4.12	38.92	
	1,3,4-Oxadiazole	70.1	−0.69	−4.12	38.92	
	1,2,5-Thiadiazole	86.1	0.34	−0.28	54.02	
	1,2,4-Thiadiazole	86.1	−0.2	0.46	54.02	

Table 7.2 (*Continued*)

Heterocycle	*Name*	*MW*	*LogP*	*pKa*[a]	*PSA*	*pKa*[b]
	1,2,3-Thiadiazole	86.1	0.96	−3.39	54.02	
	1,3,4-Thiadiazole	86.1	−0.2	−0.63	54.02	
	Tetrazole	70.1	−0.6	0.8	54.46	4.73

[a]pKa for conjugate acid,
[b]pKa for conjugate base.

Table 7.3 Physicochemical properties of commonly used benz-fused 5-membered rings.

Heterocycle	*Name*	*MW*	*LogP*	*pKa*[a]	*PSA*	*pKa*[b]
	Indole	117	2.59	−2.4	15.79	
	Benzofuran	118	2.68		13.14	
	Benzothiophene	134	3.2		28.68	
	Benzimidazole	118	1.32	5.26	28.68	13.2
	Benzoxazole	119	1.59	1.17	26.03	
	Benzothiazole	135	1.9	0.85	41.13	
	Indazole	118	1.77	1.26	28.68	
	Benzisoxazole	119	2.02	−2.03	26.03	
	Benzoisothiazole	135	1.85	4.37	41.13	
	1,2,3-Benzotriazole	119	1.44	1.17	41.57	

[a]pKa for conjugate acid,
[b]pKa for conjugate base.

incorporation of the non-bonding electron pair of the N-atom into the cyclic conjugated system of the pyrrole molecule. The pyrrole ring is also a weak acid (about the same strength as the simple alcohol) and the pKa of its conjugate base is 17 in aqueous solution.

These three rings form a platform for rest of the heteroaromatic rings that contain an additional sp^2 nitrogen atom (Table 7.2). The variation in the position of the extra nitrogen atom further adds to the structural diversity of the groups especially for heterocycles containing the two or three heteroatoms.

Extra nitrogen atoms and their placement in the ring (relative to the first heteroatom) also have an important effect on the properties of the ring system. The additional nitrogen atom has lone pair of electrons that is in plane with the ring and therefore has attributes that are quite similar to six-membered heteroaromatic rings. Like pyridine and its six-membered analogs, the increase in number of nitrogen atoms causes a decrease in the energy levels of the π orbitals so that the heterocycles are less electron rich compared with their counterparts containing only one heteroatom. This makes them less reactive relative to the pyrrole, thiophene and furan.

The effect of additional sp^2 nitrogen atoms can also be seen on the acidity and basicity of these heterocycles (Table 7.2). Even though all 'azoles' are weak bases like the pyrrole, the lone pair of electrons adds sites of protonation and therefore most 'azoles' are stronger bases than pyrrole (Table 7.2).

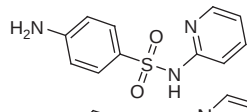
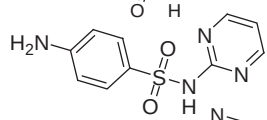
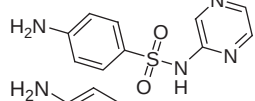
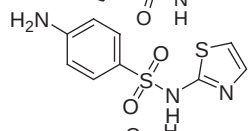
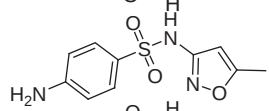
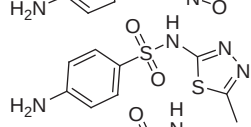
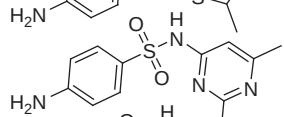
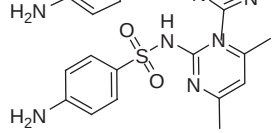
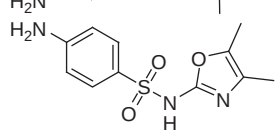
The position of the sp^2 nitrogen atom relative to the first heteroatom also influences the basicity of the rings. For example, the imidazole ring has a pKa of around 7.1 while pyrazole (nitrogen atom at the two position relative to the first nitrogen) has a pKa of 2.5. The azoles containing additional NH groups (imidazole, pyrazole, triazoles and tetrazoles) are stronger acids than the pyrrole. Thus, imidazoles and pyrazoles are stronger acids (pKa of 14) than pyrrole (pKa $\sim$17), while the triazoles and the tetrazole have pKa values of 9.5 and 4.92 (which is equivalent to a carboxy group), respectively.

The addition of nitrogen atoms also affects the log P value of these rings. The logP values of all rings are listed in Table 7.2. The additional nitrogen atom adds polarity to the ring and hence lowers the lipophilicity of all these rings relative to their one heteroatom analogs. However, the trend is similar to that observed in the rings with single heteroatom, *i.e.* $\log_{\text{Pthiazole or isothiazole}} > \log_{\text{Poxazole or isoxazole}} > \log_{\text{Pimidazole or pyrazole}}$.

An illustration of the effect of incorporation of different heterocyclic rings on physicochemical properties of compounds is depicted in Table 7.4. Replacement of a pyridine ring in sulfapyridine with pyrazine or pyrimidine results changes the clogP and PSA values significantly. Similarly, replacement of six-membered rings with thiazole, isoxazole or thiadiazole in sulfathiazole, sulfamethoxazole and sulfamethizole affects the lipophilicity and the PSA of the molecule depending upon the ring incorporated in the compound. Also, the addition of the type of substituents and the position of attachment can sometimes affect the physicochemical properties of a compound.

In addition to monocyclic heterocycles and their benz-fused derivatives, the 'hybrid' bicyclic rings resulting from annulation of two aromatic heterocycles have also become very popular among medicinal chemists.[2] These rings are comprised of either five-membered rings fused with five-membered rings or five-membered rings fused with six-membered rings. Given the number of heterocycles that belong in the five-membered rings and five-membered rings classes,

Table 7.4 Effect of heteraromatic rings on the physicochemical properties of sulfa drugs.

Compound	*Structure*	*MW*	*clogP*	*clogD*$_{7.4}$	*PSA*
Sulfapyridine		249	0.47	0.41	93.46
Sulfadiazine		250	−0.07	−0.99	106
Sulfapyrazine		250	0.05	−1.68	106
Sulfathiazole		255	0.05	−0.33	121.7
Sulfamethoxazole		253	0.66	−0.54	106
Sulfamethizole		270	0.52	−1.16	134
Sulfisomidine		278	0.3	0.004	106
Sulfadimidine		278	0.58	−1.0	106
Sulfamoxzole		267	0.68	0.32	106

several bicyclic rings can be envisioned in this hybrid class. Some members of this group are commonly used in drug design and are considered bioisosteres of the benz-fused six-membered and five-membered rings. Others are commonly observed in the natural and endogenous ligands, such as the purine ring (imidazopyrimidine) observed in DNA bases or the pteridine (pyrazinopyrimidine) observed in folic acid. Drugs containing these rings are shown in Figure 7.7.

7.3 Influence of Heteroaromatic Rings on ADME Properties of Compounds

One goal of introducing a heterocycle as a bioisostere in a compound is to improve its absorption, distribution (*e.g.* brain penetration) and clearance

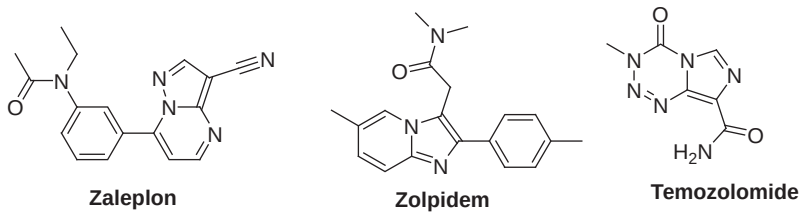


Figure 7.7 Examples of drugs with 'hybrid' bicyclic ring systems.

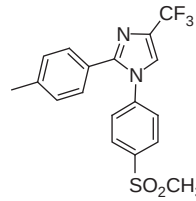
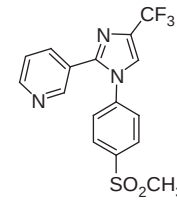
characteristics while maintaining the activity of that compound. As discussed earlier, incorporation of heterocyclic rings into drugs can affect their physical properties and hence modify their pattern of absorption, metabolism or toxicity. The following sections present some case studies that illustrate the influence of the heteroaromatic rings on the absorption, distribution and clearance (metabolism and excretion) of drugs.

7.3.1 Absorption

For most molecules, the rate and extent of absorption is largely dependent on the dissolution of the compound in the aqueous contents of the gastrointestinal tract and the permeability through the membrane. These in turn depend upon the molecular characteristics of a drug such as molecular weight/size, aqueous solubility, lipophilicity and the hydrogen bonding capacity of the molecule.[16] Since aqueous solubility dictates the amount of drug that will be available for absorption, most efforts are often directed towards the optimisation of solubility of molecules.[17] One approach involves introduction of polar solubilising groups in a molecule to modulate the lipophilicity of the compound and hence increase its absorption. Replacement of phenyl rings in the molecule with aromatic heterocycles does not change the molecular weight (MW) of the compound to a significant extent. However, it can modify the log D and pKa of compounds to improve the absorption properties of a molecule.

Pyridine ring is one of the heterocycles that is commonly used to modify the absorption properties of molecules. Replacement of a phenyl ring with this ring not only imparts unique ionising properties at the acidic pH (therefore increasing solubility), but also provides a handle for the introduction of various groups that help in modulating the solubility and lipophilicity characteristics of new molecules, which can assist in absorption of the compound. Khanna and coworkers exemplify this in their continued efforts to seek new diaryimidazoles as COX-2 inhibitors with better oral activity.[18] Replacement of a tolyl group in compound **7.1** (Table 7.5) with a pyridine ring (compound **7.2**) yielded heteroaromatic analogues with improved water solubility which resulted in excellent bioavailability ($>90\%$) and high plasma levels in the rat following a dose of $20\,mg\,kg^{-1}$ relative to **7.1**. The key to enhanced absorption of **7.2** was probably ascribed to improved physicochemical properties of this compound

Table 7.5 Physicochemical properties of COX-2 inhibitors.[18]

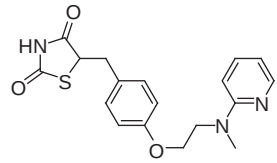
		
Property	*Compound 7.1*	*Compound 7.2*
MW	380	367
ClogP	2.68	1.13
PSA	60.34	73.23
$clogD_{6.5}$	2.68	1.13
$Sol_{6.5}$ (mg/ml)	0.005	0.05

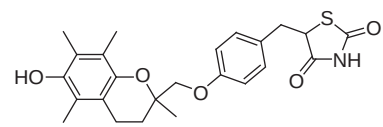
(Table 7.5). Replacement of tolyl ring in **7.1** with a polar bioisosteric pyridyl ring in **7.2** lowered the lipophilicity of **7.2** (clogP of **7.1** = 2.68 and clogP of **7.2** = 1.13) sufficiently to increase its aqueous solubility and therefore it bioavailability.

Incorporation of a pyridine ring has also been shown to alter the absorption characteristics of the glitazones. Assessment of the pharmacokinetic properties of rosiglitazone indicated complete absorption following oral administration of [^{14}C]rosiglitazone solution (8 mg) and rosiglitazone tablets (4 mg, B.I.D.), resulting in an absolute bioavailability of 95% and 99%, respectively.[19] In contrast, the oral bioavailability of troglitazone was estimated to be 40–50% following oral administration of a 400 mg dose to humans.[20] In addition, administration of troglitazone (400 mg) with high fat meal increased the plasma AUC by 30% to 80%, suggesting that oral absorption was incomplete in the fasted state. Comparison of the physicochemical properties of these two glitazones is shown in Table 7.6. Enhanced absorption of rosiglitazone is surmised in part to the improved solubility at the clinically relevant doses. The key modification is the replacement of the chroman moiety in troglitazone with an aminopyridine ring in rosiglitazone. This change resulted in lower clogP of rosiglitazone relative to troglitazone (3.02 *versus* 4.69) and increase in solubility (0.05 *versus* 0.003 mg/ml) (Table 7.6). It is important to note that the dose, which to a degree is also dependent upon its potency, also affects the solubility of the compound in the gastrointestinal tract. Thus, in addition to lipophilicity, increased solubility of rosiglitazone in contrast to troglitazone can be ascribed to a lower dose (4 mg B.I.D., or 8 mg QD) of rosiglitazone in contrast to troglitazone (200–400 mg, QD). The primary difference between the two structurally related glitazones is its affinity for the PPAR-γ receptor (332 nM for troglitazone and 36 nM for rosiglitazone).

Optimum pKa and logP value of imidazole rings also makes this ring very attractive in imparting solubilising properties in a molecule and modulating the lipophilicity. These rings with appropriate substituents are also commonly used

Table 7.6 Comparison of troglitazone and rosiglitazone.[19,20]



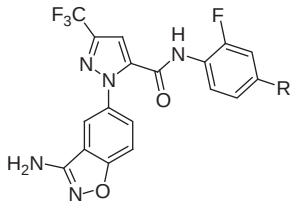


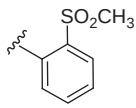
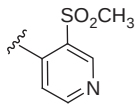
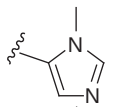
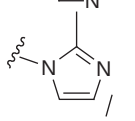
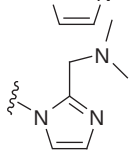
Property	*Rosiglitazone*	*Troglitazone*
MW	357.4	441.5
clogP	3.02	4.69
PSA	96.83	110.16
$clogD_{6.5}$	2.35	4.31
$Sol_{6.5}$ (mg/ml)	0.05	0.003
% Oral bioavailability	95–99%	40–50%

in the optimisation of the pharmacokinetic properties of candidates in early discovery. Quan and co-workers have demonstrated the use of imidazole ring to enhance permeability of the lead compound, which is a potent Xa inhibitor.[21] These efforts have resulted in the discovery of a clinical candidate, razaxaban (Table 7.7), which is an orally active factor Xa inhibitor. In this programme, although the lead compound (compound **7.3**, Table 7.7) was highly selective as a Xa inhibitor, it had poor permeability (Caco-2 Papp is $<0.1\times10^{-6}\,cm\,sec^{-1}$) and solubility ($<0.1\,mg\,mL^{-1}$) resulting in only 2% bioavailability in dogs. Since the clogP of the compound was moderate (3.12), low permeability characteristics were probably attributed to the high PSA of the molecule (142 $Å^2$). In an effort to address the permeability issue, the phenylsulfonamide moiety was replaced with several heterocycles as shown in Table 7.7. Although the pyridylsulfonamide group (**7.4**) showed slight improvement in the permeability in Caco-2 studies ($0.54\times10^{-6}\,cm\,sec^{-1}$), replacement of the phenylsulfonamide group in the molecule with solubilising heterocycles such as 1-methylimidazol-2-yl (**7.5**) and 2-methylimidazol-1-yl (**7.6**) groups improved the permeability in Caco-2 cells significantly ($4.7–7.4\times10^{-6}\,cm\,sec^{-1}$). The replacement of phenylsulfonamide with imidazoyl groups also resulted in the lowering of PSA. The enhanced permeability of the compound was potentially due to the reduced PSA following incorporation of the imidazoyl moieties as shown in Table 7.7. The introduction of the dimethylaminomethyl imidazole group in razaxaban further aided in optimising potency and permeability, and resulted in 84% bioavailability in dogs.

Sometimes, incorporation of a heteroaromatic moiety in a molecule can affect the pharmacokinetic properties of drugs. This is illustrated by the differences observed in the oral bioavailability of nilotinib (Tasigna) relative to imatinib (Gleevec) (Table 7.8). Both nilotinib and imatinib are tyrosine kinase inhibitors used in the treatment of chronic myelogenous leukemia (CML). Nilotinib appears to offer benefits in patients who are resistant to imatinib,

Table 7.7 Properties of Razaxaban and its analogs.[21]

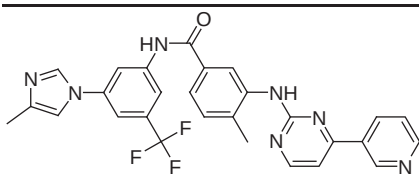


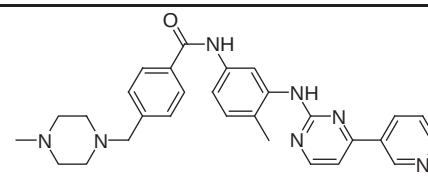
	R	*MW*	*clogP*	*PSA*	*Caco-2 Papp* $cm\ sec^{-1} \times 10^{-6}$
7.3		559	3.1	142	<0.1
7.4		560	3.0	154	0.54
7.5		485	1.9	117	4.6
7.6		485	4	117	7.4
Razaxaban		528	3.24	120	5.6

which is used as a first line treatment for CML. In humans, the absolute oral bioavailability of imatinib is 98%, indicating that absorption is almost complete.[22] In contrast, the systemic exposure of nilotinib is increased by 82% when administered 30 minutes after a high fat meal, suggesting that oral absorption of this drug in the fasted state is incomplete.[23] The variation in their absorption properties can be attributed to the differences in the lipophilicity and solubility (at pH 6.5) between the two compounds (Table 7.8). Incorporation of a 4-methylimidazole group in nilotinib increases the $clogD_{6.5}$ to 4.36 and reduces the solubility to $0.0004\,mg\,ml^{-1}$ relative to imatinib ($clogD_{6.5} = 1.77$ and solubility $0.11\,mg\,ml^{-1}$). Thus, replacement of the substituted imidazole with the piperazine side chain appears to be associated with decreased lipophilicity, increased solubility and an increase in the extent of oral absorption.

The tetrazole moiety is comparable in size and in acidity to the -COOH group and is therefore commonly used as a bioisosteric replacement of carboxylic acids.[24] Tetrazoles as isosteres of carboxylic acids are also discussed in detail in Section 3.7.2. The tetrazole moiety is 10-fold more lipophilic and

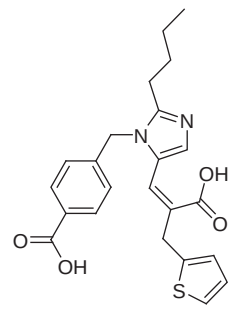
Table 7.8 Comparison of nilotinib (Tasigna) and imatinib (Gleevec).

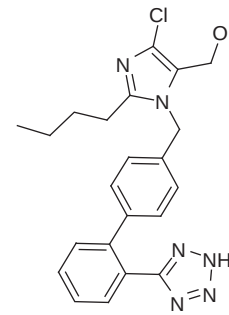




Property	*Nilotinib*	*Imatinib*
clogP	4.42	2.89
PSA	97.62	86.28
$clogD_{6.5}$	4.36	1.77
$Sol_{6.5}$ ($mg\,ml^{-1}$)	0.0004	0.11

Table 7.9 Comparison of physicochemical properties of losartan and eposartan





Property	*Eprosartan*	*Losartan*
ClogP	2.96	4.36
PSA	120	92.5
$clogD_{6.5}$	−0.53	2.6
$Sol_{6.5}$ ($mg\,ml^{-1}$)	4.6	0.04
% Oral bioavailability	13[25]	37[26,27]

masks the polarity of the carboxylate group, and hence tends to increase the bioavailability of compounds. The effect of replacing a carboxylic acid functionality with a tetrazole ring on absorption has been demonstrated in angiotensin II converting receptor antagonists, eprosartan and losartan (Table 7.9). Assessment of the pharmacokinetic properties of losartan and eprosartan indicates that, even though the bioavailability of losartan in humans was only 37%, the drug is completely absorbed through the gut following oral administration.[25] In contrast, only 15% of eprosartan was absorbed through the gut, resulting in bioavailability of 13%.[26,27] Low absorption is inherent in the physicochemical properties of eprosartan rather than incomplete dissolution. As shown in Table 7.9, the tetrazole ring in losartan and the carboxy group in

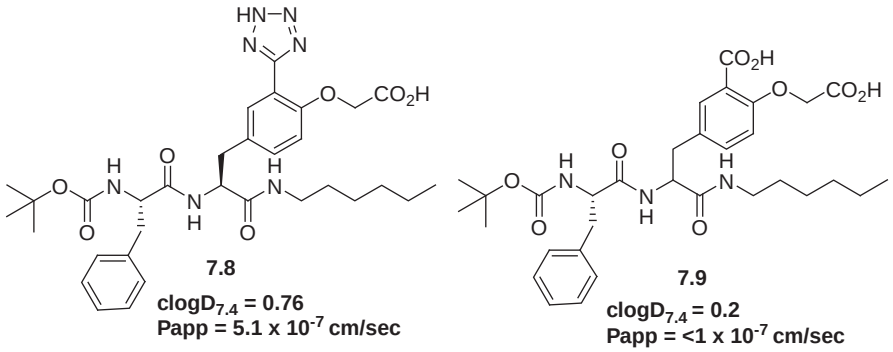


Figure 7.8 Structures of protein tyrosine phosphatases 1B (PTP1B) inhibitors and their clogD.

eprosartan have pKa values of 6 and ~4, respectively; hence both compounds are almost completely ionised at the pH in the gastrointestinal tract (~6.5). Although losartan is less soluble than eprosartan (0.04 mg/ml *versus* 4.6 mg/ml), the negative clog$D_{6.5}$ of eprosartan (log $D_{6.5}$ of −0.53) hinders the permeability of the compound through the membrane. On the other hand, replacement of dicarboxylic acid with a tetrazole moiety and a hydroxymethyl substituent in losartan results in a clog$D_{6.5}$ of 2.6, thereby enhancing the permeability through the gastrointestinal tract in spite of poorer solubility. In addition to lipophilicity, the difference in the polar surface area of the two compounds (92.5 $Å^2$ for losartan *versus* 120 $Å^2$ for eprosartan) may favour permeability of losartan through the membranes compared to eprosartan.

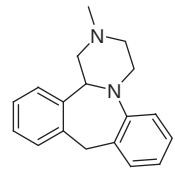
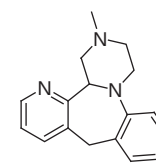
In another example, Liljebris and co workers have reported the influence of tetrazole ring on the bioavailability of small molecular weight peptidomimetics that were designed and synthesised as competitive inhibitors of protein tyrosine phosphatases 1B (PTP1B).[28] Because of the low cell permeability of this compound class, the possibility of replacing one or both of the remaining carboxyl groups while maintaining PTP1B inhibitory activity was explored. An important discovery was the ortho tetrazole analogue **7.8**, which was equipotent to the lead dicarboxylic acid analogue **7.9** (Figure 7.8). This novel monocarboxylic acid analogue revealed higher Caco-2 cell permeability and this was attributed to the increase in lipophilicity compared to the lead (Figure 7.8).

7.3.2 Distribution

Central nervous system (CNS) penetration is an important consideration in drug discovery and development for many therapeutic areas. Thus, examples that demonstrate the influence of aromatic heterocycles on the permeability of compounds to cross the blood–brain barrier (BBB) are discussed in this section.

Like absorption, permeability of a drug across the blood–brain barrier is affected by its PSA and logP, *i.e.* the polarity of a compound.[16] Since the

Table 7.10 Comparison of physicochemical properties and ratio of the brain to blood concentrations for mianserin and mirtazapine.[29]

Property	 *Mianserin*	 *Mirtazapine*
clogP	1.14	0.28
PSA	6.48	19.47
Brain–blood	9.7	3.4

presence of one or more heteroatoms in the aromatic heterocyclic ring results in variation of both parameters, this can potentially influence the permeability across the blood–brain barrier. Most often, inclusion of heteroatoms in a ring system results in an increase in the number of hydrogen bond donors (HBDs) or hydrogen bond acceptors (HBAs) (and therefore an increase in PSA) in a molecule. Therefore incorporation of aromatic heterocyclic rings into a molecule or replacement of carbocyclic rings with aromatic heterocycles can somewhat hinder the permeation of a drug into the CNS.

For example, a comparison of the ratio of concentrations in the brain to blood of the two antidepressants, mianserin (Tolvon) and mitrazapine (Remeron), indicates that mianserin has ~three-fold higher concentrations in the brain than mirtazapine (Table 7.10).[29] The only structural difference between these two compounds is the replacement of one of the phenyl rings with a pyridine ring (Table 7.10). Comparison of log P and PSA values of mianserin (clogP = 1.14; PSA = 6.48 Å^2) and mirtazapine (clogP = 0.28; PSA = 19.47 Å^2) indicates that isosteric replacement of a methine group in mianserin with a sp^2 nitrogen atom lowers the lipophilicity and increases the PSA of mirtazapine. Even though the molecules with small PSA (<20 Å^2) are completely absorbed *via* passive diffusion,[30] the relatively low permeability of mirtazapine compared to mianserin is possibly associated with the lowering of clogP value and a slight increase in PSA by isosteric replacement of a phenyl with a pyridyl group in mirtazapine.

Although lipophilicity plays an important role in BBB permeability, some reports have indicated that changes in PSA of a molecule can affect BBB permeability more than the changes in lipophilicity.[29] The presence of additional heteroatoms in heteroaromatic rings generally increases its potential for hydrogen bonding (and therefore the PSA) of the molecules. Hence, this can negatively impact the permeability of the molecule across the blood–brain barrier.

The following example illustrates this point. As shown in Table 7.11, replacement of a pyridine ring in compound **7.10** with a thiazole ring in compound **7.11** introduces another heteroatom into the molecule. Even though the

Table 7.11 Effect of PSA on the blood brain permeability of compounds.[29]

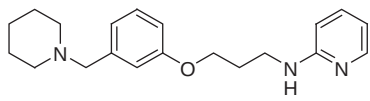
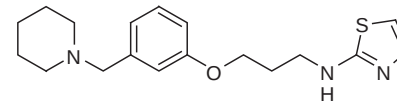
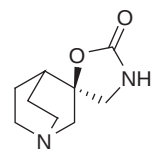
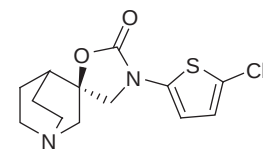
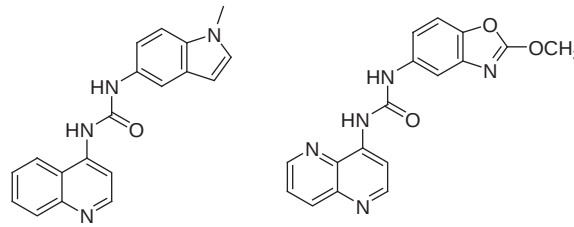
	 *Compound 7.10*	 *Compound 7.11*
clogP	3.75	3.93
PSA	37.39	65.63
Brain–blood	4.9	2.8

Table 7.12 Comparison physicochemical properties of α7 neuronal receptor agonists 1 and 2.[33]

Property	 *Compound 7.12*	 *Compound 7.13*
clogP	−1.5	0.57
$clogD_{7.4}$	−0.5	2.1
PSA	42	33
HBD	1	0
Brain to plasma ratio	0.76	59

sulfur atom is a weak hydrogen bond acceptor, its presence in the ring can affect the PSA of the molecule as observed in **7.11** (PSA for **7.10** is 37.39 *versus* 65.63 for **7.11**). However, no significant change in the lipophicity is observed with such a replacement (clogP for **7.10** is 3.75 and clogP for **7.11** is 3.93). Determination of brain to blood ratio of compounds **7.10** and **7.11** shows a 1.8-fold decrease when the aminopyridine group in **7.10** is replaced with aminothiazole moiety in compound **7.11**.[29] Since the clogP for both compounds are similar, the decrease in the brain to blood ratio in **7.11** is ascribed to the change in PSA of the two compounds.

Recent studies have shown that the permeability across the blood–brain barrier is primarily affected by the number of hydrogen bond donors than hydrogen bond acceptors present in the molecule.[31,32] Reduction in the number of hydrogen bond donors in a molecule has been attempted by addition of chlorothienyl moiety to the cyclic carbamate **7.12** (Table 7.12) by Tatsumi and co-workers.[33] Compound **7.12** was synthesised as a part of developing α7 neuronal nicotininc acetylcholine receptor agonists for the treatment of schizophrenia. Addition of the chlorothienyl group led to an increase in brain to



Compound 7.14
clogP = 4.4
clogD = 2.6
PSA = 59
HBD = 2

Compound 7.15
clogP = 3.4
clogD = 3.4
PSA = 102
HBD = 2

HBD = no of Hydrogen Bond Donors

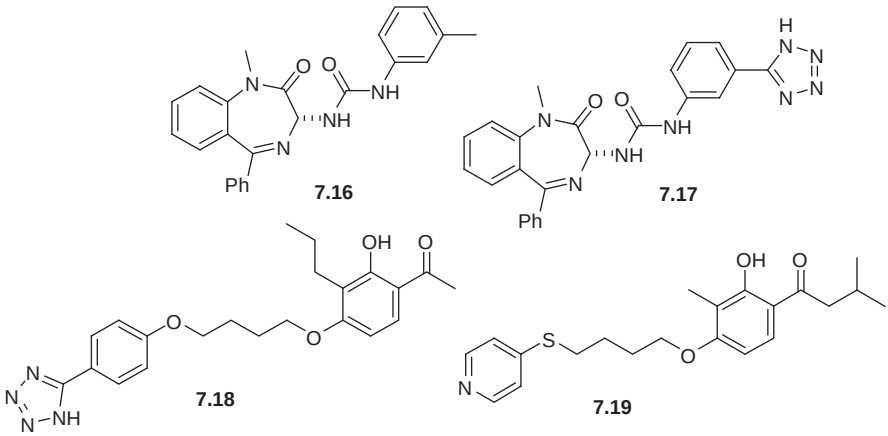
Figure 7.9 Intramolecular hydrogen bonding in the most polar compound increases the brain penetration.

plasma ratio from 0.76 for **7.12** to 59 for **7.13**. The change was rationalised in part to the reduction in hydrogen bond donating capability of **7.13**, resulting in a slightly lower PSA relative to **7.12**. This approach also led to an overall improvement of $clogD_{7.4}$ of the compound ($clogD_{7.4} = 0.57$ for **7.13** *versus* -1.5 for **7.12**). The calculated physicochemical parameters are shown in Table 7.12.

A group of oxerin-1 and 2 (OX1 and OX2) receptor antagonists, designed and synthesised by Porter and his co-workers, represents an interesting example where introduction of a polar napthyridine group in a molecule increased its brain to plasma ratio in spite of lower log P and higher PSA relative to the lead.[34] The lead compound **7.14** (Figure 7.9) showed good affinity for the receptors but exhibited poor blood–brain barrier permeability following an intravenous infusion to rats. The compound was essentially undetectable in the CNS even though it exhibited good lipophilicity (clogP, 4.4; clogD, 2.6) and a moderate polar surface area of 59 $Å^2$. Structure–activity relationship (SAR) studies to optimise potency and selectivity resulted in compound **7.15** that showed considerably higher brain to plasma ratio (0.4) relative to **7.14**. Higher ratios were observed despite the lower clogP than **7.14** (clogP 3.4) and significantly higher PSA (102 $Å^2$). So in theory, even though **7.15** has two hydrogen bond donors like the lead and unfavourable PSA, potential intramolecular hydrogen bonding between the nitrogen atom at the 5-position of napthyridine and the proton on the proximal urea nitrogen can mask its proton donor ability and therefore reduce the PSA. This in turn can help in enhancing BBB penetration.

The previous section describes the advantages of introducing tetrazolyl moiety as a bioisostere of carboxylic acid in an effort to increase in the bioavailability of losartan. However, introduction of this ring has been shown to negatively affect the brain exposure of compounds. This has been observed with some tetrazolyl analogs that were synthesised as CCK-B antagonists.[35] In this report, replacement of the methyl group on the phenyl urea (**7.16**) with a

Table 7.13 Comparison of the physicochemical properties of CCK-B antagonists (1 and 2) and mGlu2 modulators (3 and 4).[35,36]



Compound	*MW*	*clogP*	*clogD*	*PSA*	*HBD*	*HBA*
7.16	398	3.71	3.71	73.8	2	6
7.17	452	2.63	0.65	128	3	10
7.18	410	5.4	3.4	110	2	8
7.19	374	5.4	5.3	84.7	1	4

tetrazole moiety (**7.17**) (Table 7.13) resulted in poor brain exposure of **7.17**. Similarly, enhanced brain to plasma (B/P) ratios in rat were observed following replacement of tetrazole terminus in **7.18** (B/P = 0.01) with a 4-thiopyridyl group (**47.19**) (B/P = 1.1) for the modulators of mGlu2 receptor (Table 7.13).[36] In both examples, replacement of the substituent with the tetrazole moiety increased the number of hydrogen bond donors and hydrogen bond acceptors, and therefore increased PSA. Although there was a decrease in the clogD in both cases, the authors attributed the poor permeability to cross the blood–brain barrier to a high PSA and an increase in hydrogen bond donors.

7.3.3 Metabolism

In addition to enhancing absorption properties, heterocyclic rings are often incorporated into a molecule to improve the metabolic stability of newly synthesised compounds. Replacement of a carbocyclic ring with an aromatic heterocycle is a common strategy to reduce the metabolic liability and sometimes block the sites of metabolism.

Structure–metabolism relationship studies have shown that incorporation of one or more heteroatoms in an aromatic ring of a compound modulates its chemical and biochemical reactivity (in addition to changing lipophilicity), and therefore alters its metabolism. The degree of influence of heteroaromatic rings on the metabolism of a compound depends on the type of ring system that is

incorporated in a molecule and the number of nitrogen atoms present in the ring. As described in Section 7.2, compounds containing six-membered ring systems are less reactive than their corresponding carbocyclic analogs. Incorporation of one or more nitrogen atoms in the rings decreases the electron density in the aromatic ring carbons, thereby decreasing P450-mediated aromatic oxidation. Thus, the lower reactivity of six-membered rings with multiple nitrogen atoms makes these prime fragments to improve metabolic stability such as higher resistance to chemical or enzymatic degradation.

Despite low reactivity, compounds containing these rings are not devoid of P450 catalysed metabolic transformations. For example, voriconazole, an antifungal agent containing a pyrimidine ring, primarily undergoes CYP2C19 or 3A4-mediated *N*-oxidation of the pyrimidine ring (Figure 7.10).[37,38] Similarly, compounds containing a pyridine ring such as indinavir or rosiglitazone are subject to oxidative metabolism either on the pyridine ring or on the rest of the molecule by P450 (Figure 7.11).[19,39] Binding of these compounds to the P450 active site is attributed to their overall lipophilicity ($clogD_{7.4}$ for indinavir = 3.4; $clogD_{7.4}$ for rosiglitazone = 2.5 and $clogD_{7.4}$ for voriconazole = 1.9).

Alternatively, the presence of the lone pair of electrons on the nitrogen atom of the aromatic heterocycles provides a site for glucuronidation by UDP-glucuronyl transferases (UGT) for most six-membered rings. Pyridine containing compounds are readily prone to glucuronidation. Indinavir, which undergoes extensive oxidative metabolism, is also subject to *N*-glucuronidation (Figure 7.11).[39,40] Other examples include compounds such as nicotine that form the *N*-glucuronide in humans (Figure 7.12).[41] Lamotrigine, an anti-epileptic agent containing a triazene ring, is also metabolised *via N*-glucuronidation (Figure 7.12).[42] This suggests the importance of nucleophilicity of the nitrogen atom rather than the reactivity of the heteroaromatic ring in glucuronidation of the molecule. Not much is known about the interactions of the substrates with UGTs.

A unique metabolic pathway for compounds containing electron deficient azaheterocycles is oxidation by molybdenum hydroxylases such as aldehyde oxidase (AO) and xanthine oxidase (XO).[43] Unlike P450s that promote the

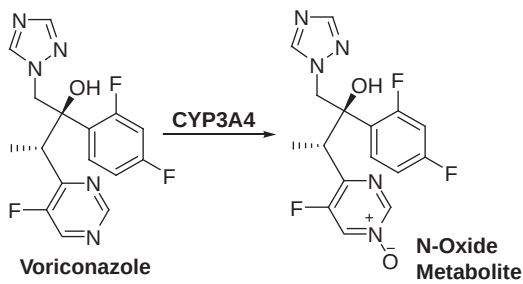


Figure 7.10 Metabolism of the pyrimidine moiety in voriconazole by CYP2C19 and 3A4 to its N-oxide metabolite.

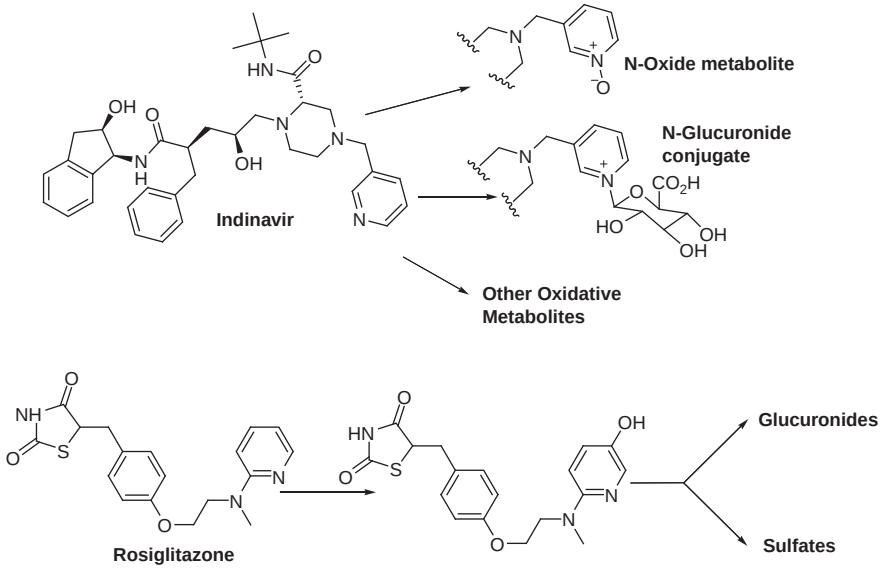


Figure 7.11 Metabolism of the pyridine ring in indinavir and rosiglitazone.

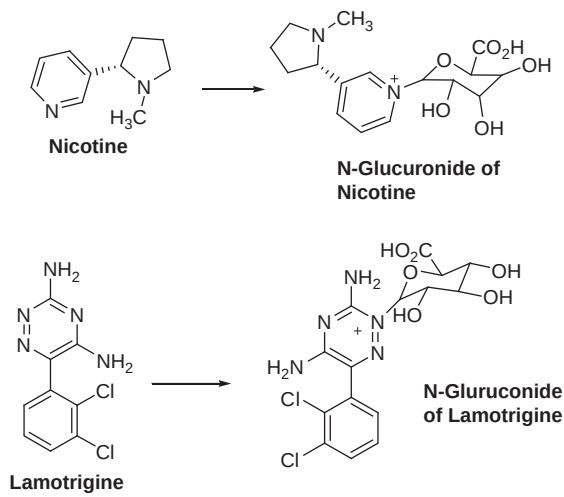


Figure 7.12 *N*-Glucuronidation of nicotine and lamotrigine.

electrophilic attack *via* the Fe=O complex on aromatic substrates, these enzymes oxygenate heteroaromatic rings *via* nucleophilic attack of Mo–OH on the electron-deficient carbon atom adjacent to nitrogen atoms in the heterocycles. Both AO and XO can oxidise several single or polyaromatic heterocycles, but AO shows broader substrate specificity than XO. Some of the

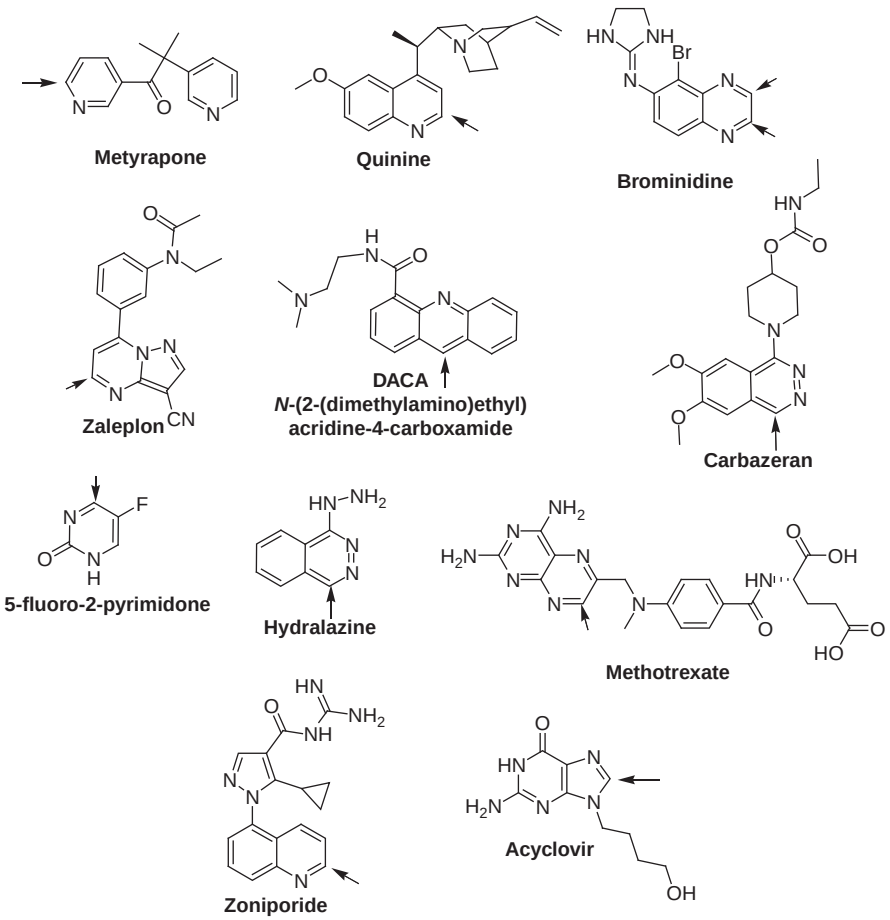


Figure 7.13 Aldehyde oxidase mediated oxidation of various drugs. Arrows indicate the site of oxidation.

representative compounds that undergo AO and XO mediated metabolism are shown in Figure 7.13. An example is the bioactivation of famciclovir to the active antiviral agent penciclovir which requires AO mediated metabolism.[44] Famciclovir is converted to deoxypenciclovir, which is oxidised by AO to penciclovir (Figure 7.14).

Hydrophobicity and electron deficiency appears to be two important factors in predicting metabolism by AO. As the number of nitrogen atoms in the ring increases, the ring becomes more electron deficient and more hydrophobic. As a result, the potential for the compound to undergo AO-mediated oxidation increases. This has been demonstrated by Hall and Kretinistsky who studied the capability of the rabbit AO to oxidise purines and their analogs.[45] In this study, the chemical nature of the 6-substituent of purine markedly influenced substrate efficiency. Substituents that were hydrophobic and electron

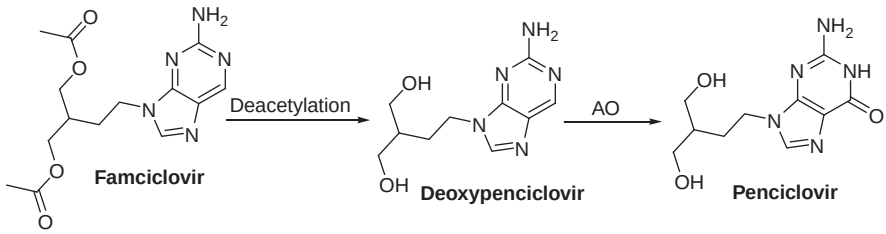


Figure 7.14 Metabolic activation of famciclovir to penciclovir.

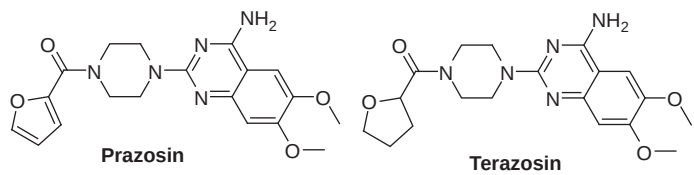


Figure 7.15 Structures of Prazosin and Terazosin.

withdrawing enhanced AO oxidation, whereas 6-hydroxy and 6-amino substituent virtually abolished substrate activity.[45]

Compounds containing five-membered heterocyclic rings behave quite differently than those containing six-membered rings. Since all five-membered heterocycles are electron rich in nature, compounds with these rings can undergo similar P450 mediated oxidative metabolism like their carbocyclic congeners. In some instances, the five-membered rings serve as alternative sites for metabolic attack and, at times, have the potential to undergo unusual metabolic transformations that can result in toxic events. The metabolic transformations for some well-known drugs containing five-membered rings result in electrophilic intermediates (see Section 7.3.4). The metabolism of five-membered heterocycles is also extensively reviewed elsewhere.[46]

There is a good correlation between lipophilicity and the metabolism of five-membered rings. Thiophenes, pyrroles and furan containing compounds, which are quite lipophilic, undergo extensive metabolism to ring opened products. Lowering lipophilicity by subtle changes in these heterocyclic rings can significantly improve the pharmacokinetic properties of the compound. For example, prazosin (Figure 7.15), a furan containing compound, is a selective $\alpha 1$ blocker that is extensively metabolised by CYP450 resulting in a half-life of 2–3 h and a bioavailability of 45–65%.[47,48] However, terazosin, in which the furan ring is replaced by a tetrahydrofuran moiety, increases the bioavailability to 90% with a half-life of 12 h.[49] The increase in exposure of terazosin is possibly attributed to the lowering in the clogP value (0.8) compared to prazosin (clogP 2.14) by replacement of a furan with a hydrophilic tetrahydrofuran moiety (Figure 7.15).

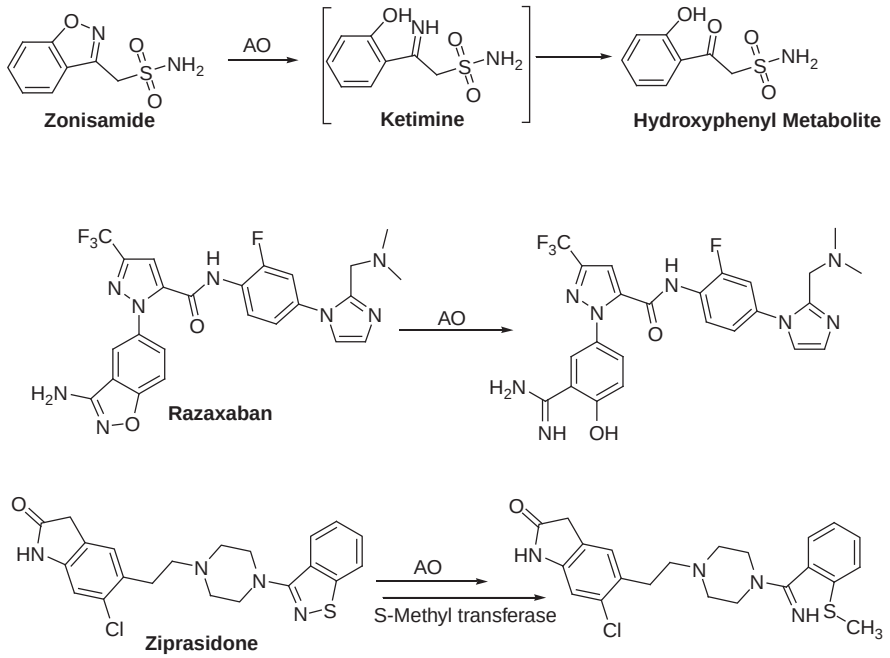


Figure 7.16 Reductive metabolism of zonisamide, razaxaban and ziprasidone by aldehyde oxidase.

Compounds containing five membered heterocycles, especially those containing two heteroatoms, undergo an AO-mediated reduction and subsequent ring opening of the heterocyclic ring. The 1,2-benzisoxazole moiety in zonisamide (Figure 7.16) is reductively cleaved to a hydroxyphenyl derivative *via* the ketimine intermediate.[50] A similar reductive cleavage has been observed in the metabolism of the anticoagulant razaxaban as well as the benzothiazole derivative ziprasidone (Figure 7.16).[51–53] Since the mechanism of AO-mediated reduction pathways is unknown, a proper correlation of the physicochemical properties and the AO reducing activity has not been reported. Comparison of the clogP values (0.72 for zonisamide and $\sim$3.0 for ziprasidone and razaxaban) and molecular weights (ranging from 212 to 528) of the three drugs suggests that lipophilicity could be a determinant in the reductive metabolism of these compounds by AO.

Like six-membered rings, the compounds are less susceptible to metabolic attack by P450 or other enzymes as the number of heteroatoms in the ring increase. For example, bioisosteric replacement of a carboxylic ester group in a molecule with an oxadiazole or a thiadiazole moiety increases the stability to hydrolytic degradation. This approach has been employed in the design of several arecoline analogs (Figure 7.17).[54,55] The ester functionality is prone to hydrolysis by a number of esterases that are present throughout the body and also subject to chemical degradation at acidic pH in the gastrointestinal tract. This liability prevents the ester containing drugs from being used orally.

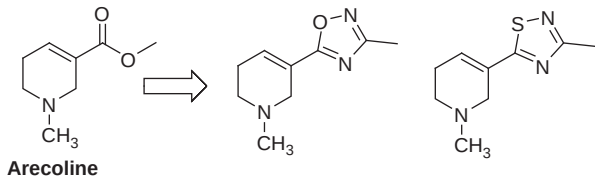


Figure 7.17 Structure of arecoline and its analogs.

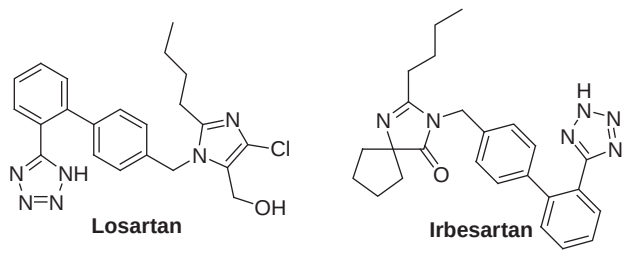


Figure 7.18 Structures of losartan and irbesartan.

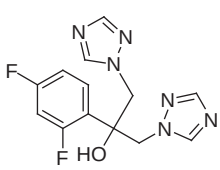
Compounds containing rings with two or more nitrogen atoms increase compound polarity and lower the log P of the molecule. Most often, these compounds are excreted unchanged in the urine as observed with diflucan, a triazole derivative (see Section 7.3.4).[56] Alternatively, more lipophilic compounds are eliminated *via* the glucuronidation pathway. Formation of a N2-glucuronide has been identified as a major route of metabolism for the tetrazole-containing losartan (Figure 7.18).[57,58] Within the same therapeutic drug class, irbesartan (Figure 7.18) has also been shown to undergo tetrazole N2-glucuronidation in humans.[59]

7.3.4 Excretion

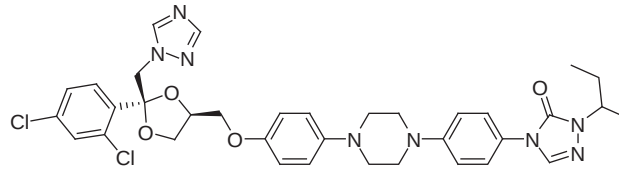
As mentioned earlier, introduction of polar heterocyclic rings systems in molecules can influence the routes of clearance. Substitution with appropriate polar heterocyclic rings during drug design that allows the drug to get absorbed and confers metabolic stability to the molecule can sometimes favour the drug to be cleared renally.

Fluconazole (Diflucan) (Table 7.14) is a classic example in which introduction of heterocyclic rings has influenced the route of elimination of this drug. Fluconazole belongs to a triazole class of antifungal agents used in the treatment and prevention of superficial and systemic fungal infections. As described before, triazoles are ring systems that impart significant polarity to a molecule and make them resistant to metabolism. However, these rings are unionised at physiological pH and therefore allow complete absorption of the drug in the

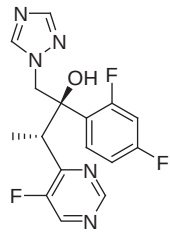
Table 7.14 Physicochemical properties of fluconazole, voriconazole, itraconazole and posaconazole



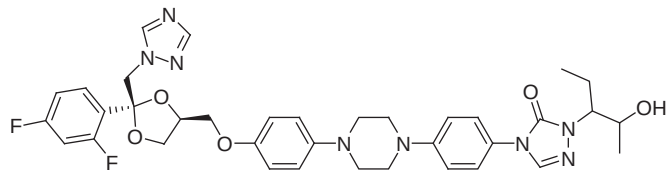
Fluconazole



Itraconazole



Voriconazole



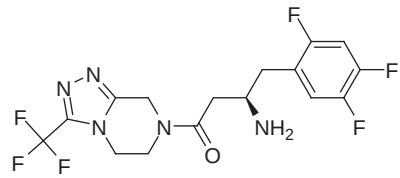
Posaconazole

Compound	*MW*	*clog$D_{7.4}$*	*% Excreted in urine*
Fluconazole	306.2	0.45	80
Voriconazole	349.3	1.21	2.1
Itraconazole	705.6	4.93	0.01
Posaconazole	702.7	3.68	Not detected

gastrointestinal tract (oral bioavailability is $>90\%$).[60] The presence of two triazole rings in fluconazole imparts polarity to the molecule ($\log D_{7.4}$ 0.45) (Table 7.14). Poor lipophilicity of the molecule renders the molecule stable to P450 and UGT mediated metabolism and results in about 80%of the drug being primarily excreted in the urine unchanged.[56] In contrast, other triazole containing compounds such as itraconazole and pasoconazole are quite lipophilic and have a $\log D_{7.4}$ value of 4.93 and 3.68, respectively (Table 7.14). Both compounds are primarily cleared *via* metabolism (P450 mediated for itraconazole and glucuronidation for pasoconazole) and no unchanged drug is excreted in the urine.[61,62]

Sometimes, subtle changes in the molecular structure can result in significant changes in the routes of clearance despite the low molecular weight and low log D. For example, replacement of the triazolomethyl moiety in fluconazole with a fluoropyrimidinyl benzyl group in voriconazole (Table 7.14) results in a $\log D_{7.4}$ of 1.2. Regardless of the low molecular weight like fluconazole (Table 7.14) and only a slight change lipophilicity ($\Delta \log D_{7.4} = 0.76$ relative to fluconazole), the compound is extensively metabolised (2% of the unchanged drug being excreted in the urine) to the pyrimidine *N*-oxide by CYP 3A4 (Table 7.14).[63,64]

Even though a correlation between physicochemical properties (log D and MW) and the renal excretion of the drugs has been well established,[16] good



Sitagliptin

MW 407.3

clogD$_{7.4}$ 1.93

clogP 2.06

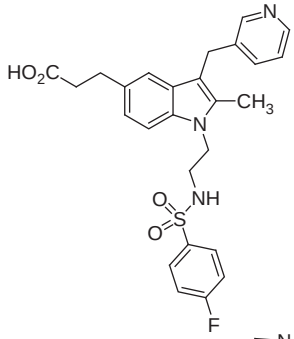
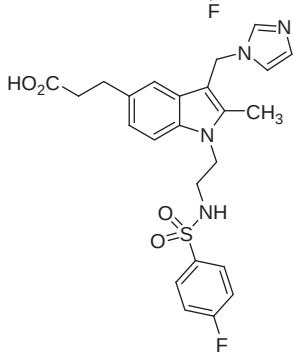
Figure 7.19 Structure of sitagliptin (Januvia), a DPP-4 inhibitor.

substrate properties of compounds for some efflux transporters can also lead to renal excretion of lipophilic drugs. Sitagliptin (Januvia) (Figure 7.19), an orally active triazolopyrazine derivative and an inhibitor of the dipeptidyl peptidase-4 (DPP-4) enzyme, presents an interesting case.[65]

Despite modest lipophilicity ($clogD_{7.4} = 1.93$) and molecular weight ($MW = 407$), approximately 79% of sitagliptin is excreted unchanged renally and metabolism is a minor pathway of elimination.[65] Following a [$^{[14]}$C]sitagliptin oral dose, only 16% of the radioactivity was excreted as metabolites of sitagliptin. Metabolic stability is achieved by the presence of triazolopyrazine and trifluorophenyl group in the molecule. Reports indicate that sitagliptin is a low affinity substrate of human organic anion transporter-3 (hOAT-3).[66] Organic anion transport systems such as human organic anion transporters (hOAT1 and 3) are predominantly expressed in the kidney and play an important role in the transport of organic anions across the basolateral membrane of human proximal tubules.[67] Hence, clearance of sitagliptin primarily involves its active tubular secretion from the body into the urine.

Like the kidney, many transporters are expressed on the canalicular membrane of the liver. These transporters can mediate the excretion of selected compounds into the bile. Lipophilic compounds and high molecular weight compounds with a considerable hydrogen bonding functionality and which show poor permeability can be potentially cleared *via* this active efflux process. Incorporation of heterocyclic rings in molecules can alter the route of clearance for high molecular weight lipophilic compounds from metabolism to active efflux (and therefore biliary excretion of unchanged drug). The change is dependent upon the hydrogen bonding capability that is incorporated into the molecule by the newly introduced heterocyclic ring system. Gardner and coworkers have demonstrated this for inhibitors of thromboxane A_2 synthase (**7.20** and **7.21**, Table 7.15).[68] Both the compounds showed high hepatic extraction ratio ($E = 0.9$) in the isolated perfused rat liver. Comparison of their physicochemical properties showed that both compounds had molecular weight close to 500 (Table 7.15). Compound **7.20** was mainly eliminated from the body by metabolism whereas **7.21** was excreted into the bile unchanged. This suggested that **7.21** was a substrate for one of the hepatic efflux

Table 7.15 Effect of replacement of heterocyclic ring systems in a compound on the route of elimination.[68]

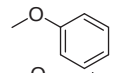
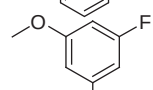
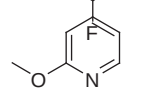
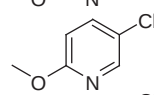
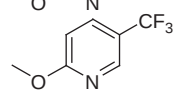
Compound	*Structure*	*PSA*	*clogD$_{7.4}$*	*clogP*	*HBD*	*HBA*
7.20		109	0.053	2.78	2	7
7.21		114	−0.73	1.93	2	8

transporters. The change in the route of clearance for **7.21** was attributed to its high polarity (negative clogD$_{7.4}$) relative to **7.20** and increase in the number of hydrogen bond donors and acceptors in the molecule. It should be noted that the above inference is merely speculative since the influence of the physicochemical properties on the SAR of efflux transporters is not fully understood.

7.4 Influence of Heteroaromatic rings on Toxicity of Compounds

Heteroaromatic rings can influence the toxicity of compounds in a desirable or an undesirable manner. Lipophilic compounds containing phenyl rings (especially phenolic or aromatic amines) are bioactivated to electrophilic quinoid intermediates that covalently bind to macromolecules and possibly cause untoward side effects. A strategy to mitigate the potential risk of bioactivation involves replacement of the labile motifs with polar and less reactive bioisosteres. Since the aromatic heterocycles can influence the reactivity and lipophilicity of a molecule, these rings are attractive fragments that can be incorporated into new compounds to prevent metabolite activation.

Table 7.16 Incorporation of substituted aromatic heterocycles reduces the level of covalent binding of a molecule to microsomal proteins.[69]

Compound	*Analog*	*Covalent binding (pmol-equiv/mg protein)*
7.22		3870
7.23		1690
7.24		911
7.25		303
7.26		88

An example to illustrate this approach was reported by Samuel and co-workers.[69] Studies with radiolabelled phenoxy analog (**7.22**) exhibited extensive covalent binding (3870 pmol-equiv mg^{-1} protein) to microsomal proteins following incubation with NADPH supplemented human liver microsomes (Table 7.16). Replacement of this group with a pyridinyl group resulted in a significant decrease in covalent binding to the microsomal protein when the analog was incubated under similar conditions as the lead. Additional decrease in covalent binding was observed when the pyridine ring was substituted with electron withdrawing group (CF_3 group). Although not described by the authors, the decrease in covalent binding of the analogs is probably related to the decrease in electron density (therefore the reactivity) of the moiety in the molecule that is susceptible to metabolic activation.

Other possible applications of heterocyclic rings have been proposed to avoid bioactivation of compounds. For instance, metabolic activation of carboxylic acids *via* formation of acyl glucuronide and subsequent acyl migration is a matter of concern due to the potential for these metabolites to react covalently with other biological molecules including proteins. These reactions have been implicated in severe toxicity, resulting in the withdrawal of multiple drugs from the marketplace in the USA and other countries. To reduce the risk of metabolism related liabilities associated with acyl glucuronides, tetrazoles can be used to replace carboxylic acid moieties.[70] Though the tetrazolyl derivatives are metabolised *via* glucuronidation like the carboxylic acids, the corresponding glucuronide conjugates do not undergo acyl rearrangement to form a reactive metabolite.

Although heterocyclic rings have been widely used to reduce undesirable effects of a compound, these rings can also result in unwanted adverse effects such as heptotoxicity or inactivation of P450. Compounds containing thiophenes

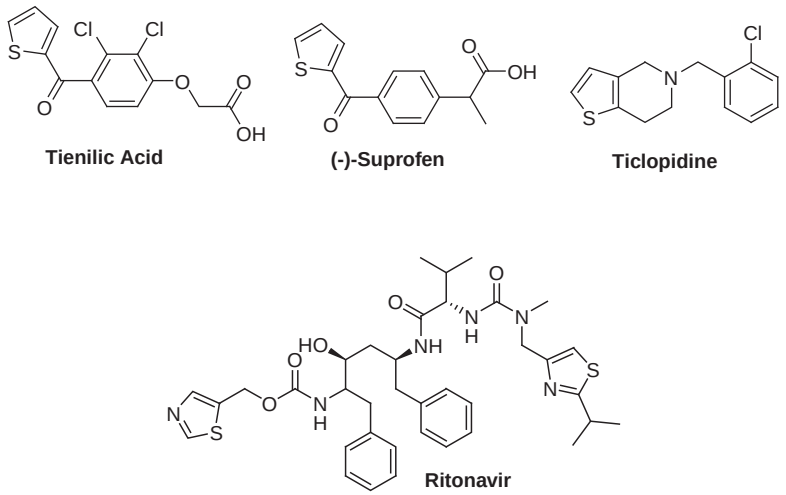


Figure 7.20 Structures of drugs containing thiophene and thiazole rings.

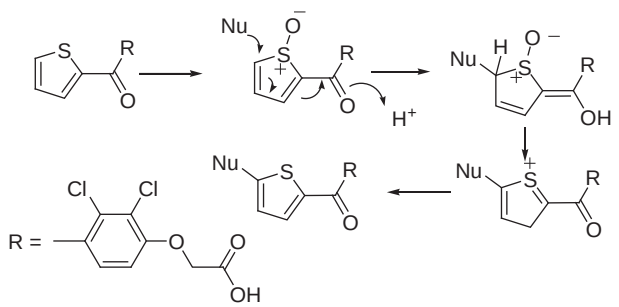


Figure 7.21 Mechanism of metabolic activation of thiophene ring containing tienilic acid.

and furans commonly fall into this category.[46,71] Several therapeutic agents with these rings have been withdrawn from the market or have warnings on their labels due to specific organ toxicities and related idiosyncratic reactions, and are inactivators of drug metabolising enzymes. Tienilic acid, suprofen and ticlopidine (Figure 7.20) are examples of thiophene containing compounds that have been associated with adverse drug reactions as a consequence of metabolic activation.

Tienilic acid was withdrawn from the market because of hepatotoxicity. It has been proposed that tienilic acid undergoes sulfoxidation to a reactive electrophilic, tienilic acid-S-oxide by CYP2C9, which inactivates the enzyme (Figure 7.21).[72]

Tienilic acid specific autoantibodies (called anti-LKM2) directed against CYP2C9 have been detected in patients treated with tienilic acid and suffering

with tienilic acid-induced hepatitis. More recently, the structural analog (−)-suprofen was shown to also be a mechanism-based inactivator of P450 2C9.[73] Tienilic acid and (−)-suprofen are reported to cause mechanism-based inactivation by a similar mechanism, leading to covalent modification of the CYP2C9 apoprotein within the active site.[74] Although ticlopidine is still utilised clinically as an inhibitor of ADP-induced platelet aggregation, its use is associated with a relatively high incidence of agranulocytosis, aplastic anaemia and thrombocytopenia. The thiophene ring in ticlopidine is known to undergo CYP2C19 and 2B6 mediated catalysis to reactive intermediate(s) that causes enzyme inactivation.[72,75] Other examples of sulfur containing heterocycles include the potent mechanism-based inactivator of CYP3A4 by the protease inhibitor, ritonavir (Figure 7.20).[72] Ritonavir has been speculated to undergo bioactivation of one or both of its thiazole rings and therefore result in inactivation of the enzyme.

Furan-containing compounds such as furosemide, ipomeanol and L-739010 (Figure 7.22) also cause hepatic and renal necrosis in mouse and humans or develop potentially lethal pulmonary lesions in rat.[46] Oxidative ring opening of furan and irreversible protein binding of the corresponding substituted ketoenals has been reported during metabolism studies on many furan-containing biologically active compounds (Figure 7.23).[76–79]

Several compounds containing furan rings are inactivators of P450 enzymes.[76,77] Evidence for P450 inactivation by reactive intermediate(s) derived from furan ring scission has also been presented for the experimental HIV

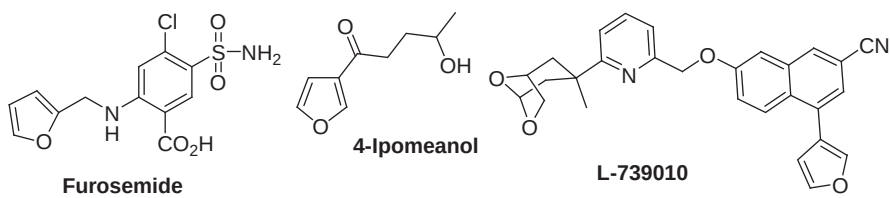


Figure 7.22 Structures of compounds containing a furan ring system.

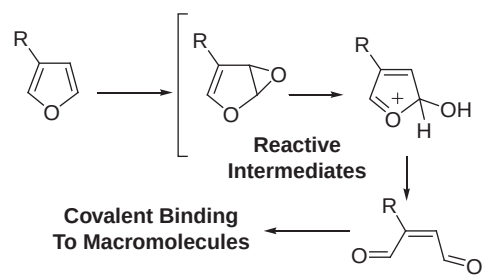


Figure 7.23 Metabolic activation of ring opening of furan rings.

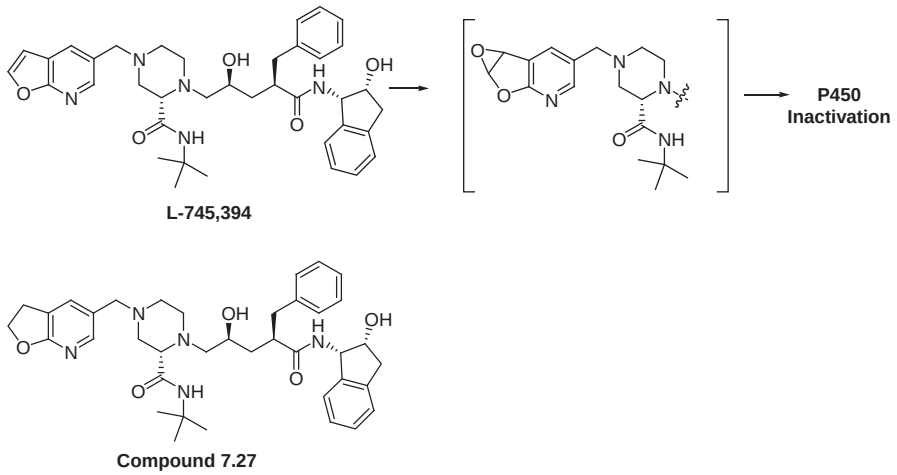


Figure 7.24 Proposed mechanism for inactivation of CYP450 by L-745,394.

protease inhibitor L-745 394 which inactivates rat CYP2C11 and human CYP3A4 enzymes (Figure 7.24).[80,81] Removal of the furan ring or reduction of 2,3-double bond in L-754,394 led to compound **7.27** (Figure 7.24) which did not inactivate CYP3A4, implicating that the furan ring was involved in the bioactivation sequence leading to enzyme inactivation.[82] For this reason furan, thiophenes and thiazoles (especially the amino thiazole ring) are considered as structural alerts and are generally excluded by the medicinal chemists when considering the design of new drugs candidates.

Some six-membered heterocycles with substituted alkyl, amino or hydroxyl substituents can also undergo metabolic activation. For instance, 2,3-diaminopyridine containing compounds (**7.28**) (Figure 7.25) that were designed as bradykinin B1 receptor antagonists undergo CYP3A4-catalysed bioactivation and covalent binding to liver microsomal proteins and glutathione.[83] The presence of two amino groups on the pyridine ring increases its susceptibility to P450-mediated two-electron oxidation and consequent formation of the reactive pyridine-2,3-diimine **7.29** (Figure 7.25). Reaction of this intermediate with glutathione afforded the glutathione adduct **7.30**. The bioactivation liability of the 2,3-diaminopyridine moiety was addressed by replacement of the 2-amino group on the pyridine nucleus with an oxygen atom (**7.31)** or addition of a methyl group on the 2-aminopyridine (**7.32)** (Figure 7.25).

Compounds possessing a sterically unhindered nitrogen heterocycles are known to act as reversible inhibitors of P450 enzymes. These compounds have been shown to coordinate with the heme iron inside the CYP catalytic pocket. Known as type-II ligands, these compounds can inhibit CYP by displacing a sixth (weak) ligand, water, and stabilising the iron in its low spin state. This spin state change is accompanied by an increase in the redox potential of P450, which makes the P450 reduction (by NADPH P450 reductase) more difficult.

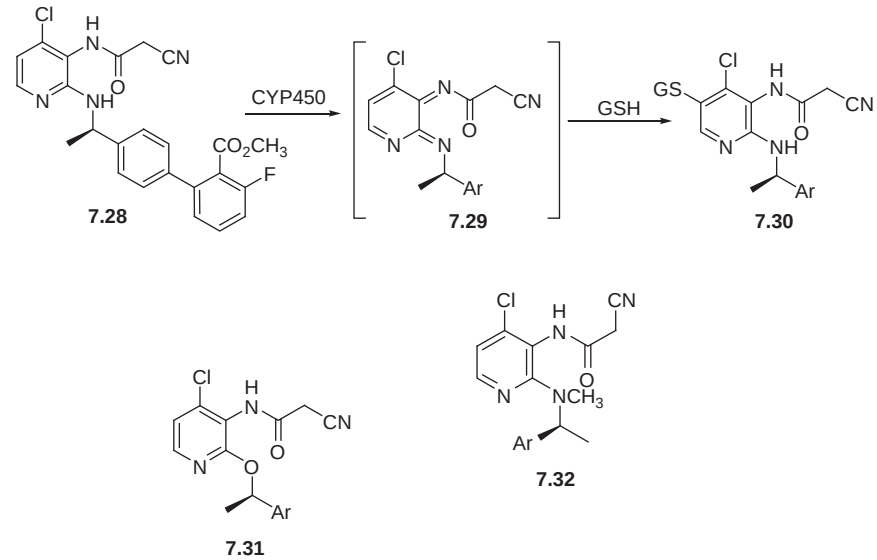


Figure 7.25 Metabolic activation of bradykinin B1 receptor antagonist (1) containing 2,3-diaminopyridine ring.

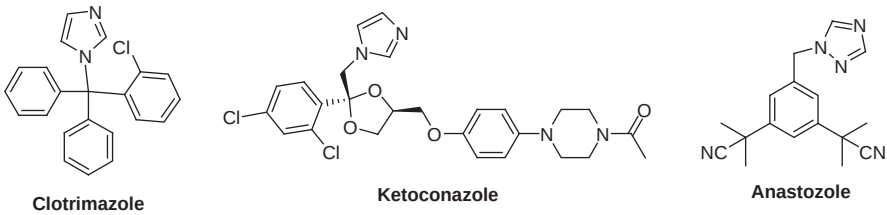
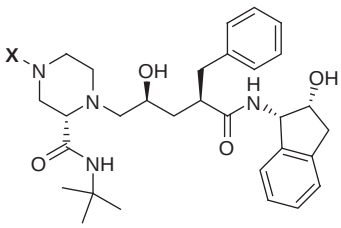


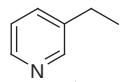
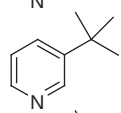
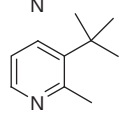
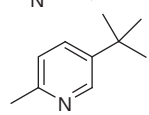
Figure 7.26 Structures of additional azole containing compounds that can inhibit CYP450.

The formation of type-II complexes may inhibit the metabolism of co-administered drugs and result in drug–drug interactions.

A few classic inhibitors that are known to inhibit P450 by coordinating with the heme include azole antifungals such as clotrimazole (Figure 7.26), ketoconazole (Figure 7.26), itraconazole (Table 7.14) and fluconazole (Table 7.14).[84] The primary mode of action of all antifungal agents is to inhibit the fungal P450 (CYP 51s). Since the selectivity of the compounds to inhibit CYP51s over human drug metabolising P450s is low, these compounds are associated with significant drug interactions. Aromatase inhibitors are another class of compounds that inhibit CYP450. Triazole derivatives in this class such as anastrozole (arimidex) (Figure 7.26) can inhibit CYP1A2, 2C9, and 3A-mediated

Table 7.17 Influence of structural modification of indinavir on P450 binding spectra and CYP3A4 inhibitory potency.[86]



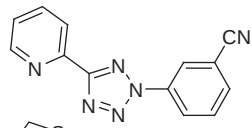
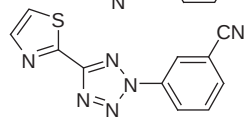
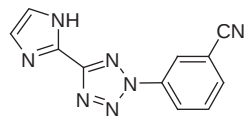
Compound	*X*	*P450 binding spectra*	*IC_{50} (μM)*
Indinavir		II	0.45
7.33		II	0.87
7.34		I	15.1
7.35		I	8.7

catalytic activities.[85] The strength of the bond between their heteroatomic lone pair electrons and the prosthetic heme iron governs the inhibitory character of these compounds in addition to hydrophobicity.

Minor structural modifications dramatically change the CYP3A4 potency of these compounds as illustrated by some analogs of indinavir (Table 7.17).[86] Although addition of a *gem*-dimethyl group (**7.33**) did not change the inhibitory characteristics of indinavir, incorporation of a methyl group on the carbon atom adjacent to nitrogen atom of the pyridine ring (**7.33** to compounds **7.34** or **7.35**) dramatically decreased CYP3A4 inhibitory potency by >10-fold relative to indinavir (Table 7.17).[86] The decrease in enzyme inhibition was presumably due to the change in the interaction of substrate and P450 active site.

In another example, Smith and co-workers have conducted SAR relationship studies focused on bioisosteric replacements of 2-pyridyl group (**7.36**) mGlu5 receptor antagonists to reduce inhibition of CYP1A2 (Table 7.18).[87] Compared to **7.36,** which inhibited CYP1A2 with an IC_{50} of 3.8 μM, the thiazolyl derivative **7.37** showed an undesired increase in CYP1A2 inhibition. On the other hand and more importantly, CYP1A2 inhibition was greatly reduced (IC50 >14 μM) with 2-imidazolyl derivative **7.38** compared with **7.36**. The change in the potency correlated with the log P value of the three compounds. Thus, the

Table 7.18 Influence of clogP on the inhibition of CYP1A2 by mGlu5 receptor antagonists.[87]

Compound	*Structure*	*CYP1A2 IC_{50} (μM)*	*clogP*
7.36		3.8	2.16
7.37		1	2.31
7.38		>14	1.63

least lipophilic compound with an imidazole ring was the least potent while the incorporation of a thiazole ring increased the lipophilicity of the compound and hence its affinity for P450.

Many *N*-substituted azoles also have the ability to induce hepatic microsomal mixed-function oxidases. Some *N*-substituted imidazoles such as clotrimazole have been characterised as high magnitude inducers of rat hepatic CYP. Although the number of clinically used drugs which induce P450 enzymes in humans is limited, studies with nilotinib (Table 7.8) have shown that this drug may induce CYP2B6, 2C8 and 2C9 in humans and thereby decrease the concentrations of drugs eliminated by these enzymes.

It is now well recognised that that adverse events (either idiosyncratic toxicity or induction) caused by drugs correlates well with the dose administered. Reducing the dose size either by increasing the potency of the compound or by improving its pharmacokinetic properties can potentially reduce the toxic events of a compound. Thus the impact of the administered dose should not be underestimated.

A classic example of the impact of dose on the toxicity of compounds can be observed with glitazones. As described earlier, troglitazone, which is administered at a relatively high dose was withdrawn from the market due to hepatotoxicity. The toxicity was attributed to the ring opening of thiazolidinone ring in the molecule. Furthermore, this drug was associated with induction of CYP3A4. In contrast rosiglitazone, which has the same thiazolidinone functionality and is administered at a much lower dose, is devoid of hepatotoxicity; neither does it show evidence of enzyme induction.

7.5 Summary

This chapter illustrates the influence of heteroaromatic rings on the ADME properties of a compound. The examples reviewed here demonstrate that

incorporation of heteroaromatic rings in a compound can have major effect on its disposition in the body. However, it is important to note that such a correlation is not straightforward. The disposition of a compound can be quite complex and is affected not only by addition of heterocyclic rings but other groups and substituents on the molecule. Further, other physiological factors (*e.g.* protein binding and transporters) can also affect the ADME properties of compounds. Nevertheless, it is hoped that this overview provides a flavour of the impact that various aromatic heterocycles have on the ADME properties of the compound.

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CHAPTER 8

Peptidomimetics and Peptides as Drugs: Motifs Incorporated to Enhance Drug Characteristics

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8.1 Introduction

The human genome is projected to have 5000–10 000 potential drug targets, of which it is speculated that 3000–6000 are amenable to small molecule drugs and another 1500–3000 to biopharmaceuticals.[1–3] Currently marketed drugs are directed towards less than 500 targets encoded by the genome.[3,4] Thus, it would appear that a large pool of potential therapeutic targets remain untapped. Much of this has to do with a significant lack in detailed understanding of the biological roles played by most of these genome products. For some, where roles have been established, their mechanisms of action can involve protein–protein or peptide–protein interactions for which a detailed understanding of the epitopes and specifics of their molecular interactions remain unknown. For a few peptide–protein and protein–protein interactions, such molecular specifics have been resolved from crystal structures of complexes or approaches using targeted mutagenesis to identify critical amino acids or peptide sequences that are involved in an interaction. The interactions are generally based on complementary structural features at the interaction site and can include

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hydrogen bonding, polar and hydrophobic effects between proximal amino acids, and restricted conformations in the bound state. For systems where a detailed understanding of the interactions is known, peptides or proteins as oral pharmaceuticals have not been successfully compared with small molecule pharmaceuticals due to their intrinsic properties which include molecular size, absorption across biological membranes, and rapid systemic digestion by proteolytic enzymes.

For over three decades, medicinal chemists have taken a peptidomimetic approach to overcoming these limitations. Several definitions have been ascribed to peptidomimetics. Giannis and Kolter[5] gave a functional definition of a peptidomimetic as: 'a compound that, as the ligand of a receptor, can imitate or block the biological effect of a peptide at the receptor level'. Wiley and Rich[6] defined peptidomimetics as 'chemical structures designed to convert the information contained in peptides into small non-peptide structures' and Gante[7] defined a peptidomimetic as 'a substance having a secondary structure as well as other structural features analogous to that of the original peptide from receptors or enzymes'. These definitions confine peptidomimetics to design features of molecules related to optimal binding to a receptor or enzyme. The progress of research in the area of peptidomimetics as pharmaceutical molecules encompasses a broader definition to include physicochemical properties compatible with current understanding of the parameters that constitute a drug molecule.

Here we define a 'peptidomimetic' as any small molecule whose structural base is derived from the peptide and defined by the minimum number of interactions that provide binding at a receptor or enzyme active site with equal or better affinity than the original peptide sequence, effects the same physiological response as the original peptide, and possesses drug-like properties for absorption and pharmacokinetics consistent with an appropriate therapeutic index.

In this chapter we have selected a few examples from the literature where drug design originated from a peptidomimetic approach to yield therapeutic agents, and examples where peptidomimetics show promise with *in vitro* systems but need further testing for drug properties consistent with therapeutic agents. We examine a few functional groups used as isosteres for the peptide bonds, amino acid side chains and spatial interaction within binding sites, and finally the drug properties of the resulting molecules. This chapter includes some topics, transition state analogues, which are also covered in Chapter 9, although here we study the absorption, distribution, metabolism and excretion (ADME) properties from the viewpoint of the peptidomimetic properties of the drugs.

8.2 Peptidomimetics for Aspartic Acid Proteases

Aspartic acid proteases are particularly interesting because of the development of marketed antiviral drugs from peptidomimetic approaches in the past two decades and the potential for drugs to address mammalian aspartic acid

proteases important in physiological processing of the cell. The human immunodeficiency virus-1 (HIV-1) and hepatitis C retroviruses use virally encoded aspartic acid proteases which digest the *pol* precursor proteins from the immature virions to produce the mature viral particles which are then released from the cell.[8] The HIV-1 protease (HIV-PR) is a homo-dimer with 99 amino acids per monomer. NMR and X-ray crystal structures of HIV-PR for the unbound and substrate-based inhibitor-bound complexes have provided insights into the dynamics associated with catalysis and inhibition.[9–11]

Figure 8.1(a) shows the sequence of an HIV-PR substrate peptide with a scissile amide bond between the two methionines. Replacing the methionine residues with norleucine resulted in a peptidomimetic with an affinity to the HIV-PR comparable to the substrate peptide. To produce a non-hydrolysable peptide, methylene-amine was chosen as the isostere to replace the scissile amide bond. The transition state for aspartic acid proteases involves a tetrahedral intermediate from nucleophilic addition of water to the scissile amide bond (Figure 8.1b), catalysed by the carboxylate residues of Asp25 in the protease sequence. Further modification of the substrate peptide by replacing the scissile amide bond with the methylene-amine isostere (Figure 1c, boxed) resulted in MVT-101, the first non-hydrolysable peptidomimetic inhibitor of the HIV-PR with a Ki of about 0.8 μM which is substantially higher than the Km for the hydrolysable substrates.[12] The methylene-amine isostere provides a tetrahedral carbon adjacent to the amide nitrogen and a molecular volume that is smaller than either the amide bond or its hydrated transition state. However, the methylene hydrogens lack the hydrogen bonding capability required for stabilization of a tetrahedral geminal diol transition state. Furthermore, this isostere has free rotation about the C–N bond which is lacking in the amide bond, thus allowing MVT-101 greater conformational flexibility than the original substrate peptide. These factors may account for the lower affinity of MVT101 for the HIV-PR than the substrate peptides.

In the ligand-free structure, two flexible β-hairpin loops between amino acids 45 and 55 of each monomer were identified in the vicinity of the active site. These loops showed considerable motion that allowed the substrate or substrate-based inhibitor access to the active site aspartates (residues 25 and 25′). Molecular dynamic simulations suggested a flexible active site with an 'induced fit' that drives the catalytic power of this protease.[9,11] This interpretation was consistent with the crystal structure, which showed that the β-hairpin loops become ordered and closed; placing residues Ile 50/50′ over the cleavage site.[12] Peptidomimetics of the *pol* protein sequence between Leu165 and Ile169, where the scissile amide bond between Phe167 and Pro168 of the penta-peptide sequence was replaced by either the methylene-amine or hydroxyethylamine amide bond isosteres, showed good HIV-PR inhibition but failed to advance due to poor bioavailability.[13]

Figure 8.2 shows the hydroxyethylamine transition state isostere for the amide bond hydrolysis. While this isostere is larger in volume than the transition state for hydrolysis of the amide due to the methylene group interspaced between the geminal diol and the amine in the transition state, it has a

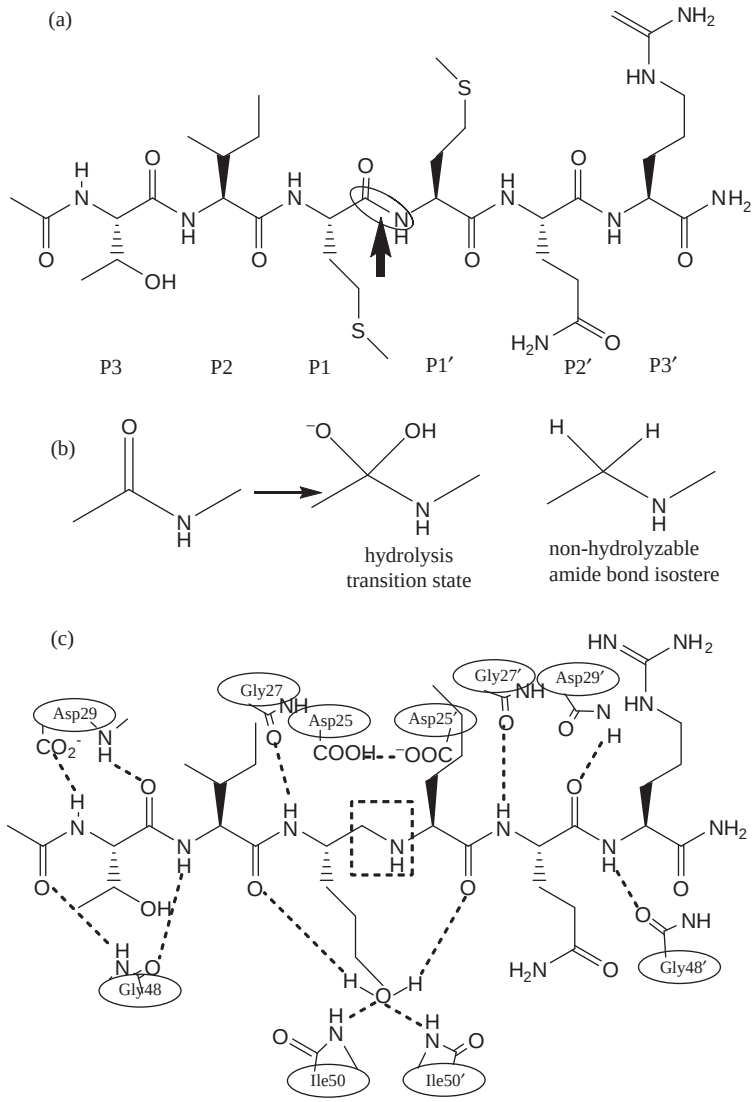


Figure 8.1 Schematic representations of: (a) the substrate hexapeptide with the arrow showing the scissile amide bond; (b) the transition state for hydrolysis of the amide bond by aspartic acid proteases and the non-hydrolysable methylene-amine amide bond isostere; and (c) the peptidomimetic MVT101 that has the methylene-amine amide bond isostere between residues P1 and P1′ (boxed) bound to the enzyme, showing the flap bound water and hydrogen bonds from protein residues to the peptide.

tetrahedral carbon with a hydroxyl group capable of hydrogen bonding equivalent to the tetrahedral geminal diol intermediate of the transition state, but incapable of hydrolysis. This isostere has been successfully incorporated into the nine peptidomimetic anti HIV-PR drugs shown in Figure 8.2 and

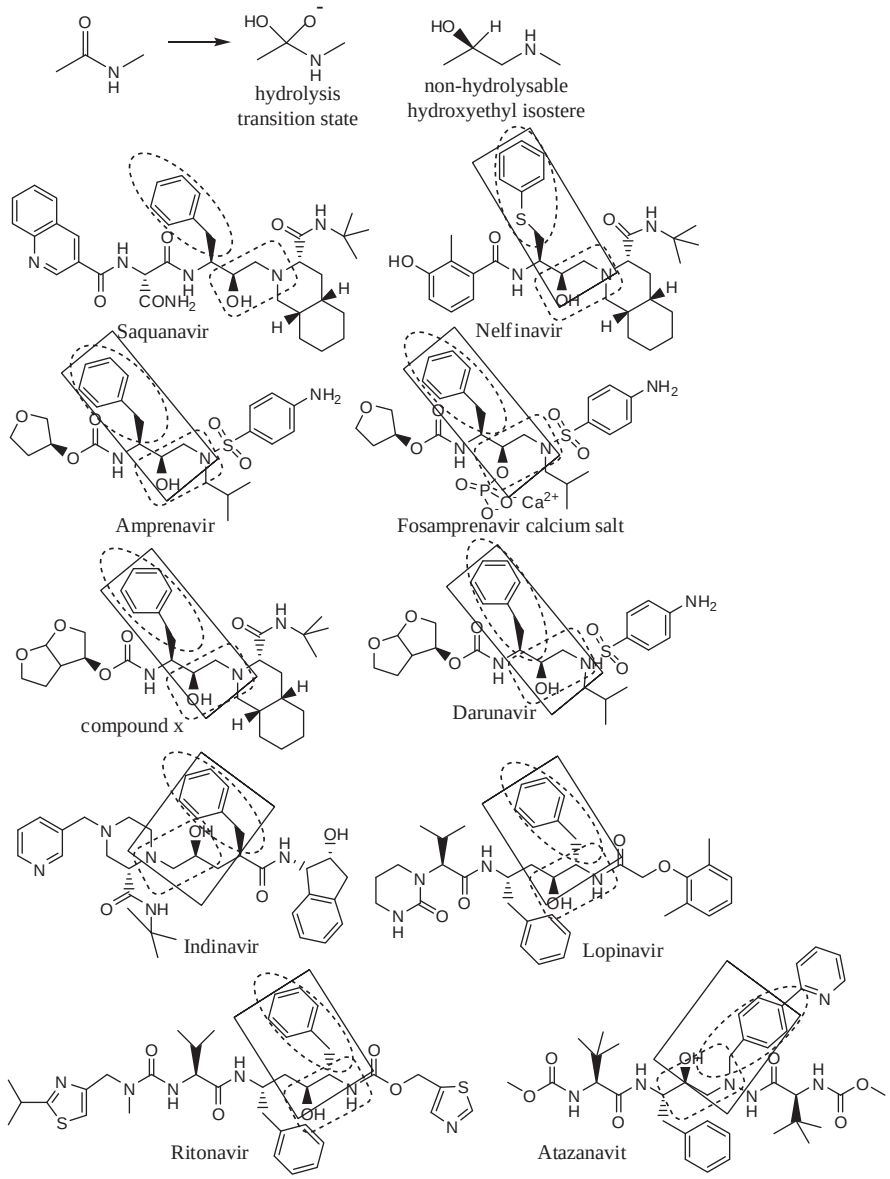


Figure 8.2 Hydroxyethyl transition state isostere for hydrolysis of an amide bond: ten approved peptidomimetic HIV-1 protease inhibitors incorporating the hydroxyethyl isostere.

which were approved by the US Food and Drug Administration (FDA) between 1995 and 2006.

The common motifs among the nine peptidomimetic drugs shown in Figure 8.2 are the hydroxyethylamine transition state isostere (dashed box)

and a phenethyl equivalent group as an isostere of the phenylalanyl residue in the pentapeptide (dashed circle). These two groups constitute the core pharmacophore of this class of HIV-PR inhibitors (solid box). Except for darunavir, the affinities of these drugs for the HIV-PR are comparable, yet their overall structures have distinct differences at the flanks of the core pharmacophore. The nine peptidomimetic structures show little resemblance to the original peptide from which they were derived and contain variable numbers of amide bonds, yet they possess affinity for the protease that is comparable or higher than the original peptide. In this series, the hydroxyethylamine transition state isostere appears crucial for the tight binding with the protease. Its hydroxyl group is suggested to be hydrogen bonded to the catalytic aspartic acid residue in the active site, mimicking the bound water molecule in the native enzyme which serves as the nucleophile for hydrolysis of the amide bond. In saquinavir, a bicyclic amine serves as an isostere for the prolyl residue in the peptide sequence. In addition to providing the structural constraint that proline provides in peptides, the bicyclic amine isostere is believed to provide additional hydrophobic interactions. A quinaldic acid serves as a hydrophobic replacement for the carboxy terminal leucine.

Nelfinavir was structurally derived from saquinavir by truncating the peptide at the *N*-terminus by replacing the leucine–asparagine sequence with the less peptidic hydroxymethyl benzoyl moiety, resulting also in a decrease in molecular weight and an increase in solubility. Nelfinavir showed comparable affinity for HIV-PR to saquinavir. In compound-X, the leucine–asparagine sequence was replaced by a bicyclic *bis*-tetrahydrofuranyl carbamate. The compound was similar in affinity to saquinavir with the protease but with greater solubility, suggesting that the *bis*-tetrahydrofuranyl isostere provided comparable binding properties as the quinaldic acid moiety in saquinavir.

Amprenavir is structurally distinct from saquinavir and nelfinavir. It contains a *p*-aminosulfonamide in place of the prolyl isostere of saquinavir and nelfinavir. Additionally, the asparaginyl end was replaced by a furanocarbamate. Amprenavir is significantly more soluble than nelfinavir, with comparable affinity for the protease.

Indinavir is structurally distinct from saquinavir, nelfinavir and amprenavir at both flanks of the core pharmacophore. An amidohydroxy-indan group replaces the prolyl-isoleucine motif of the pentapeptide and a pyridyl-methyl-piperazine motif replaces the asparaginyl end. These substitutions provide indinavir with even greater solubility.

Ritonavir contains a methyl thiazole carbamate at the prolyl end and, at the asparaginyl end, a substituted urea containing an isopropylthiazole group. Atazanavir, approved by the FDA in 2003, contains this core pharmacophore except that the hydroxyethyl amine is replaced with the hydroxyethyl hydrazine isostere, which results in a decreased number of stereochemical centres in the drug structure and increased ease of manufacture. The core pharmacophore in this molecule is symmetrically flanked by carbamates.

Modification of amprenavir with the bicyclic *bis*-tetrahydrofuranyl isostere of the asparaginyl moiety in compound X yielded darunavir which was

approved by the FDA for therapy in 2006. Despite the very high structural similarity of darunavir to amprenavir, it shows almost two orders of magnitude tighter binding to the wild-type protease and a 1.5 order of magnitude tighter binding to the multi-drug resistant mutants of the HIV-PR.[14–17]

Since all the peptidomimetic structures approved for drug use (except darunavir) demonstrate comparable affinity for the HIV-PR, the large structural diversity flanking the core pharmacophore in these molecules suggests a high degree of tolerance in the active site cavity for the prolyl–isoleucyl carboxyl end and the leucyl–asparaginyl amino end of the peptide sequence from which they were derived. These structures vary in amide bond content from one to four, which appears to be the only common feature to the peptide from which they were designed. Thus, the differences in therapeutic potential observed for these compounds must derive from differences in their physicochemical properties which impact absorption, distribution and metabolism, and suggests room for further enhancements based on physicochemical and drug metabolism properties of groups attached to either flank of the core pharmacophore.

From a drug design strategy point of view, it is of interest to mention tipranavir (Figure 8.3), the only non-peptidomimetic HIV-PR inhibitor approved by the FDA for therapy. This compound was designed by a traditional structure activity approach from 4-hydroxycoumarin and 4-hydroxypyrone leads identified from traditional library screening approaches.[18] This structure lacks amide bonds and has no apparent similarity to any of the peptidommetically derived structures shown in Figure 8.2.

From a drug metabolism perspective, saquinavir shows poor bioavailability; this may possibly be due to poor absorption resulting in low solubility and extensive metabolism by CYP3A4. Nelfinavir, in contrast, shows greater bioavailability than saquinavir which may be a consequence of its decreased molecular weight and increased solubility. Nelfinavir and saquinavir have short *in-vivo* half lives due to extensive metabolism by CYP3A4.

The primary metabolic pathway for these drugs involves hydroxylation at the bicyclic prolyl isostere and at the *N-t*-butyl methyl carbon. It is unclear if the metabolites are pharmacologically active and if they contribute to the overall potency of these drugs. Amprenavir has higher solubility than either saquinavir or nelfinavir and has higher plasma exposure with a longer half-life (10 h), permitting twice daily dosing regimens. Amprenavir is also metabolised

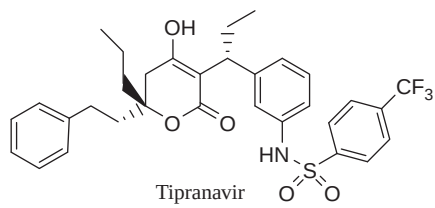


Figure 8.3 Structure of the approved anti HIV-PR drug, tipranavir, designed by a non-peptidomimetic strategy.

primarily by CYP 3A4. Fosamprenavir is a prodrug for amprenavir with higher solubility and is rapidly hydrolysed at the intestinal brush border to release amprenavir, thus allowing formulation of higher strength tablets. Indinavir shows high bioavailability and plasma exposure, probably due to the hydroxyindan and pyridyl-methylpiperazine motifs which offer greater solubility. Indinavir is a substrate and inhibitor of CYP 3A4. Ritonavir is a potent CYP 3A4 inhibitor; consequently, its primary use in antiretroviral therapy has evolved as a low dose co-drug to increase the plasma exposure of co-administered antiretrovirals.[17,19] Atazanavir has high bioavailability providing high oral exposure and a longer *in-vivo* half-life that can support once daily dosing for treatment. Darunavir is metabolised by CYP 3A4 but is a weak inhibitor of 3A4. It is co-administered with low dose ritonavir to achieve plasma exposure necessary for therapy. The high affinity of darunavir for the HIV-PR and its higher solubility than the other anti HIV-PR drugs allows for doses in the range of 300–400 mg twice daily as opposed to doses of 800–1000 mg twice or thrice daily for the other protease inhibitors.

As these anti HIV-PR drugs are generally metabolised by CYP3A4, a clear understanding of drug interactions between co-administered antiretrovirals in clinical treatment of HIV is critical where multi-drug therapy is considered the standard of care. For example, darunavir/ritonavir co-administered with saquinavir resulted in a significant decrease in steady state exposure of darunavir, suggesting that these drugs should not be co-administered.[20] The development of anti HIV-PR antivirals is an excellent example of successful application of the peptidomimetic approach to the discovery of new therapeutic agents.

Renin is another example of an aspartic acid protease where peptidomimetic approaches have been tried for the development of antihypertensives. However, with renin, the peptidomimetic approach has not met with success comparable to that with the HIV-PR. Renin cleaves angiotensinogen to the decapeptide angiotensin I, which is further digested by a non-specific dipeptidyl carboxypeptidase, agiotensin-converting enzyme (ACE), to the octapeptide angiotensin II which is a potent vasopressor. Renin and ACE are popular targets for the development of antihypertensive drugs.

Figure 8.4 shows a commonly used retro inversion of an amide bond as a strategy in peptidomimetic design to prevent protease digestion. This strategy was used to design the three peptidomimetic inhibitors of renin shown in Figure 8.4. Histidine is the only residue that was not changed in the inhibitors, as this residue provides specific binding and hydrogen bonding interaction with Ser233. The P4 prolyl residue was replaced with piperidinyl or morpholino rings to better fit the S4 renin binding site.[21] The P3 phenylalanine was replaced by 2-methyl-naphthyl-succinic acid, with a retro inverso amide to the amino group of piperidine or morpholine at P4 and the α-amino group of histidine. The naphthyl aromatic ring was selected to prevent recognition of a Phe–His-like amide bond by chymotrypsin. The P1 isoleucine was replaced with norstatine (3-amino-2-hydroxy propionic acid) having either an isobutyl or methylcyclohexyl group at P3. Compound **8.3** (Figure 8.4) was found to be

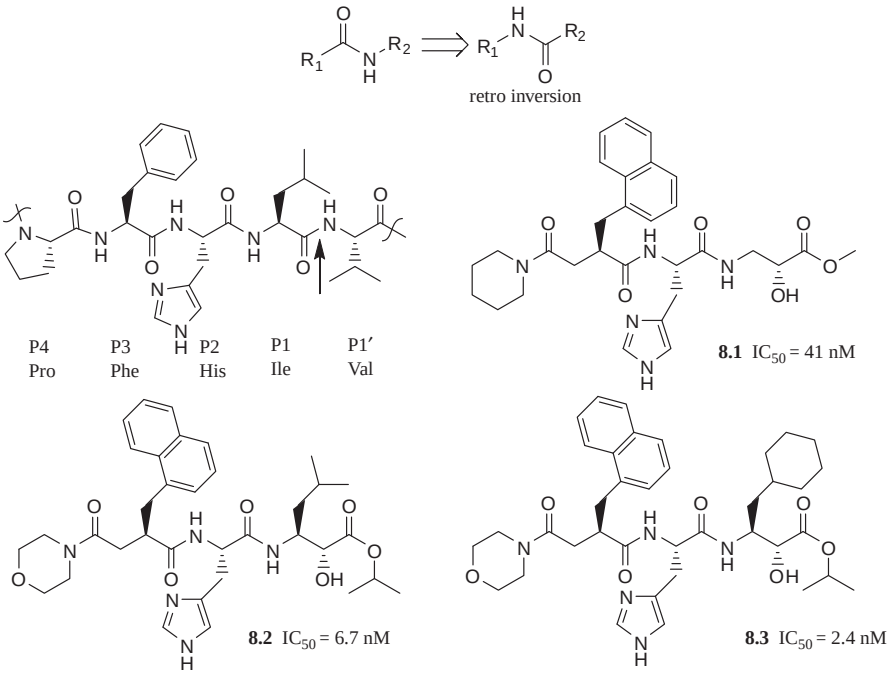


Figure 8.4 Retro inversion of the amide bond used in peptidomimetics to decrease protease susceptibility of peptidomimetic inhibitors of renin derived from the angiotensinogen pentapeptide sequence (arrow shows the scissile amide bond).

stable in monkey liver homogenates, human plasma and chymotrypsin, and was pharmacologically active in monkey at an oral dose of 10 mg kg^{-1} causing a 10–20 mm Hg fall of blood pressure over a five-hour period.[22] Clearly, the pharmacological effect for 5 h at a 10 mg kg^{-1} dose suggests that the pharmacokinetic properties of the peptidomimetic are not optimal for potential therapeutic use.

Much effort has been spent by several pharmaceutical companies in developing peptidomimetics for renin.[23] The frustrations described by Ciba-Geigy scientists with the peptidomimetic approach for the search of renin inhibitors starting in the early 1980s with CGP29287, a nona-peptide peptidomimetic (FW 1495) that had high affinity (7 nM) for renin but poor absorption and pharmacokinetics. Its next generation peptidomimetic derivative CGP38560 in the late 1980s, with a lower molecular weight (FW 729) and an order of magnitude higher affinity (0.7 nM) but equally poor bioavailability and pharmacokinetics, led the Ciba-Geigy/Novartis group to abandon the traditional peptidomimetic approach in favour of a structure-based approach. But, as noted by Cohen,[23] the lowest energy conformational model of the peptidomimetic, CPG38560, docked to the homology model of renin served as the starting point for the *de novo* structure-based design approach used in the

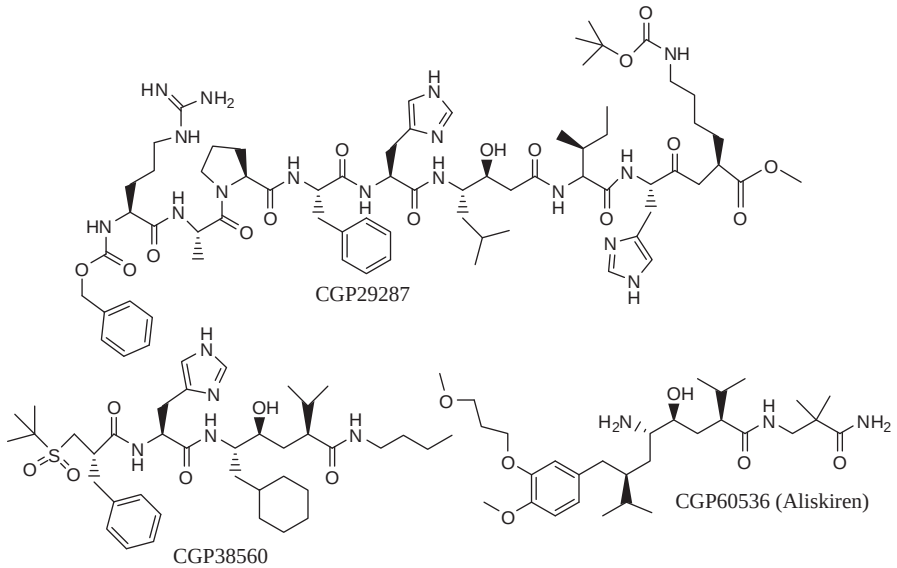


Figure 8.5 Potent *in vitro* peptidomimetics for human renin.

development of non-peptide based structures that ultimately yielded aliskerin, which was approved in 2007 as the first renin targeting antihypertensive drug. Figure 8.5 shows the structures of aliskerin and the peptidomimetics that failed as pharmaceuticals because of their ADME properties, not because of their ability to inhibit renin.

The efforts undertaken by many pharmaceutical companies to develop renin inhibitors since the mid-1970s reinforces the critical need to incorporate physicochemical properties, components of drug metabolism such as metabolic stability, permeability and pharmacokinetics early in any of several strategies in used for the development of pharmaceuticals. While aliskerin is the first commercialised renin inhibitor, its human bioavailability is only 3%. This suggests room for significant enhancements in next generation renin inhibitors based on knowledge of aliskerin's ADME properties in humans.

8.3 Anticancer Peptidomimetics

Mammalian farnesyl transferase plays a critical role in translocation of *ras* gene products from the cytosol to the plasma membrane where they play an important role in mitogen-activated cell proliferation. Inhibition of the pathway is considered a target for anticancer therapy.[24,25] CAAX is the signal recognition sequence for farnesylation of these gene products where cysteine is the fourth residue in from the carboxy terminal, the second and third residues (AA) are typically valine or isoleucine, and X can be any amino acid but is usually serine or methionine. The tetrapeptide CVIM is a potent *in vitro*

inhibitor of farnesyltransferase, but has poor activity in cell culture due to its lack of intracellular access.[26] Crystal structure analysis of CVIM bound to the transferase shows that the tetrapeptide is bound in an extended conformation through hydrophobic interactions with the Ile–Met residues and a hydrogen bond to the isoleucine carbonyl group.[27] A peptidomimetic approach has been used to develop potent inhibitors of farnesyl transferase.

As shown in Figure 8.6, when the Val-Ile residues were replaced by p-aminobenzoyl spacer the peptidomimetic **8.4** showed a higher affinity than the inhibitory peptide CVIM (IC50 340 nM). Given the extended conformation in which the peptide is bound within the active site and the enhanced affinity of peptidomimetic **8.4**, analog **8.5** was examined and found to bind with comparable potency as **8.4**, eliminating the chiral components of the P1 methionine. Crystal structure analysis showed a hydrophobic space adjacent to the biphenyl spacer. Attachment of a phenyl substituent to the amino phenyl ring of structure **8.5** resulted in a more potent inhibitor (**8.7**, IC_{50} 15 nM). Placement of the phenyl ring on the 4-amino-benzoic acid spacer at the 3-position resulted in the most potent peptidomimetic in this series.[28,29] While these compounds show excellent *in vitro* potency against farnesyl transferase and would be expected to enter cells because of their lipophilic characteristics, lack of drug metabolism

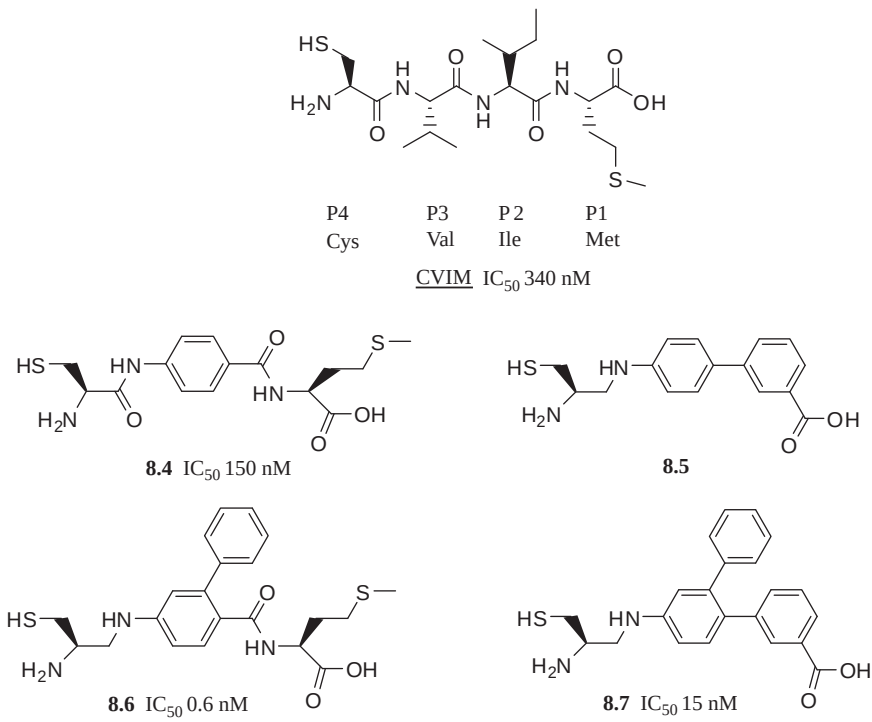


Figure 8.6 Peptidomimetics of farnesyltransferase.

information makes judgment of these structures as potential therapeutic agents impossible.

Cancer cells are compromised in their ability to undergo apoptosis;[30] many cancer cell lines and tumors isolated from patients have high levels of expression levels of the X-linked inhibition of apoptosis protein (XIAP).[31] XIAP functions by binding and inhibiting three members of the caspase family of enzymes.[32] Mitochondrial proteins, Smac/DIABLO, released into the cytosol in response to an apoptotic stimulus such as TNFα, bind to XIAP and caspase-9 reversing the inhibition by XIAP of caspases.[33–36] As XIAP blocks apoptosis at the convergence of multiple signalling pathways, it is particularly attractive as a target for the design of drugs aimed at reversing the apoptotic resistance of tumor cells. Crystal and NMR solution structures reveal that the *N*-terminal tetrapeptide (AlaValProIle) of Smac binds to a surface groove on XIAP. Figure 8.7 shows a schematic representation of the interaction between the tetrapeptide with residues on the BIR3 domain of the XIAP protein.

As with peptides in general, Smac based peptides show good *in vitro* binding but have poor cell permeation. However, when tethered to a carrier peptide, they were shown to sensitise tumor cells establishing the validity of the target for cancer therapy.[37–39] The peptidomimetics shown in Figure 8.6 show strong but comparable binding to the XIAP protein and represent the early stage in the discovery of novel drugs for targeted chemotherapy similar to the early stages of anti HIV-protease anti retroviral peptidomimetics. Lessons from the failure to develop peptidomimetic inhibitor drugs for renin should help guide approaches to the development of a new generation of anticancer drugs directed at these novel targets.

8.3.1 Summary

As shown by the examples above, peptidomimetics hold promise as another means of developing novel therapeutics based on a clear understanding of the biochemical pathway and the mechanisms underlying peptide–protein or protein–protein interactions. As was shown for the development of HIV-PR inhibitors, the structures of the final drug products had little in common structurally with the peptide from which they were derived, and with exception of the core pharmacophore, have little in common with each other. Furthermore, a potent antiretroviral, tirpanavir, was developed by a traditional iterative structure–activity–drug properties screening approach from lead matter identified in high throughput screening, without structural knowledge of the binding to the active site but a robust assay for activity. At the core of discovering new chemical matter for therapeutic use is a clear understanding of the biochemical basis for target selection, clear design strategies for new chemical matter with high potency and early evaluation of drug properties for absorption, pharmacokinetics, and metabolism, which lead to the selection of optimal structures to progress through the drug discovery pipeline.

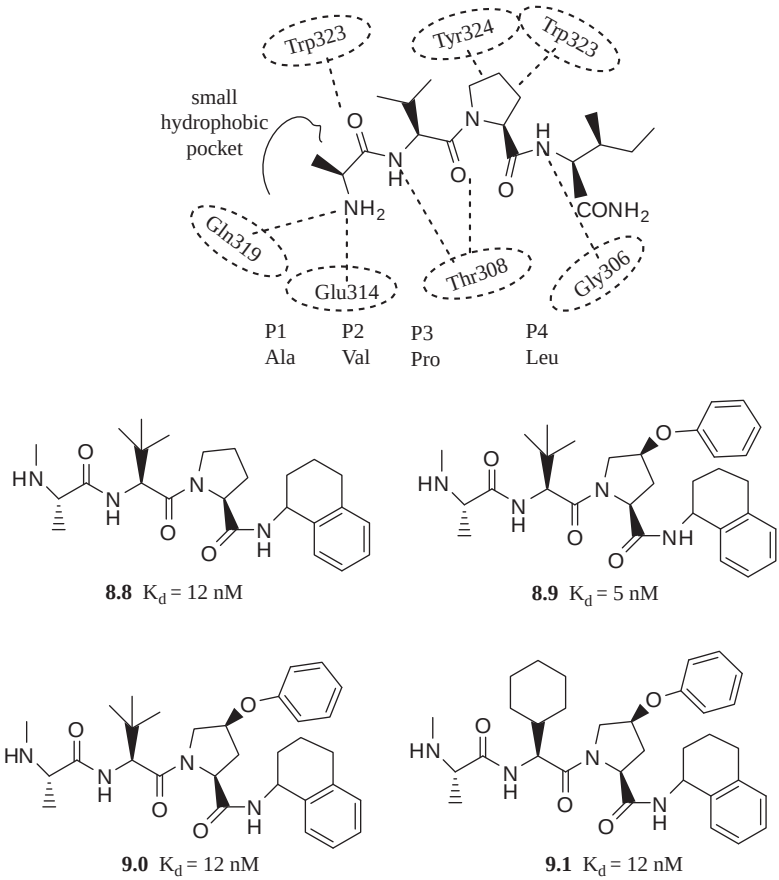


Figure 8.7 Schematic representation of the *N*-terminal tetrapeptide (AlaValProLeu) of Smac bound in the BIR3 binding groove of XIAP showing the interactions of peptide backbone and side chains with amino acids of the XIAP protein and four potent Smac peptidomimetics with dissociation constants.

8.4 Peptide Drugs

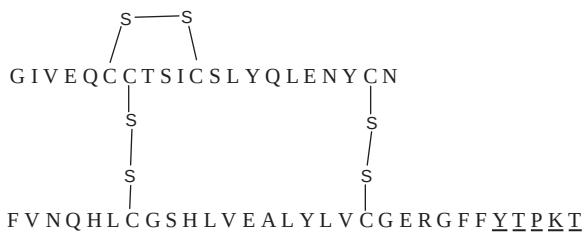
8.4.1 Insulin and Insulin Analogs

The discovery of insulin in the first quarter of the 20th century[40] has led to the treatment of diabetes mellitus for over 70 years, with early treatments using partially purified extracts from porcine or bovine spleen that had highly variable efficacy. With progress in purification of animal spleen-derived insulin, efforts focused on enhancing the action duration of insulin preparations. Treatment initially involved subcutaneous injections of crystalline insulin just prior to meals and typically showed an action onset between 0.5 and 1.0 hour, with peak activity between 2 and 3 h and between 6 and 8 h of duration.

Early efforts to increase the action duration were achieved by a complex between insulin and protamine, with further enhancements achieved by adding zinc ions to stabilize the protamine–insulin complex (PZ insulin) and further modifications to produce a neutral protamine insulin complex termed NPH insulin.[41] Proteolysis of protamine in the complex at the deposition site resulted in the slower release of insulin and a doubling of the duration of insulin's action. Another form of extending the action duration of insulin was by the addition of excess variable amounts of zinc ions, which produced amorphous crystalline suspensions called lente insulins.[42]

In the 1980s, through recombinant technology human insulin replaced bovine and porcine pancreatic insulin. Crystalline insulin, PZ-insulin, NPH-insulin and lente insulins were the main therapeutic forms of insulin up to the 1990s. In the 1990s, two major clinical studies, the UK Prospective Diabetes Study Group (UKPDS33) and the Diabetes Control and Complications Trial Research Group, established a clear link between glycemic control and microvascular complications in diabetics.[43,44] These findings, along with the observation of the basal and postprandial secretion pattern of insulin in normal subjects,[45] suggested that the goal of insulin therapy should be to mimic the rhythmic pattern observed for insulin secretion.[46] The finding that pancreatic insulin secretion was directly into the hepatic portal circulation and knowledge of the structure and physicochemical properties of native insulin made it evident that subcutaneous deposition of insulin was not ideal to mimic the observed physiological secretion pattern of insulin. Efforts were directed towards developing short acting analogs to mimic postprandial secretion and long-acting analogs to mimic basal secretion.

Figure 8.8 shows the sequence of human insulin. Soluble human insulin is a hexameric aggregate which dissociates slowly to monomers within the



Short acting insulin analogs
Insulin lispro: B chain P_{28} K_{29} inversion to KP
Insulin aspart: B chain P_{28} replaced by D
Insulin glulisine: B chain N_3 replaced with K;K_{29} replaced with E

Long acting insulin analogs
Insulin glargine: A chain N_{21} replaced with G, RR added to position 30 in the B chain
Insulin determir: B chain T_{30} removed; K_{29} acylated with myristic acid

Figure 8.8 Structure of human insulin and the structure modifications to create short- and long-acting analogs.

subcutaneous tissue with the resultant slow appearance in blood. Structural characterization of human insulin showed that residues 26–30 (YTPKT) were not critical for binding of insulin to the insulin receptor, but were critical for aggregation.[47] Inversion of proline–lysine at position 28–29 in the B chain of human insulin results in an analog (insulin lispro) with comparable insulin receptor affinity but decreased self association. This in turn results in faster dissociation of aggregates into monomers and consequently faster absorption from the subcutaneous deposition site, leading to an earlier and greater peak serum level for a shorter duration of time compared with unmodified human insulin. Pharmacokinetics of insulin lispro shows an onset of activity between 0.2 and 0.5 h, with maximal activity between 0.5 and 2 h and a duration of 3–4 h.[48,49] Insulin lispro was the first insulin analog approved for human use in 1996.

Insulin aspart was derived from human insulin by substituting proline 28 in the B chain with aspartic acid. Like insulin lispro, this analog results in weak dimeric and hexameric aggregation leading to comparable rapid absorption into serum after subcutaneous injection. The pharmacokinetic behaviour of insulin aspart is similar to insulin lispro[50] and it was approved for human use in 2000.

Insulin glulisine is the third approved rapid onset insulin analog approved for human use. Insulin glulisine was obtained by substituting B chain lysine 29 and aspargine 3 with glutamic acid and lysine, respectively. The pharmacokinetic properties of insulin glulisine are comparable with those of the other two fast acting insulin analogs, with rapid onset of activity within 20 minutes and maximal activity in 1.5 h.[51,52]

The pharmacological properties of the insulins lispro and aspart are comparable to human insulin in terms of insulin receptor affinity, receptor off rate, metabolic potency, insulin like growth factor-1 receptor affinity, and mitogenic potency. Insulin glulisine has somewhat lower affinity for the human insulin receptor and is significantly weaker in affinity to the insulin-like growth factor-1 receptor and mitogenic potency.[53,54] In general, the three fast-acting insulin analogs are very similar in pharmacokinetic and physiological function and more closely mimic the normal physiological insulin response following prandial subcutaneous administration when compared to subcutaneously administered human insulin.

Long-acting insulin analogs have been developed by shifting the isoelectric point towards neutral pH and by increasing the hydrophobicity by covalent modification with a fatty acid. Insulin glargine was developed by replacing asparagine 21 in the A chain with glycine and adding two arginine residues at position 30 in the B chain. These amino acid substitutions result in an isoelectric point shift from pH 5.4 to 6.7.[55] This analog is injected subcutaneously as an acidic solution at pH 4.0 and forms a precipitate. The precipitated insulin glargine dissolves slowly and is absorbed into the serum without any significant peak. It has a duration of action of about 20 h at physiological doses and is significantly longer acting than NPH insulin.[56] Insulin glargine (lantus) was the first long-acting insulin analog to be approved for human use.

Another long-acting insulin analog approved for human use is insulin determir. This analog is formed by removing threonine 30 in the B chain and acylating the lysine at position 29 in the B chain with myristic acid. Insulin determir forms soluble hexameric and di-hexameric complexes at the site of injection *via* aggregation of the fatty acid chains. These self-associated complexes are thought to equilibrate with serum albumin. The equilibrium between self-association and albumin binding are thought to be the reason for the long depot residence time. The binding to albumin is also thought to slow its distribution to peripheral tissues resulting in an increased duration of activity.[57] Binding of insulin determir to serum albumin does not appear to compete with other albumin bound compounds or to show kinetic differences in low albumin states, suggesting that albumin may serve as a reservoir for insulin determir forming a buffer for any rapid changes in insulin absorption.[58,59]

Long-acting insulin analogs show comparable reductions in HbA1c to NPH insulin.[60,61] However, these analogs show a significantly lower risks of nocturnal hypoglycemia.[62–64] Combination therapies with long- and short-acting insulin analogs, as well as long-acting insulin analogs and oral antidiabetic drugs are being considered as therapeutic options to achieve the rhythm of insulin observed in normal humans.

8.4.2 Incretin Hormones

Plasma insulin levels are substantially increased upon oral glucose administration compared with intravenously administered glucose. This effect is termed the *incretin effect*[65] and is caused by the release of peptides from endocrine cells in response to ingestion of food. Several such incretin peptides have been identified and their plasma levels are known to be differentially affected by nutrients in the digestive track. These include:

- glucagon-like peptide-1 (GLP-1)—this is released by intestinal L-cells, plasma levels of which remain elevated for a while after cessation of feeding;
- amylin—a 37 amino acid polypeptide co-secreted with insulin by pancreatic β-cells;
- cholecystokinin (CCK)—a peptide released by I-cells in the upper intestinal track in response to the presence of digestive products of fats and proteins;
- peptide YY—like GLP-1 this is released by intestinal L cells slowly during meals and remains elevated in plasma for several hours;
- ghrelin—produced by gastric endocrine cells is a 28 amino acid peptide that serves as a signal of energy depletion and acts to initiate food intake.

GLP-1 and amylin analogs have become therapeutic agents whereas CCK, PYY and ghrelin receptors are still investigational targets for therapy.

Most of the incretin effect on pancreatic function is derived from GLP-1.[66] Glucose homeostasis in the postprandial period is regulated by GLP-1 through several mechanisms that include inhibition of glucagon secretion, increase of insulin synthesis, delay in gastric emptying and promotion of satiety.[67] Type 2 diabetic patients have a decreased incretin effect due to a decrease in secretion of GLP-1, which results in lowered insulin secretion and disruption of glucose homeostasis.[68] Administration of GLP-1 to type 2 diabetic patients results in an increase in insulin secretion and lowering of postprandial blood glucose.[69] GLP-1 is, however, rapidly degraded by dipeptidyl peptidase 4 (DPP-4), a protease that is widely distributed in multiple cell types including the capillary bed of the gut mucosa and so rapidly inactivates GLP-1.

Exendin-4 (Bayetta, Exenatide) a 39 amino acid peptide extracted from the venom of the Gila monster (*Heloderma suspectum*) with a high affinity for GLP-1 receptors and has 53% homology with GLP-1. Exenatide is the first GLP-1 receptor agonist approved for adjunctive therapy for type 2 diabetic patients receiving sulfonyl ureas or methformin treatment but who have not achieved adequate control. Clinical trials have demonstrated that exenatide improves glycemic control when combined with methformin and sulfonylureas. It may be an alternative to insulin glargine for patients who require non-traditional therapy.

Amylin's site of action is the area posterma—a hind brain circumventricular organ with a porous blood–brain barrier. Peripheral administration of amylin analogs have been shown to reduce food intake in non human primates.[70] Pramlintide is a human amylin analog that has been approved for human therapy co-administered with mealtime insulin for patients who have not achieved glucose control. Clinical studies have demonstrated reductions in HbA_{1c} and body weight.[71]

Most currently used peptide-based drugs are currently administered parenterally by subcutaneous or intramuscular injection. This is a major limitation to the administration of such medications. Efforts to develop inhaled forms of such drugs have met with limited success. Peptides and peptide-based drugs show no oral bioavailability due to gastric digestion as well as poor absorption across the intestinal wall.

Some efforts to develop methods for oral delivery of such drugs including protein drugs have focused on the dietary uptake mechanism of vitamin B12.[72] In this approach, vitamin B12 is either conjugated directly with the protein–peptide of interest for oral administration or is encapsulated in a vitamin B12 coated dextran nanocapsule. Success has been achieved using this technology for some proteins and peptides including insulin and erythropoietin.[73,74] The development of such approaches including exploring other carrier-mediated transport processes across the intestinal wall needs further research and technological innovations to circumvent the oral gastric barrier when delivering peptide and protein drugs. Such developments will dramatically enhance the use and development of peptide-based drug molecules based on protein–protein or protein–peptide interactions.

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CHAPTER 9

Pharmacokinetics and Metabolism of Compounds that Mimic Enzyme Transition States

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9.1 Enzyme Transition States

Enzymes are catalysts that function by enhancing the rate of covalent bond formation and breaking. Inhibition of enzymes has been a fruitful source of drugs effective in treating disease, with 300 drugs targeting more than 70 different enzymes approved for therapeutic use in humans.[1]

A central tenant of the catalysis of biological reactions by enzymes is that the enzyme–substrate complex exists in equilibrium with a higher energy transition state which sits at the top of an energy maximum where the chances of the enzyme–substrate complex reverting to substrates or proceeding to products

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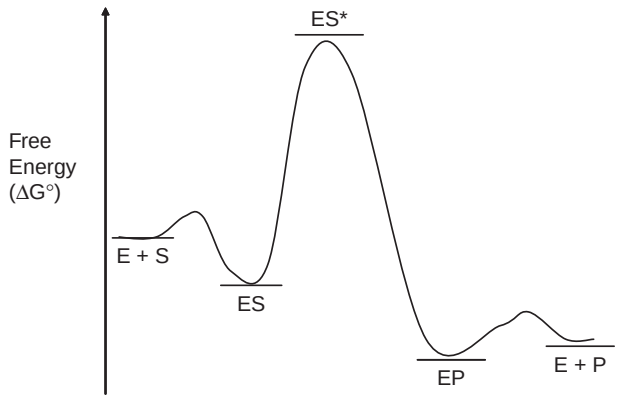


Figure 9.1 Graphical representation of energy changes along the reaction coordinate as enzyme (E) binds to substrate (S) forming an enzyme-substrate complex (ES), followed by formation of a high energy transition state (ES*) with eventual formation of an enzyme–product complex (EP) and release of product from the enzyme (E + P).

are equal (Figure 9.1). In reality, enzymatic transition states are dynamic with lifetimes (a fraction of a picosecond) defying direct physical observation.[2]

The catalytic power of enzymes can be viewed to lie in the very high affinity that enzymes have for the transition state relative to the affinity with which they bind substrate in the ground state.[3] As an example, calf intestinal adenosine deaminase has a dissociation constant for the transition state approximately 10^{12} times smaller than it has for the substrate adenosine.

Transition state analogue inhibitors (TSAIs) are a class of competitive enzyme inhibitors that are designed to take advantage of this very high affinity interaction between an enzyme and the transition state of the reaction it catalyses. By mimicking the chemically unstable transition state, whilst being chemically stable themselves, TSAIs can bind to the enzyme with much higher affinity than substrate.[4–8] Although in most cases substrates and transition state analogues bind in a similar fashion, the transition state analogue can not be converted to a product and as a result is a potent enzyme inhibitor. The theoretical potency of a perfect transition state analogue inhibitor can be calculated as shown in eqn (9.1):[6]

$$\text{Theoretical maximum Ki} = \text{substrate Km}/\text{catalytic rate enhancement} \quad (9.1)$$

As catalytic rate enhancements by enzymes are typically of the order of $10^{10–15}$ and can be as high as 10^{19}, then a perfect transition state analogue inhibitor can bind to an enzyme with a dissociation constant of approximately 10^{-22} M.[8] As it is not possible to exactly replicate the bond angles, bond distances and electronic distribution of a transition state in a stable molecule, it is unusual to see TSAIs with potency increases of 10^{15} or more compared to substrate. However, even picking up some of the structural features of the transition state

can yield potent inhibitor compounds that combine sufficient chemical stability to be therapeutically useful as enzyme inhibitors.

To prove that an enzyme inhibitor is acting as a transition state analogue inhibitor and not simply as a potent competitive or covalent inhibitor is not trivial because binding kinetics alone (including slow inhibition and/or offset) are not diagnostic for a compound being a transition state analogue inhibitor.[2] Experimental techniques to confirm that a compound is a transition state analogue inhibitor include:[9–14]

(1) Deriving the free energy relationship for the interaction between inhibitor and enzyme
(2) Kinetic isotope experiments where individual atoms close to the bonds being broken are replaced with isotopes (*e.g.* H is replaced with D or T, and ^{13}C replaces ^{12}C and ^{15}N replaces ^{14}N) and the rate of catalysis determined
(3) X-ray structural analysis
(4) NMR.

The potential to inhibit enzymes with high affinity and specificity using transition state analogue inhibitor approaches has attracted much attention and TSAIs have been described for all major classes of enzymes and for more than 130 individual enzymes.[7,15] However, the number of TSAIs that have been successfully tested in humans as potential therapeutic agents is relatively small.

In this chapter we discuss the impact of the structural features needed to mimic the transition state of an enzymatic reaction on the disposition of drugs in the body and some of the strategies used to successfully develop TSAIs as therapeutic agents. In considering the effects of chemical structure and physicochemical properties on oral bioavailability (F), we have used eqns (9.2) and (9.3) to separate the effects on absorption and first pass clearance/metabolism:

$$F = Fa \times Fg \times Fh \tag{9.2}$$

where:

F = bioavailability
Fa = fraction absorbed
Fg = fraction escaping gastrointestinal tract first-pass metabolism
Fh = fraction escaping first-pass clearance in the liver.

$$Fh = [1 - (CL_h/Q)] \tag{9.3}$$

where:

CL_h = hepatic blood clearance
Q = hepatic blood flow.

Q is assumed to be 90, 70, 40, 40 and 20 $ml\,min^{-1}\,kg^{-1}$ in mouse, rat, dog, monkey and human, respectively. Unfortunately, Fg is unknown for many compounds and in these cases we have assumed Fg to be 1.

As the majority of pharmacokinetic studies in the literature report plasma (rather than blood) clearance data, we have converted the plasma clearance to blood clearance by dividing it by the blood : plasma ratio (BP). Wherever possible, we have used measured values of BP; if an assumed value is used this is noted.

9.2 Physicochemical Properties of Transition State Analogues

The atoms that mimic the bond(s) breaking/forming in the transition state of the enzymatic reaction actually represent only a small fraction of those present in TSAIs. The rest of the transition state analogue inhibitor molecule functions as a scaffold to position the transition state mimicking group correctly within the active site of the target enzyme and makes interactions with the amino acid residues present in the active site of the enzyme to increase the potency and specificity with which the TSAI binds the target enzyme. In this way, selective inhibition of the target enzyme can be achieved without affecting the function of closely related enzymes. In addition, the scaffold of the TSAI can be used to modify the pharmacokinetics and metabolism of transition state analogue inhibitor compounds.

The general physicochemical properties of TSAIs such as molecular weight (MW) and lipophilicity are largely reflective of the scaffold of the transition state analogue inhibitor and vary considerably depending on the lipophilicity and size of the active site of the enzyme target. The TSAIs discussed in this chapter have molecular weights ranging from 228 (zebularine) to 1500 (CGP-29827), with clogP values spanning a 16 log unit range (from -5.6 for zanamivir to +11 for BAY-793) and encompass acidic, basic and neutral compounds.

The one physicochemical property shared by TSAIs is a relatively high polar surface area (PSA). Figures 9.2–9.4 show the calculated PSA *vs*. molecular weight, the hydrogen bonding count normalised by molecular weight *vs*. PSA and the PSA *vs*. clogP, respectively, for the TSAIs discussed in this chapter together with the same data for a number of approved small molecule human drugs.

Only 14% of the approved drug dataset have a PSA $>140\,Å^2$ (a value often used as a cut-off for predicting good oral absorption); this value increases to 52% when TSAIs are considered. That TSAIs have a high PSA is not unexpected since the majority of transition states involve the intermediacy of high energy, highly polar, charged species such as a tetrahedral intermediate following nucleophilic attack on an amide bond. By mimicking these polar, short-lived transition states, TSAIs can achieve great potency, but the cost is that TSAIs have high PSAs that can limit the passive membrane permeability and hence the oral absorption of the compounds.

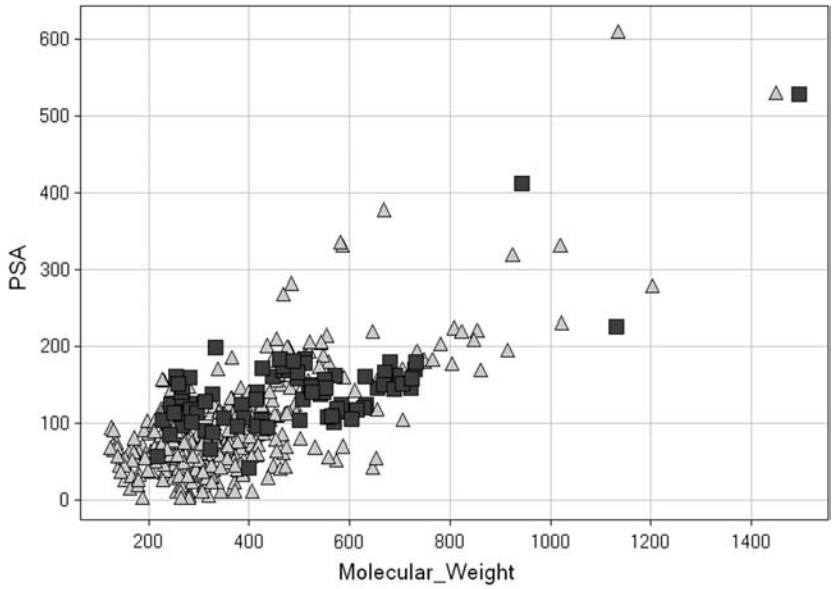


Figure 9.2 Molecular weight *vs.* polar surface area (PSA) for human drugs (triangles) and the transition state analogues discussed in this chapter (squares).

This apparent conundrum has been tackled by a number of strategies:

- the use of prodrugs;
- the intermediacy of hemi-acetals or internal hydrogen bonding networks that mask polarity and allow better lipid solubility;
- the use of functional groups that only form the polar transition state analogue following attack by the enzyme (*e.g.* some boronic acids).

Another strategy utilised to increase absorption has been to increase clogP (and, in turn, the non-polar surface area of the molecules) to try and overcome the high polar surface area of TSAIs and achieve sufficient oral exposure for the compounds to have therapeutic efficacy. While this strategy has improved the absorption of some transition state analogue inhibitor compounds, it also tends to result in rapid metabolism and high clearance of the compound; so although the compound's absorption is improved, the bioavailability is often still low due to high first-pass clearance. Achieving a balance of permeability and absorption, whilst maintaining low enough clearance to attain oral exposure of the drug, has been a major challenge for a number of classes of TSAI.

The volume of distribution at steady state (Vss) of the TSAIs discussed in this chapter is shown in Figure 9.5. As with other drugs, lipophilicity (which largely reflects the property of the scaffold of the molecule) and charge (which in some cases is provided by the transition state mimicking group) are the two main factors influencing Vss. Acid compounds generally have low Vss even at high

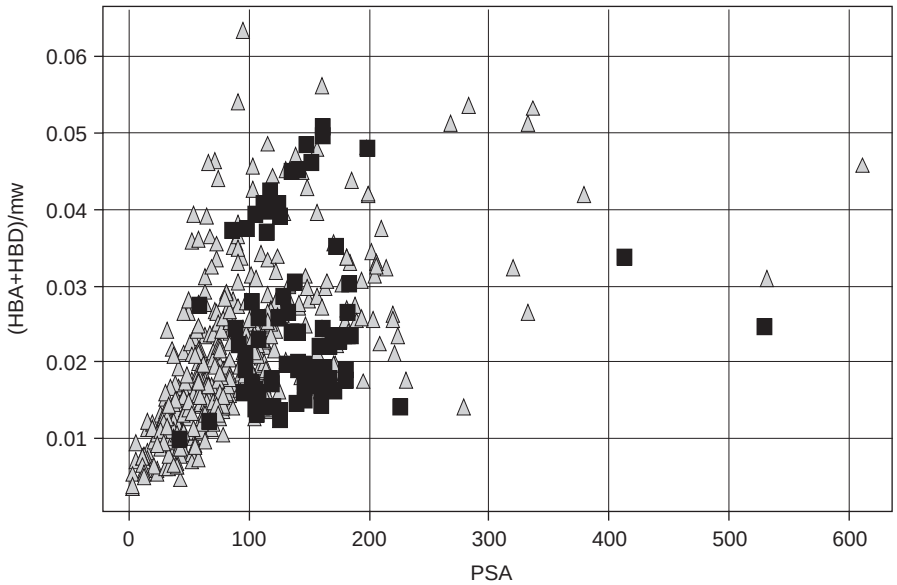


Figure 9.3 Sum of hydrogen bond acceptors (HBA) and donors (HBD) divided by molecular weight *vs.* PSA for human drugs (white triangles) and TSAI discussed in this chapter (black squares).

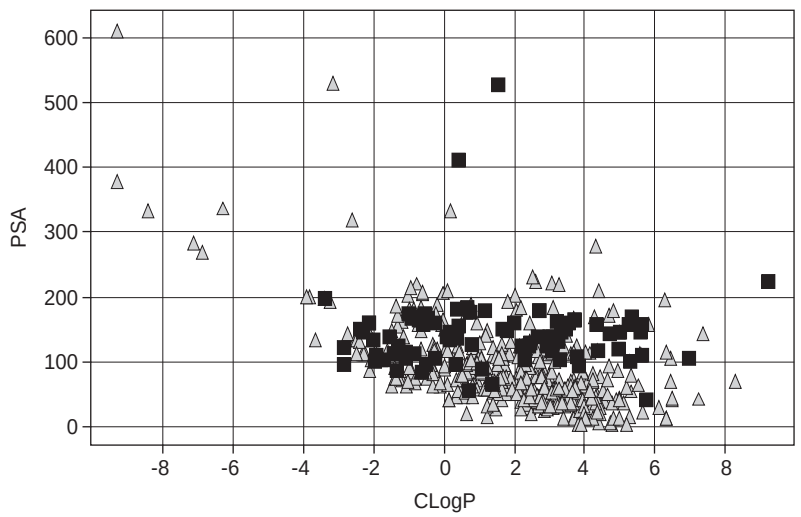


Figure 9.4 Polar surface area (PSA) *vs.* clogP for human drugs (white triangles) and TSAI discussed in this chapter (black squares).

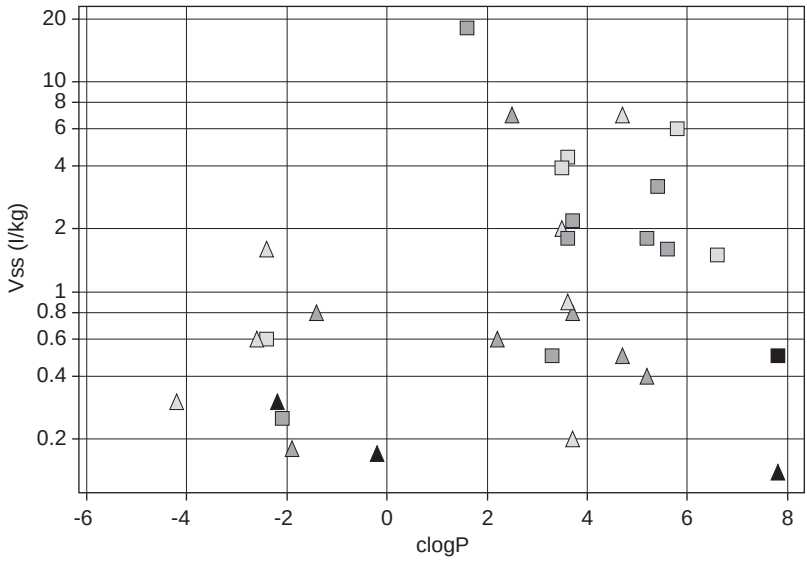


Figure 9.5 Vss ($L\,kg^{-1}$) of transition state analogue inhibitors *vs*. clogP. Triangles represent human data and squares rat data. Basic compounds are light grey, neutral compounds (basic pKa <5; acid pKa >8) are dark grey and acidic compounds are coloured black.

clogP values, whilst the basic and neutral TSAIs tend to have larger Vss than acidic compounds. The basic compounds show a trend for increased Vss with an increase in clogP whilst the trend is not as obvious for neutral compounds. The other thing to note from Figure 9.5 is that there are relatively few acidic TSAI compounds.

The widely differing physicochemical properties of transition state analogues present vastly different challenges to the drug discovery scientist depending on the physicochemical properties of the TSAI needed to potently inhibit the enzyme target. These are illustrated in the next section where specific enzyme targets that have been successfully targeted by TSAI therapeutics are considered.

9.3 ADME Properties of Transition State Analogue Inhibitors against Different Enzyme Targets

9.3.1 Proteases

Human proteases cleave peptide (amide) bonds of polypeptides with specificity that is controlled by the accommodation of specific peptide side chain residues into the pockets neighbouring the active site.

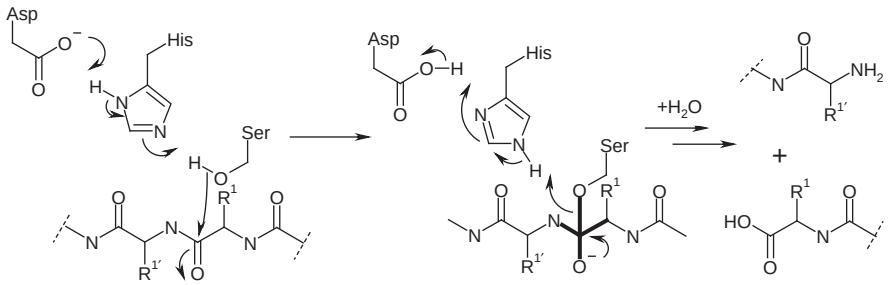


Figure 9.6 The first step in the catalytic mechanism is attack of the active site serine onto the substrate peptide's scissile bond, forming a tetrahedral intermediate which is stabilised by further interactions by the protease prior to hydrolysis to release amine and acid products.

The mechanism of proteolysis by serine proteases relies upon a catalytic triad of amino acids comprising aspartic acid, histidine and serine. Figure 9.6 shows the initial steps of this process—the deprotonation of the hydroxyl of serine by histidine's imidazole assisted by a neighbouring aspartic acid. The resultant nucleophile attacks the carbonyl of the scissile bond to form a tetrahedral transition state which subsequently collapses following hydrolysis by water. An equivalent mechanism using a nucleophilic thiol from cysteine is exploited by cysteine proteases, whilst aspartyl proteases and metalloproteases can use a nucleophilic water molecule to generate the tetrahedral transition state.[16] The protease enzymes lower the energy required for amide hydrolysis by stabilising the (high energy) tetrahedral transition state. Figure 9.7 shows a number of chemotypes that have been used to mimic the tetrahedral transition state of proteases in TSAI. Each of these chemical classes is discussed in the following sections.

9.3.1.1 Alcohol Containing TSAI of Proteases

A number of aspartyl protease enzymes (including HIV protease and renin) are important therapeutic targets and have been targeted with alcohol (and enol) containing TSAIs.[16,17] The first step in the catalytic mechanism and the proposed tetrahedral transition state of aspartyl proteases,[18] along with the different alcohol (and enol) groups that have been used to mimic the transition state, are shown in Figure 9.8. An alternative catalytic mechanism has also been proposed for HIV protease whereby cleavage occurs in a one-step process in which nucleophile (water) and electrophile (acidic proton) attack the scissile bond in a concerted manner.[19]

The substrates of HIV protease (GP-160, gag, gag-pol) and renin (angiotensinogen) are fairly large polypeptides; consequently TSAI of these enzymes also tend to be large and have high clogP (average 5.1) in order to make enough interactions with the enzyme to effectively compete with substrate (see Table 9.1 for structures, physicochemical properties and pharmacokinetic data). This

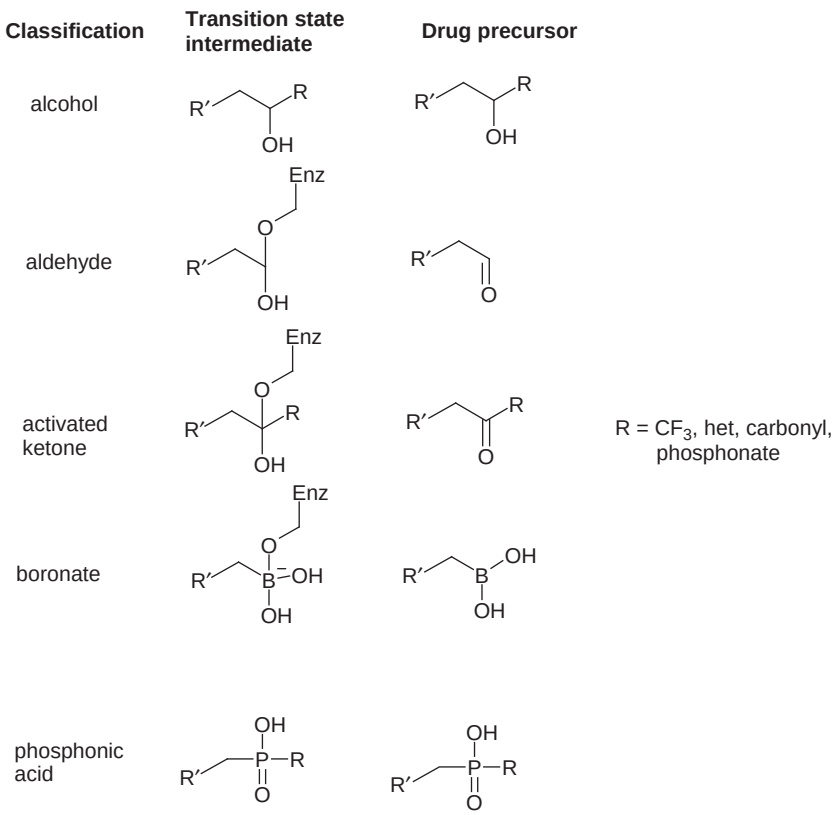


Figure 9.7 Tetrahedral transition state mimetics used in protease inhibitors (het = heterocycle).

high lipophilicity together with the high PSA (average 174 $Å^2$) and high hydrogen bond acceptor (HBA) and donor (HBD) count has a large impact on the pharmacokinetics of the compounds. So whilst these TSAIs are highly potent inhibitors of their enzyme target (*e.g.* the renin inhibitors discussed have Ki in the 0.7–14 nM range and the HIV protease inhibitors have Ki's of <10 nM),[20] achieving potency and oral bioavailability in the same molecule is often challenging.

The pharmacokinetic behaviour of the peptidic renin inhibitors such as remikiren is fairly typical for compounds with these physicochemical properties. Although oral administration of the peptidic renin inhibitors to pre-clinical species often resulted in pharmacological efficacy, oral F% was usually low requiring high doses to be used, plasma levels were often highly variable (for instance the plasma levels of remikiren varied up to 100-fold in rats given the same dose) and the concentration time profiles often showed secondary maxima peaks or were an irregular shape. In addition, there is evidence that the renin inhibitor compounds could inhibit their own metabolism after oral

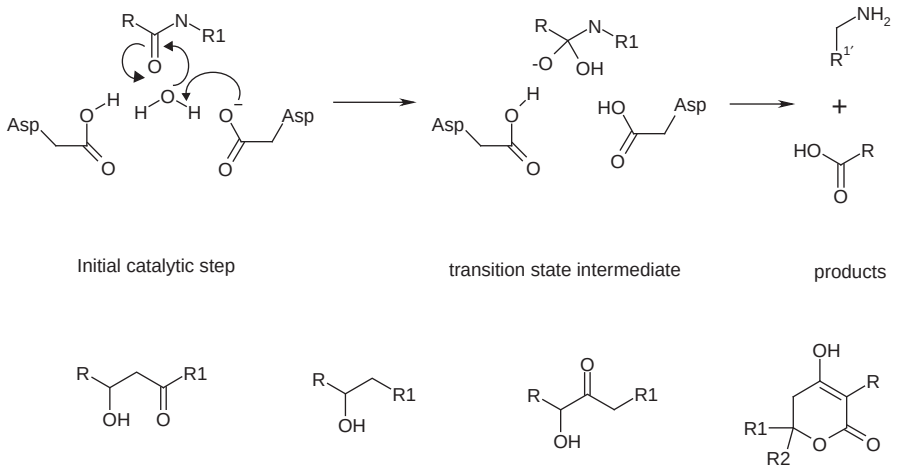
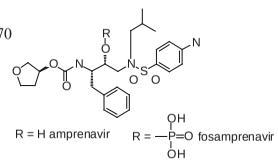
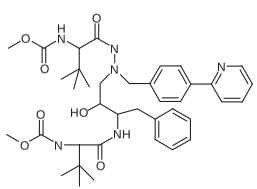
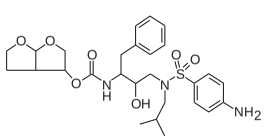
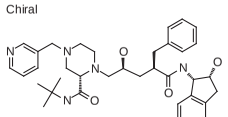


Figure 9.8 Proposed catalytic mechanism and transition state for aspartyl proteases where the active site aspartic acids and substrate (RCONR1) are shown, together with alcohol and enol groups that have been used to inhibit aspartyl proteases.

dosing.[21–23] The human pharmacokinetics of remikiren[24] were characterised by high blood CL (1500 ml min^{-1}; 21 ml min^{-1} kg^{-1}) that approximates to liver blood flow. Consequently when remikiren was dosed orally in humans, plasma concentrations were highly variable and bioavailability was very low (<1%) and variable.[25] Ultimately, none of the peptidic renin inhibitor compounds had a sufficient balance of potency and absorption, distribution, metabolism and excretion (ADME) properties to be developed as a drug. As the chemical synthesis of many of the peptidic renin transition state analogue inhibitor compounds was complicated, the low bioavailability and subsequent high doses needed for efficacy meant that the cost of these compounds also became too high to be commercially attractive.

The three properties that need to be balanced in order to achieve high oral bioavailability for alcohol containing TSAIs are solubility (as only drug in solution can be absorbed), permeability and first-pass clearance. The interplay of these three factors can be seen with the HIV protease inhibitors. The solubility of HIV protease inhibitors is low, leading to erratic absorption (often with multiple absorption peaks being observed) and high variability after oral dosing. Other consequences of the low solubility are that the absorption of the compounds is heavily influenced by whether subjects are fed or fasted and on the particular drug formulation administered. The effects of food on the HIV protease inhibitors vary from compound to compound with atazanavir, darunavir, saquinavir (F% increases 18-fold), lopinavir, nelfinavir and tipranivir showing an increased AUC in the fed state (presumably due to increased solubility in the presence of the gastric secretions and increased gastric motility of the fed state),[248] while the AUC of indinavir and amprenavir are decreased

Table 9.1 Chemical structure, physicochemical properties and clearance, bioavailability and absorption estimates of alcohol (and enol) containing TSAI.

Compound	*Structure*	*Target*	*MW*	*clogP*	*TPSA* [a] *HBA HBD*	*pKa*	*Vss (L/kg)*	*PPB (%)*	*CLp (ml/min/kg)*	*F%*	*Fa (%)*
Amprenavir Refs. 20, 33, 270		HIV protease	506	3.3	140 3 4	Neutral	6–10 h (V/F)	90–96 h	~11–20 h (CL/F)		>90 h
Atazanavir Refs 26, 271		HIV protease	705	5.2	171 13 5	Neutral (4.7 B)		86h	6–8 h (CL/F)		>80 h
Darunavir 27, 264, 272		HIV protease	548	2.9	149 4 4	Neutral	1.3 h	95 h	8 h 1.4 with ritonavir	37 h 82 with ritonavir	>90 h
Indinavir Refs. 31, 52, 246, 263, 273–275	Chiral 	HIV protease	614	3.7	118 7 4	Weak base (5.2)	2.2 r 0.7 d 1.5 mk 0.8 h	70 r* 70 d* 70 m* 64h	107 r 16 d 36 mk 10–18 h	16–24 r 16 d (pH 6.5) 72 d (pH 2.5) 19 mk 60–65 h	81 r 100 d 100 mk >80 h

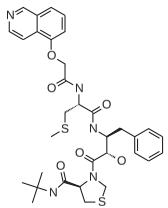
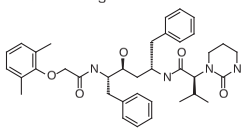
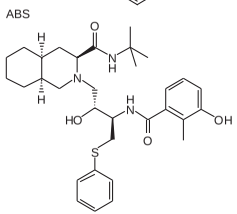
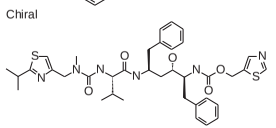
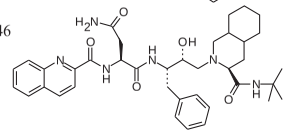
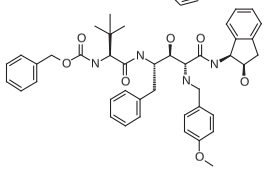
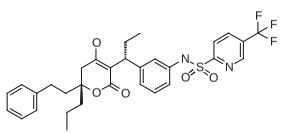
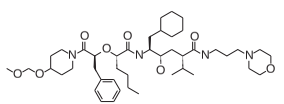
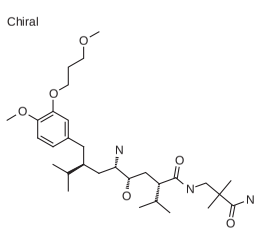
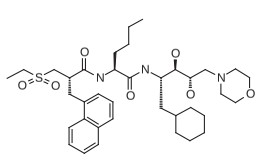
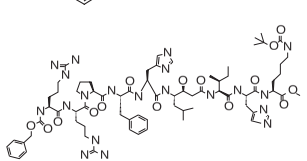
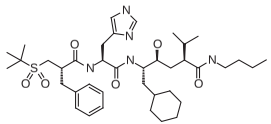
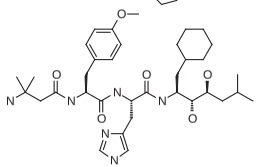
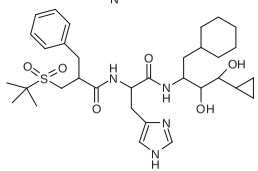
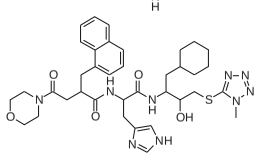
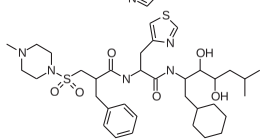
KNI-272 Refs. 276–278	Chiral 	HIV protease	668	4.7	201 9 4	Weak base (4.9)	5–21 r 0.9 mk 0.11 hp	98–99 h	18–48 r 42 mk 11 hp 10–11 h	12 hp 16–55 h	32–100 h
Lopinavir Ref 30		HIV protease	629	6.1	120 5 4	Neutral		98–99 h	1.4–1.7 h (CL/F in presence of ritonavir)	25 r	> 70 h
Nelfinavir Refs 82, 279, 280	ABS 	HIV protease	568	5.8	127 6 4	Basic(7.5)	3–7 r 1.7 – 4 d 2.7 mk 10 h (V/F)	98–99 h	27–60 r 22–60 d 20–28 mk 10–12 h (CL/F)	30–43 r 29–43 r 30–59 d 9–42 mk	100 r 100 d 63 mk > 80 h
Ritonavir Refs 34, 281–284	Chiral 	HIV protease	720	5.2	202 11 4	Neutral	1 r 0.3 d 2.2 mk 0.4–0.5 h (V/F)	97–99 r 99 d 96–99 mk 99–99.5 h	11–17 r 4–5 d 11–12 m 1–2.1 h (CL/F)	71–78 r 21–100 d 30–70 mk	> 70 h
Saquinavir Refs 34, 51, 246		HIV protease	670	4.7	167 7 6	Basic (7.6)	4–10 h	98–99 h	13–17 h	4 h hgc 12 h sgc	~ 30 hge ~ 100 sgc
SDZ-PRI-053 Ref 285		HIV protease	723	5.6	158 7 6	Weak base (6)	2.7 mo 1.6 r		19 m 34 r 9.5 d (CL/F)	47–61 m 22–54 r	60–77 m 43–100 r

Table 9.1 (*continued*)

Compound	*Structure*	*Target*	*MW*	*clogP*	*TPSA* [a] *HBA HBD*	*pKa*	*Vss (L/kg)*	*PPB (%)*	*CLp (ml/min/kg)*	*F%*	*Fa (%)*
Tipranavir Refs 29, 286–288		HIV protease	603	7.8	114 5 2	Acid (4.5)	0.5 r 0.13 d 0.11–0.14 h (V/F with ritonavir)	> 99.9 in all species	20 m 2.8 r 1.3 d 0.2–0.3 h (CL/F with ritonavir)	11 m 28–30 r 10 rab 7–8d	53–60 r >18 h
A-74273 Ref. 289		Renin	787	5.5	139 7 3	Basic(7.5)			12–20 d (CL/F)	26 r 31 f 54 d 1.9 mk	
Aliskiren Refs. 36, 53, 290	Chiral 	Renin	552	3.5	146 7 6	Basic (9.8)	2 h	50 h	~2 h	~2.6 h	~5 h
BW-175 Ref. 23		Renin	688	6.6	154	Basic (6.8)	1.5 r		22 r	3–10 r	<15 [c] r
CGP-29827		Renin	1495	0.3	528 20 20	Neutral					

CGP-38560 Ref. 291		Renin	730	5.0	179 7 5	Base (6.7)				<2 h	
Enalkiren Ref. 291		Renin	657	3.7	192 9 8	Basic (8.9)	0.2 h	94 h		Not measurable	Only pharmacologically active by IV route
Remikiren Ref. 22, 24, 291,292		Renin	631	3.6	170 7 5	Basic(6.7)	1.8 m 4.4 r 1.5 d 2.7 ma 0.9 h	86–96 h	119 r 48 ma 25 d 20 mk 13 h	3 r 6 ma 1.1 d <0.3 mk <2 h	~30 [b] r ~56 [b] d <2 [d] mk
YM-21095 Ref. 293		Renin	732	3.5	206 9 4	Basic (6.7)	3.9 d		22 r 37 d	3–10 r 0.2 d	<15 [c] r
Zankiren		Renin	706	5.6	189	Basic (6.2)			148 r 28 f 10 d 27 mk	24 r 32 f 53 d 8 mk	100[b] r 72 [c] d 25 [c] mk

d = dog, f = ferret, h = human, hp = human paediatric population, ma = marmoset, mk = monkey, mo = mouse, r = rat, rab = rabbit, hgc = hard gelatin capsule, sgc = soft gelatin capsule

[a] Topological polar surface area

[b] Based on urinary and biliary recovery of radioactivity

[c] Assuming blood : plasma ratio = 1

[d] Assuming blood : plasma ratio = 0.6 (as in human)

* = blood binding.

by food.[26–30] The requirement for some compounds to be given with food and others to fasted subjects complicates the usage of these compounds in the multiple drug regimes needed to treat HIV infection. In addition to food, the absorption of some compounds is very dependent on intestinal pH. For instance the low solubility of atazanavir at high pH means that this compound can not be co-administered with antacids or proton pump inhibitors that raise the pH of the gastrointestinal tract[26] and similar pH dependent effects were seen when indinavir was dosed orally in the dog.[31]

The commercial formulations of the protease inhibitors often require organic excipients to maintain the compounds in solution and to achieve sufficient exposure after oral dosing; the oral solution formulation of lopinavir contains 40% v/v ethanol, tipranavir is formulated as a fatty emulsion[29] and the commercial dosage form of amprenavir requires the use of organic excipients (including PEG 400 and propylene glycol) and a low drug loading (150 mg), meaning that pill burden for the patient was high (eight capsules have to be taken twice a day).[32]

To try and overcome the problems caused by in solubility, the use of more soluble prodrugs has been investigated.[32] The alcohol group that mimics the tetrahedral transition state in amprenavir has been conjugated with phosphate to give a prodrug (fosamprenavir) that is 10-fold more soluble (0.4 mg ml^{-1}) than parent amprenavir (Table 9.1). The advantage of this increased solubility was that fosamprenavir could be formulated at a higher drug loading (700 mg tablet), reducing the pill burden for the patient (two capsules dosed once or twice a day). Once dosed orally, fosamprenavir is converted almost entirely to amprenavir at or near the intestinal epithelium by alkaline phosphatase in both animals and humans; the amprenavir is then absorbed and equivalent systemic exposures are achievable using fosamprenavir and the standard amprenavir capsules.[33] In contrast to amprenavir, fosamprenavir pharmacokinetics are unaffected by food and this together with the lower pill burden mean that fosamprenavir has been commercially successful.[32] Another advantage for fosamprenavir is that it should be possible to combine fosamprenavir in a single tablet with other classes of HIV drugs; the organic excipients and low drug loading meant it was not easy to do this with amprenavir.

Saquinavir also has low solubility and formulation changes have been used to increase its bioavailability. Originally marketed as a hard gelatine capsule (HGC) formulation, saquinavir had low bioavailability (F% 4) partly as a result of incomplete absorption. When saquinavir was reformulated in a soft gelatine capsule as a lipid formulation containing mono- and di-glycerol medium chain fatty acids (SGC) the F% increased 3–4 fold (12–16%) and was further increased in the presence of food.[34] The SGC formulation presumably increases the solubility and dispersion of saquinavir and in some ways mimics the effect of food on the compound. However, it is also possible that the excipients have other effects (*e.g.* changing intestinal leakiness, inhibiting P-gp) that contribute to the higher F%. Some evidence to support this theory is that, when boosted with ritonavir, the plasma levels from the HGC formulation are higher than those with the SGC formulation;[35] this is hard to explain if the

difference between formulations is only due to solubility effects. Adding a basic centre to molecules is another strategy that has been employed to try and increase the solubility and hence absorption of HIV protease inhibitors (the discovery of indinavir is discussed in Chapter 4).

Once in solution the absorption of compounds depends on the passive permeability across the intestine and the action of drug transporters that can act to increase (influx) or decrease (efflux) the rate and extent of drug passage across the intestine. Ideally absorption of compounds is estimated from a comparison of intravenous and oral pharmacokinetics of the compound. Many of the compounds in Table 9.1 are too lipophilic to formulate in a vehicle that can be administered intravenously. For these compounds, an estimate of human absorption has been made by assuming that the compounds are not metabolised in the luminal contents of the gastrointestinal tract and calculating the Fa from the recovery of unchanged drug in faeces after oral dosing. Although this may underestimate absorption (as material absorbed and excreted unchanged in bile or by exsorption of unchanged drug back into the intestine is considered not to have been absorbed), it allows the absorption of more of the compounds in Table 9.1 to be compared.

PSA is one factor known to limit passive membrane permeability and hence oral absorption of compounds. A plot of Fa (%) against PSA for the compounds in Table 9.1 is shown in Figure 9.9. Clearly there are compounds with high PSA and low absorption in this dataset. For example aliskiren (the only marketed renin inhibitor) is highly soluble ($>350\,\mathrm{mg\,ml^{-1}}$) at acidic pH but has high molecular weight (552) and PSA ($146\,\mathrm{\AA^2}$). Despite low clearance in humans ($9\ \mathrm{L\,hr^{-1}}$; $\sim 2\,\mathrm{ml\,min^{-1}\,kg^{-1}}$), the compound has low oral

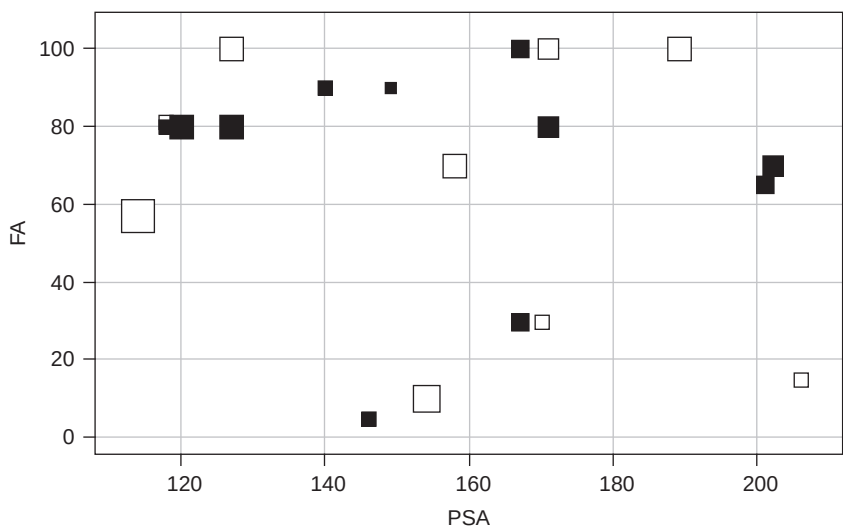


Figure 9.9 Absorption (FA as percentage) of alcohol TSAI compounds in human (black) and rat (white) *vs.* PSA (larger symbols indicate larger clogP).

bioavailability (~2.6%) and absorption (~5%).[36,37] Following a 300 mg oral dose of ^{14}C-labelled aliskiren, the majority (91%) of the dose was eliminated in the faeces (77.5% as unchanged drug reflecting elimination of unabsorbed drug).[37]

But there are also compounds in this dataset with high PSA and high absorption in both rats and humans. This is true even for compounds (atazanavir, KNI-272 and ritonavir) with very high PSA (>170 Å^2). Where these compounds do have low bioavailability, it is due more to high metabolic clearance than to poor absorption *per se*. The number of HIV protease inhibitors that can be administered effectively in combination with ritonavir (see later) also suggests that they have sufficient intrinsic membrane permeability to be orally absorbed when the contribution of P-gp and CYP 3A to first-pass clearance of the compounds is inhibited. One possible explanation for the high absorption of compounds despite high PSA is that the molecules are large enough to adopt conformations where intramolecular hydrogen bonds form, effectively hiding some of the polarity of the molecule whilst exposing the non-polar groups of the molecule to the lipophilic membrane. This would act to minimise the free energy of solvation and facilitate the passive membrane permeability of the compounds.[38]

Small changes in structure and physicochemistry can also tip the balance in favour of oral exposure. Enalkiren and zankiren are structurally related renin inhibitors. The compounds have similar molecular weight (706 *vs.* 657) and PSA (192 *vs.* 189 Å^2) but zankiren is more lipophilic (clogP 5.6 *vs.* 3.7; log $D_{7.4}$ 4.6 *vs.* 2.6) and has lower numbers of HBA (six *vs.* nine) and HBD (four *vs.* eight) groups. Whilst enalkiren has no oral bioavailability and only showed pharmacological efficacy after intravenous dosing, zankiren has high oral bioavailability and absorption in pre-clinical species.[39]

P-glycoprotein (P-gp) is expressed in the intestine and acts to efflux compounds from the intestinal cells back into the gastrointestinal tract contents and to limit absorption/bioavailability of compounds (particularly at low doses). Structural features that typically result in high affinity for P-gp are clogP >2.9, an 18 atom or longer molecular axis, a basic charge (although many neutral compounds are also P-gp substrates) and a molecular weight <800. In addition, most P-gp substrates possess two or three electron donor groups with a fixed spatial separation of 2.5–4.6 Å. Compounds with more of these elements tend to have higher affinity for the transporter.[40–41,260]

The alcohol transition state analogue inhibitor compounds contain many of these structural and physicochemical features (Table 9.1) and many are substrates for P-gp. Some of the compounds also interact with other intestinal efflux transporters that may limit the absorption/bioavailability of the compounds. For example, lopinavir is a substrate for MRP-2 and saquinavir is known to inhibit BCRP (IC_{50} 75 μM).

The effect of P-gp on disposition of HIV protease inhibitors has been studied *in vitro* and *in vivo*. Efflux of these compounds (basolateral → apical transport > than apical → basolateral transport) is seen in Caco-2 cell monolayers, in cell lines over-expressing human P-gp, and in human and rat intestine tissue

mounted in Ussing chambers.[42] The efflux ratios in these systems can be reduced with P-gp inhibitors (*e.g.* GF-120918, cyclosporin) and often saturate as substrate concentration is increased.[31,43] In addition the plasma levels of saquinavir (1–1.5 fold increase), indinavir and nelfinavir are all higher following oral dosing in P-gp knockout mice than they are in wild-type mice.[42,43] P-gp also plays a major role in limiting saquinavir absorption in the rat *in vivo*.[261]

Even though many compounds are shown to be substrates in *in vitro* P-gp expressing cell line experiments, the degree of efflux and the rate of apical to basolateral flux vary considerably showing that this parameter is also influenced by the passive membrane permeability of the compound.[44] This becomes important *in vivo* because many compounds identified as P-gp substrates *in vitro* are still well absorbed in humans.[262]

Aliskiren is an example of a compound where human absorption *in vivo* appears to be influenced by efflux transporters. The AUC of aliskiren increases in a supra-proportional manner with increasing oral doses suggesting saturation of a gut efflux mechanism, and compounds known to inhibit P-gp increase the oral exposure of aliskiren in humans *in vivo* (AUC is increased 50, 80 and 500% by atorvastatin, ketoconazole and cyclosporine, respectively),[36,45] Although these two findings are suggestive of a role for P-gp in the disposition of aliskiren, the inhibitors that increase aliskiren AUC are known to inhibit other ADME enzymes/transporters (*e.g.* CYP 3A4, BCRP OATPs) and these other enzymes/transporters might also play a role in the non-linear disposition of aliskiren.

Clearly other compounds that are P-gp substrates *in vitro* are still well absorbed *in vivo*. This probably reflects a high enough intrinsic membrane permeability of some compounds, allowing them to overcome the effects of P-gp, and also the fact that many of the compounds are given at high doses and the concentrations achieved *in vivo* saturate P-gp and limit its impact.

Many of the compounds that are P-gp substrates are also substrates of CYP 3A4 (and 3A5); P-gp and CYP 3A in the intestine act in consort to limit the oral bioavailability of compounds that are substrates for both entities. P-gp slows down the absorption of compounds and the repeated absorption and efflux cycles increase the likelihood of metabolism by the intestinal CYP 3A isozymes resulting in high first-pass intestinal extraction.[46] Because of their affinity for P-gp and CYP 3A, many of the compounds shown in Table 9.1 have the potential to undergo significant intestinal first-pass metabolism ($Fg \ll 1$). Although it is not easy to accurately estimate Fg for many of the compounds in Table 9.1, there are enough data to do this for some of them.

Pre-clinical studies have been conducted with indinavir dosed intravenously, orally and *via* infusion into the hepatic portal vein.[31] Based on data in the rat, hepatic extraction was 70% ($Fh = 0.3$) and intestinal first-pass extraction was $<10\%$ ($Fg > 0.9$). Studies in the dog showed that the intestine had minimal contribution to saquinavir metabolism after intravenous or oral dosing and that F% was low (8–20%), mainly due to high hepatic extraction ($Fh = 0.14$–0.25).[249]

Obtaining estimates of human intestinal first-pass extraction is more difficult,[47,48] but methods to estimate this parameter from *in vitro* data have been used with some success.[49] Another method that can be used, although it is not without caveats, is to compare the effects of grapefruit juice (a relatively selective inhibitor of intestinal CYP 3A) on the pharmacokinetics of the compound in question after intravenous and oral dosing.[48] Using these methods led to estimates of human intestinal first-pass extraction of 0.3–0.5 for saquinavir ($Fg = 0.5–0.7$) and 0.05 for indinavir ($Fg > 0.9$).[31,43,48–51]

The high clogP of the alcohol containing TSAI of proteases often results in rapid hepatic clearance (low values of Fh) and low bioavailability (Table 9.1). As would be expected from their physicochemical properties, the compounds tend to be cleared by metabolism or biliary excretion in the liver, with only indinavir having appreciable renal clearance (12% of total clearance) which slightly exceeds GFR, suggesting an active tubular secretion component.[52] The actual enzymes and transporters responsible for the clearance of alcohol protease TSAI vary from compound to compound and in many cases their identity is not known. Early renin inhibitors (*e.g.* CPG-29827) were peptidic in nature and rapidly hydrolysed by proteases, while later renin inhibitors were metabolised by CYP isozymes and underwent biliary excretion. When alcohol TSAI do undergo metabolism, it generally does not involve the atoms that mimic the transition state of the enzyme but more usually reflects metabolism of the lipophilic scaffold that holds the transition state mimic in place.

Compounds that undergo significant biliary excretion include remikiren ($\sim$30% biliary excretion of unchanged drug in rats and dogs) and CGP-38560, a compound that had poor oral absorption and rapid biliary clearance (half-life $\sim$7 min) in humans,[53] although no information on the transporters involved is published. As well as parent drug, an additional 60% of a remikiren dose was excreted in to bile of rats (18 radioactive peaks identified) and dogs as metabolites.[54] The AUC of radiolabelled material was lower in the bile duct cannulated dog (about 30%) than in intact animals, suggesting that the radiolabelled material also undergoes significant enterohepatic circulation.

Aliskiren is less lipophilic and more basic than many of the other renin inhibitors and is known to be a substrate for at least one member of the organic anion polypeptide (OATP) family *in vitro*; it is likely that aliskiren undergoes active uptake in to the liver *in vivo*. Following uptake into the liver, aliskiren is slowly metabolised by CYP 3A4.[45]

The HIV protease inhibitors are generally metabolised by CYP 3A4 in humans though, for some compounds, other isozymes are involved (Table 9.2). The HIV protease inhibitors also interact with a number of uptake transporters (OATP family) in both rats and humans. *In vitro* data suggest that transporters control the clearance (and subsequent metabolism) of saquinavir, nelfinavir and ritonavir in rat hepatocytes and by extension are likely to play a similar role *in vivo*.[55]

The influence of these transporters on human pharmacokinetics is less clear but indinavir, nelfinavir, ritonavir and saquinavir all inhibit uptake of estradiol-17β-glucuronide by human OATP 1B1[56–58]and there is some evidence that

Table 9.2 Effect of ritonavir co-administration on plasma AUC of HIV protease inhibitors.

Compound	*Enzymes involved in human metabolism*	*Ritonavir dose (mg, frequency)*	*Fold-increase in AUC*	*Reference*
Amprenavir	CYP 3A4	100, BD	2–4	63–65
		200, QD	4	
		300, BD	3.3	
Atazanavir	CYP 3A4	100, QD	3-4	26,66–68
		100, BD	5.9 (Cmin)	
			2.7 (Cmin)	
Darunavir	CYP 3A4	100, BD	6.6	69
Indinavir	CYP 3A4	100, BD	1.6–2.4	70–74
		200, BD	2.8	
		300, BD	3.3–5	
		400, BD	5.5	
Lopinavir	CYP 3A4	50 mg single dose	77	30
Nelfinavir	CYP 3A4 (52%) also 2C19, 2C9, 2D6	100, BD	1.3	75
		200, BD	1.5	
Saquinavir	CYP 3A4	100, single dose	30	76–79
		200, BD	18	
		300, single dose	58	
		300, BD	22	
		400, BD	22	
		600, single dose	112	
Tipranavir		100, BD	4.6–5.5	80,81
		200, BD	12.4–15.7	

the HIV protease inhibitors can also be transported by human OATPs. To date no information has been published showing transport by the major human liver OATPs (1B1, 1B3 and 2B1),[59] but saquinavir is transported by OATP 1A2 which is present in the central nervous system (CNS) and cholangiocytes in the liver and HIV protease inhibitors also appear to be substrates of OATP 3A1 and 4A1.[60]

A number of HIV protease inhibitors including saquinavir (F% 4–12), indinavir (F% 60–65) and nelfinavir (F% 45 fasted, 30% fed) have higher oral F% than would be expected if all the systemic clearance of the compounds occurred only in the liver, so it is possible that other organs are also involved in the systemic clearance of these compounds. The low and variable F% and short half-lives (mainly due to high clearance rather than low volume) of many of the HIV protease inhibitors means that they need to be dosed two or three times a day at high doses to have therapeutic efficacy. As well as being CYP 3A4 substrates, most of the HIV proteases are also potent inhibitors and often inducers of the enzyme and, at the high doses used, these compounds inhibit and/or induce their own metabolism (resulting in non-linear pharmacokinetics with increasing dose and time) and alterations in the pharmacokinetics of many

other compounds co-administered with them.[61,263] Because of its potent CYP 3A inhibition, ritonavir has been used at low doses to enhance (boost) the pharmacokinetics of other protease inhibitors allowing a lower pill burden and less frequent administration of the inhibited compound in HIV patients.

This is currently the main clinical use of ritonavir as, at the high doses needed for the compound to exert its antiviral effect, ritonavir has an unacceptable safety and toleration profile. The changes in AUC when protease inhibitors are co-dosed with ritonavir are shown in Table 9.2 and in some instances can be quite dramatic; for example, the AUC of saquinavir can increase up to 100-fold in the presence of ritonavir. Although plasma levels are higher when co-dosed with ritonavir, the pharmacokinetics of saquinavir are still highly variable.

The pharmacokinetic boosting strategy for HIV proteases is so well accepted that a number of protease inhibitors (*e.g.* lopinavir, darunavir, tipranavir) are only used in combination with ritonavir as they do not achieve sufficiently high plasma exposure alone. As well as increasing F%, ritonavir also increases the half-life of a number of compounds including saquinavir and darunavir.[27,28] Interestingly ritonavir has been shown to change the disposition of darunavir in humans. When radiolabelled darunavir was dosed alone, it was rapidly metabolised and recovery of unchanged drug was low (7% faeces, 1% urine), in the presence of ritonavir excretion of unchanged darunavir was extensive (41% in faeces and 8% in urine), suggesting that in the presence of ritonavir, transporters play a role in darunavir clearance.[27,264]

Although ritonavir boosts the levels of all the HIV protease inhibitors, its interactions with other drugs are more complex. In addition to inhibiting CYP 3A4, ritonavir also inhibits 2D6 and 2C9 and 2C19 and P-gp, and induces levels of a number of CYP isozymes (including 2B6, 2C9, 2C19) and some UGT isozymes with full induction requiring two weeks of ritonavir dosing.[62] Depending on the length and dose of ritonavir administration and the co-administered substrate, administration with ritonavir can result in inhibition or induction.

The distribution of the alcohol TSAI of proteases within the body is largely as would be expected on the basis of the physicochemical properties of the compounds (Figure 9.5, Table 9.1). The high lipophilicity of these compounds means that they tend to show extensive binding to plasma proteins. All the HIV proteases apart from the least lipophilic compound, indinavir, show extensive protein binding in humans ($>98\%$). Amprenavir, darunavir and nelfinavir bind mainly to α1-acid glycoprotein (AAG).[20,27,28,82] Amprenavir also changes the levels of AAG on chronic dosing and so changes its own protein binding with time. Atazanavir and lopinavir bind to both albumin and AAG.[26,30]

The distribution of alcohol transition state analogue inhibitor compounds has been investigated using whole body autoradiography techniques. Immediately following intravenous dosing of ^{14}C-remikiren in the rat,[83] high levels of radioactivity were seen in the liver, kidney, stomach, small intestine, choroid plexus, thyroid, pancreas, pituitary and myocardium of all three species studied (rat, guinea pig and monkey). After one hour, tissue radioactivity levels were still high but radioactivity was not detectable in the blood. At 24 h post-dose, radioactivity was still seen in the gastrointestinal tract, kidney and some brain

areas. The kidney appears to act as a reservoir for remikiren because further analysis of radioactive material in the kidney after 24 h showed that the majority of radioactivity was unchanged remikiren. A possible explanation for this retention is the material enters the kidney (possibly *via* an uptake transporter), but unlike in the liver, there is no comparable excretory transporter to remove the compound from the kidney. As metabolic capacity for this compound is low in the kidney, the compound remains in the kidney predominantly as unchanged drug.

HIV protease inhibitors in general have limited and variable ability to enter the cerebrospinal fluid (CSF) of humans treated with the drugs (Table 9.3). One reason for this is that P-gp (and possibly other transporters such as MRP 1 and 2) limits the permeability of the compounds across the blood–brain barrier.[267,268] Whilst in the intestine compounds can reach high concentrations and inhibit (and overcome) P-gp, the free systemic concentrations of these compounds are much lower than those seen in the intestine and thus P-gp at the blood–brain barrier is not inhibited.

The effect of P-gp on limiting CNS penetration of HIV protease inhibitors has been extensively studied in mice. Increases of between 5–36 fold were seen in the brains of P-gp knockout mice compared to wild-type mice dosed with amprenavir, saquinavir, indinavir, ritonavir and nelfinavir .[31,44,84,85] Increases of brain concentrations of a similar magnitude are seen for these compounds when wild-type mice are dosed with P-gp inhibitors such as GF-120918 or cyclosporine.[31,44] As well as influencing the CNS penetration of protease inhibitors, transporters (including P-gp and MRP proteins) also play a role in modulating the levels of HIV protease inhibitors in lymphocytes, a predominant site of HIV replication.[89–92]

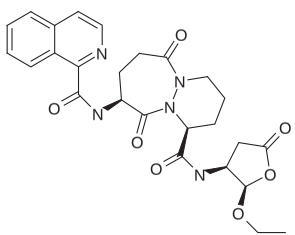
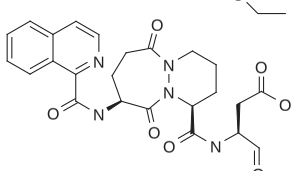
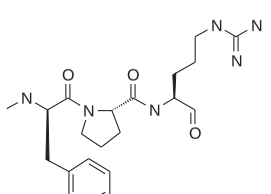
Inhibition of transporters may play a role in some of the side effects seen with HIV proteases inhibitors. Saquinavir (1.2 μM) and indinavir (6.8 μM) both inhibit human OATP 1B1, which has a physiological role in bilirubin transport. When the Fu in plasma and therapeutic plasma concentrations were considered it was concluded that, *in vivo*, the inhibition of OATP-1B1 would be 40% for indinavir and 7% for saquinavir, and that this may explain the unconjugated hyperbilirubinemia seen in some patients dosed with indinavir.[58] Ritonavir and

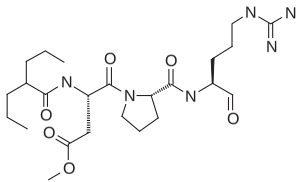
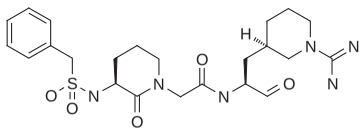
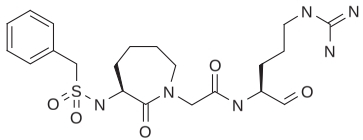
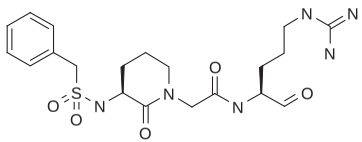
Table 9.3 CSF : free plasma concentrations of HIV protease inhibitors in human.

Compound	*Average CSF : free plasma ratio*	*Reference*
Atazanavir	~0.05	26,86
Amprenavir	~0.02	87
Darunavir	0.17	88
Indinavir	~0.2[a]	87
Saquinavir	0.07–0.2	87
Ritonavir	0.05–0.25	295
Lopinavir	Undetectable, 0.8	30, 294

[a]Occasional instances of CSF : free plasma >1 reported for indinavir.

Table 9.4 Chemical structure, physicochemical properties and clearance, bioavailability and absorption estimates of aldehyde containing TSAI.

Compound	*Structure*	*Target*	*MW*	*clogP*	*TPSA*[a]	*pKa*	*HBA*	*HBD*	*F%*
Pralnacasan Ref. 100		Caspase-1	524	2.2	147	Neutral	7	2	40–60 r
RU-36384 Ref. 100		caspase-1	496	1.2	166	Acid (4.2)	8	3	4r
Efegatran Ref. 269		Thrombin (phase 2)	417	-0.5	140	Neutral	7	6	3 r 30 d

CVS-1123 Ref. 269		Thrombin (phase 2)	511	0.8	184	Neutral	8	6	79 d 36 mk
AL 1 Ref. 98, 99		Thrombin	509	0.1	168	Weak acid (9.0)	7	6	15 d
AL 2 Ref. 98, 99		Thrombin	481	0	183	Weak acid (9.0)	8	6	66 d
AL 3 Ref. 98, 99		Thrombin	467	-0.6	183	Weak acid (9.0)	8	6	35 d

d = dog, mk = monkey, r = rat.
[a]TPSA = topological polar surface area

saquinavir both inhibit the uptake (*via* NTCP) and excretion (*via* BSEP) of bile acids in human liver.[93] If the balance of these competing actions *in vivo* results in bile acid accumulation in hepatocytes, it may lead to toxicity.

A side effect common to many HIV protease inhibitors is a partial lipodystrophy characterised by a redistribution of adipose, with a loss of fat in the face, arm and legs and the gain of fat around the back of the neck leading to a characteristic buffalo hump. This has been linked to a lack of specificity of some of the HIV protease inhibitors which also inhibit ZMPSTE24, a human protease involved in the conversion of farnesyl prelamin A to lamin A, a key nuclear structural protein.[94–96]

9.3.1.2 *Aldehyde Containing TSAI of Proteases*

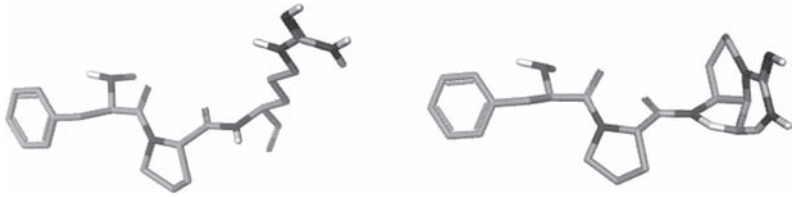
Aldehydes are not frequently incorporated into drugs as a result of their poor chemical stability and because they carry the risk of toxicity; for example, from the *in vivo* generation of covalent protein adducts through Schiff base formation. However, there are some examples of medicinal chemistry programmes that have focused on aldehyde containing compounds as potential protease inhibitors since reaction with the catalytic triad of serine proteases leads to the formation of tetrahedral (transition state analogue) intermediates (Figure 9.7). The enzymes for which aldehyde TSAI have been tested *in vivo* include thrombin and caspase-1. Caspases hydrolyse peptide bonds on the carboxyl side of an aspartate residue using a thiolate anion formed from Cys285 by His237 through a mechanism equivalent to the serine protease shown in Figure 9.6.[97] Caspase-1 cleaves pro-IL-1β and IL-18 to release the active cytokine.

Two aldehyde containing thrombin inhibitors (efegatran and CVS-1123) have reached the clinic (Table 9.4)[269] and pre-clinical data on a related set of aldehyde thrombin inhibitors (AL 1, 2 and 3) has been published.[98,99] It is likely that these compounds exist as hemi-aminals rather than true aldehydes, through reversible cyclisation between aldehyde and guanidine. This is illustrated for efegatran in Figure 9.10. The cyclisation stabilises the aldehyde functionality and could enhance oral bioavailability by reducing both the basicity of the guanidine and the overall PSA and may explain the reasonably high bioavailability of these compounds in the dog (15–79%). It is interesting that changing the ring size in AL 2 and 3 resulted in a doubling of bioavailability, although no further pharmacokinetic data were presented to explain this finding.

The free aspartate hemi-acetal caspase inhibitors have poor bioavailability due to poor absorption, but this can be improved through the use of prodrugs. Pralnacasan is an ethyl acetal prodrug of RU-36384 and increased oral bioavailability in rodents from 4% to 40–60%.[100] Pralnacasan also has good absorption in humans and is in phase II trials for osteoarthritis.[101]

9.3.1.3 *Activated Ketone Containing TSAI of Proteases*

The dipole moment of the carbonyl group makes a ketone susceptible to nucleophilic attack. If correctly presented within the active site of a protease,



	Open chain efegatran	Cyclic hemi-acetal efegatran
TPSA	140	92
cpKa	14	12
HBD*	6	3

* excluding internal H-bonding network from cyclic acetal

Figure 9.10 Open chain and cyclic hemi-acetal forms of efegatran.

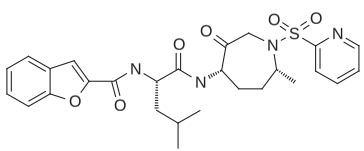
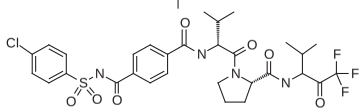
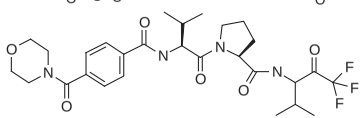
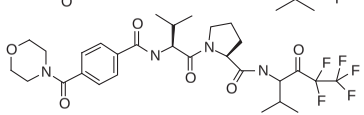
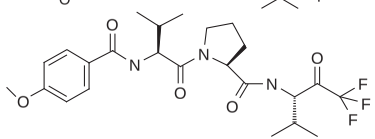
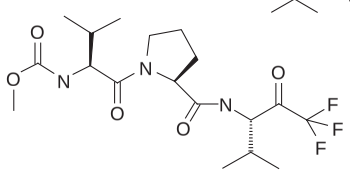
it can be converted to a tetrahedral intermediate. The addition of an α-electron withdrawing group (heterocycles, carbonyl and fluorinated alkyls) will both enhance nucleofugicity and stabilise the tetrahedral transition state thereby increasing binding affinity of the activated ketone compound. Provided that these activating groups are poor leaving groups, this can result in potent inhibitors that are not substrates of the protease.

The enzymes targeted with activated ketones include cathepsin-K, elastase, HCV NS3-protease and tryptase. The chemical structure, physicochemical properties and pharmacokinetic data for the activated ketone compounds discussed in this chapter are shown in Table 9.5.

ZD-8321 and ZD-0892 are lipophilic (clogP 3-4) trifluoromethyl activated ketone TSAIs with a PSA in the region associated with good oral absorption. In pre-clinical species these compounds, as would be expected, had high oral absorption and bioavailability was also high. ONO-6818 is a less lipophilic elastase inhibitor (clogP) with a higher PSA (144 $Å^2$), but the compound also shows reasonable bioavailability in pre-clinical species.[102]

Relacatib, a potent cathepsin inhibitor (Ki 0.04 nM), is an example of a heterocycle activated ketone and boceprevir is a carbonyl activated ketone inhibitor of the NS3-4A protease enzyme of hepatitis C virus (HCV). Both compounds are large, lipophilic, neutral compounds with a PSA on the edge of where good oral absorption is expected (relacatib PSA 147 $Å^2$, boceprevir PSA 151 $Å^2$). Despite this both compounds show reasonable exposure following oral dosing (Table 9.5). Relacatib is well absorbed in both rat and monkey, and has high bioavailability in the rat and moderate bioavailability in the monkey. Removal of the methyl substituent on the azepane ring of relacatib led to a significant poorer pharmacokinetic profile in the rat, with clearance doubling and bioavailability halving to 42% whilst protein binding was unchanged at ~97%. Addition of the methyl group may change the conformation of the

Table 9.5 Chemical structure, physicochemical properties and clearance, bioavailability and absorption estimates of activated ketone containing TSAI.

Compound	*Structure*	*Target*	*MW*	*clogP*	*TPSA* *HBA* *HBD*	*pKa*	*Vss (L/kg)*	*PPB (%)*	*CL (ml/min/kg)*	*F%*	*% absorbed*
Relacatib Ref. 103		Cathepsin-k	541	3.6	147 4 2	Neutral	1.8 r 1 mk	97 r	20 r 12 mk	89 r 28 mk	100 r ~40 (BP = 1)mk
ICI-200,880 Ref. 105, 106		Elastase	687	5.6	167 7 3	Acid (3.7)					
K-1		Elastase	583	2.8	125 5 2	Neutral					
K-2		Elastase	633	3.4	125 5 2	Neutral					
ZD-0892 Ref. 109		Elastase	500	3.9	105 5 2	Neutral			122 ham 98 r 52 d	82 r 39 d 26 ham	
ZD-8321 Ref. 109		Elastase	423	2.7	105 4 2	Neutral			58 ham 25 r 18 d	84 r 75 ham 70 d	

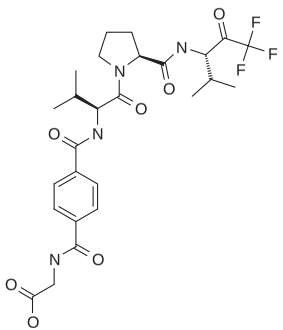
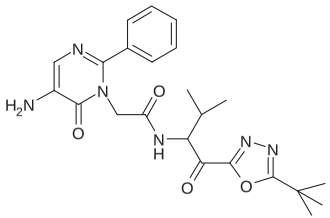
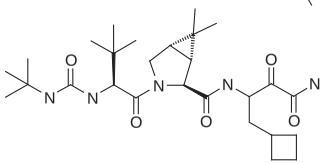
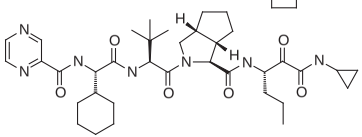
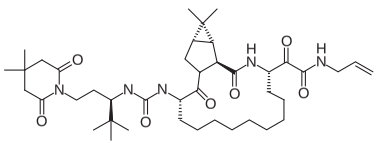
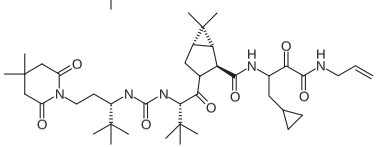
FK-706 Ref. 104	Chiral		Elastase (phase 2)	593	2.7	162 7 4	Acid (3.5)		86 h[a] 73 hs[a]		
ONO-6818 Ref. 102			Elastase	453						51 r 31 d 18 mk	
Boceprevir Ref. 10, 111			NS-3 protease (phase 3)	520	3.3	151 5 5	Neutral	0.5 r	~33 r	34 mo 26 r 30 d 4–11 mk	36 r
Telaprevir (VX-950) Ref. 111, 265, 296, 297			NS-3 protease (phase 3)	680	5.4	180 8 4	Neutral	0.5–5.8 r 1–1.8 d	18–53 r[b] 42 d	25–35 r 41 d	>45 r[c] Complete d

Table 9.5 (*continued*)

Compound	*Structure*	*Target*	*MW*	*clogP*	*TPSA HBA HBD*	*pKa*	*Vss (L/kg)*	*PPB (%)*	*CL (ml/min/kg)*	*F%*	*% absorbed*
K3 Ref. 113		NS-3 protease	740							AUC ($1\,\mu M\,h^{-1}$) at $10\,mg\,kg^{-1}$	$740\,ng\,h^{-1}\,ml^{-1}$
K4 Ref. 114		NS-3 protease	726						43 r 21 d 32 mk	22 r 35 d 4 mk	~80

d = dog, h = human, ham = hamster, hs = human smokers, mo = mouse, mk = monkey, r = rat.
[a]After inhalation administration 100 mg.
[b]non-linear with increasing dose ($0.95–5\,mg\,kg^{-1}$). [c] Telaprevir BP ratio is 0.56 (unpublished observations).

azepane ring and increase its metabolic stability.[103] In the monkey the compounds had similar Cl, but again the desmethyl analogue had poorer F% (7%).

Telaprevir (VX-950), like boceprevir, is a carbonyl activated ketone inhibitor of HCV NS3-4a protease but it has physicochemical properties considerably outside those typically seen for orally bioavailable compounds (MW 680, clogP 5.4, measured log $D_{7.4}$ 4, PSA 180 $Å^2$). Yet telaprevir has reasonable bioavailability and absorption (>45–100%) in pre-clinical species (Table 9.5) and flux through Caco-2 cells is observed *in vitro*.[265,266] The poor solubility of telaprevir does lead to variable plasma levels following high oral doses in the rat (30 mg kg^{-1}), and in both rats and dogs the oral half-life ($\sim$3 h) was longer than after intravenous dosing ($\leq$1 h) suggesting that the rate of absorption telaprevir is slower than the rate of elimination. One possible explanation for the good absorption of telaprevir despite its physicochemical properties is that the highly peptidic structure of the compound results in a well-defined secondary structure held together with intramolecular hydrogen bonding. In turn this will reduce the hydrogen bonding with solvent and lowers the desolvation penalty required for the compound to penetrate membranes.

FK-706 (clogP 2.7, MW 593, PSA 162 $Å^2$) and ICI-200 880 (clogP 5.6, MW 687, PSA 167 $Å^2$) are high molecular weight elastase TSAI with similar (particularly ICI-200 880) physicochemical properties to telaprevir. However, both FK-506 and ICI-200 880 have little oral bioavailability and need to be administered by the intravenous or inhaled route to show biological effects in humans. In the case of ICI-200 880, the low F% is due in part to high clearance while absorption of FK-706 in pre-clinical species is low.[104] Following inhalation of nebulised FK-706 in otherwise healthy humans, the AUC was similar in smokers and non-smokers; however, the Cmax was higher, the Tmax earlier, the half-life shorter and the rate of absorption about 10-fold higher in smokers reflecting the higher pulmonary permeability in people who smoke.[104] ICI-200 880 shows flip-flop kinetics following inhalation administration in the hamster (intravenous half-life 1 h, inhaled half-life 10 h)[105,106] possibly reflecting low solubility and tissue permeability of the compound.

Incorporation of electron withdrawing groups α- to a carbonyl in order to gain potency also has consequences for the behaviour of these compounds in biological systems. As well as reducing the pKa of the carbonyl group and increasing its reactivity towards nucleophiles, the electron withdrawing group increases the propensity for racemisation leading to the formation of a mixture of diasteroisomeric compounds.[107] For example, the half-life for epimerisation of K-2 in human blood serum at 37 °C is 30 min,[108] ZD-0892 and ZD-8321 are also susceptible to rapid epimerisation in plasma, in this case to give a 6 : 4 mixture in favour of the SSS isomer,[109] FK-706 exists as two epimers (the pharmacologically active SSS epimer and an SSR epimer[104]) and ICI 200,880 exists as two epimers that undergo facile interconversion in aqueous solution.[106]

It has also been proposed that differences in oral efficacy of K-1 and K-2 could be due to the measurably different levels of hydration of the two compounds.[108] Despite equal *in vitro* potencies, oral efficacy was only observed with

pentafluoroethyl derivative K-2. ^{19}F NMR studies in buffered saline showed that, while K-1 was fully hydrated, a significant proportion (20%) of K-2 existed in the ketone form. It was not ascertained whether these differing levels of hydration affected clearance rates or oral absorption, but since further examples highlighted that merely incorporating a pentafluoroethyl group is not sufficient to guarantee *in vivo* efficacy, it is more likely that oral bioavailability is driven by the overall physiochemical properties of the molecule (most notably the hydrogen bond burden and the lipophilicity) than by the degree of hydration of the ketone group.[110]

In addition to undergoing epimerisation, the lipophilic activated ketone transition state analogue inhibitor compounds are also susceptible to metabolism, often involving the activated ketone group. For instance, the major metabolite of ICI-200 880, ZD-0892 and ZD-8321 was the trifluoromethyl alcohol.[105,109] Other metabolites of ICI-200 880 were also identified in rat bile including a metabolite where hydrolysis of the right hand side amide group had occurred to liberate the whole of the trifluoromethylketone group.[105] Both Boceprevir and Telaprevir are metabolised by CYP 3A isozymes *in vitro* and the metabolism of these compounds in rat and human liver microsomes can be inhibited with the HIV protease inhibitor compound ritonavir.[111] Cmax (>3-fold) and AUC (>8-fold) of both compounds were increased when the compounds were dosed orally ($5\,mg\,kg^{-1}$) to rats with ritonavir ($5\,mg\,kg^{-1}$).[111]

As well as primary structure, the conformation of peptides is also important for recognition by protease enzymes. The finding that only the extended 'saw-tooth' conformation of peptides appears to bind to the active sites of proteases has led to the proposal that 'macrocyclisation' can be used as a general strategy to design protease inhibitors. Macrocyclic inhibitors mimicking the transition state of many enzymes (including HIV protease, renin) have been described.[112]

In terms of ADME properties, macrocyclic compounds also have some advantages. Unlike linear peptides, the macrocyclic peptides have high resistance to proteolytic degradation and, if sufficiently lipophilic, the compounds can show membrane permeability and oral bioavailability. K3 (Table 9.5) is a potent (Ki 2 nM, HCV replicon EC_{90} 20 nM) macrocyclic ketoamide inhibitor of HCV NS3 protease and shows oral exposure in rat following oral dosing.[113] Although there is no intravenous pharmacokinetic data for K3, the exposure after oral dosing in the rat ($1\,\mu M\,h^{-1}$) is very similar to that achieved with the structurally related but non-macrocyclic compound K4 (Ki 4, replicon EC_{90} 30 nM) (Table 9.5), which is a back-up compound to boceprevir and has high absorption and good bioavailability in the rat.[114]

9.3.1.4 Boronic Acid Containing TSAI of Proteases

Boronic acids are Lewis acids with the ability to accept electrons thereby completing the open shell of boron resulting in a shift from a neutral trigonal planar sp^2 boron to an anionic tetrahedral sp^3 boron (Figure 9.11). Phenyl

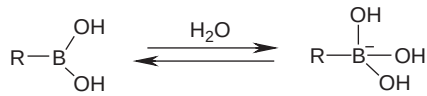


Figure 9.11 Conversion from neutral sp^2 boron to tetrahedral anionic sp^3 boron.

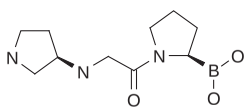
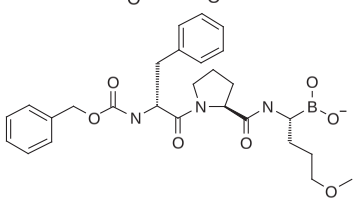
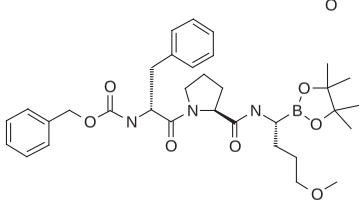
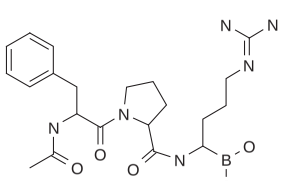
boronic acids typically have a pKa in the 4.5–8 range and butylboronic acid has a pKa of 10.6.[250]

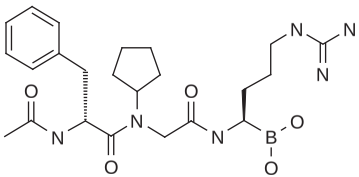
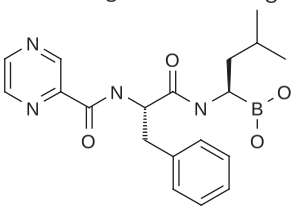
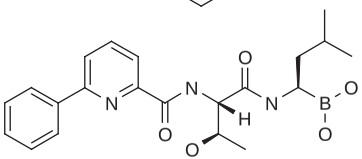
As amide (peptide) cleavage requires conversion of a neutral sp^2 carbonyl carbon to a tetrahedral sp^3 carbon, boronic acid compounds can mirror this transition and make the same interactions with the protease enzyme as the tetrahedral transition state thereby acting as potent TSAI of a number of protease enzymes.[115] Boronic acid inhibitors of serine proteases were first synthesised in the early 1970s.[116,117] Since that time boronic acid compounds have been shown to be effective inhibitors of a number of enzymes including thrombin,[118,119] Factor Xa,[120] trypsin, chymotrypsin, pancreatic elastase, leukocyte elastase, thrombin, β-lactamases,[121–125] DPP IV and HCV NS3 protease,[126,127] and have been explored for use as therapeutic agents in various disease states.[115,128]

There are two different mechanisms by which the tetrahedral boronate anion is formed. In the case of arginase inhibitors, the tetrahedral boronate anion is formed by reaction with water.[129] In contrast for other enzymes such as serine proteases, the hydroxyl anion of the catalytic amino acid typically attacks the boronic acid to form the boronate intermediate, resulting in the formation of a covalent bond to the enzyme which, while reversible, typically gives slow off-rates of hours or days thereby giving a significant pharmacodynamic advantage to this class of inhibitor.[130] Because of this requirement for boronic acids to form boronates to mimic the transition state of protease enzymes, it has been argued that boronic acids are not formal transition state analogues but should be designated as either 'reaction coordinate analogues' or covalent inhibitors depending on the mechanism of boronate formation (water *vs.* protein).

The boronic acid TSAI of proteases discussed further in this chapter are shown in Table 9.6. The enzyme targets of these compounds include thrombin, dipeptidylpeptidase IV (DPP-IV) and the 20S proteasome. DPP-IV cleaves dipeptides from the *N*-terminus of numerous polypeptides including incretins such as GLP-1 that have an important role in blood glucose homeostasis. Small, polar (clogP −2.2 to −5.3) boronic acid containing compounds are effective TSAIs of DPP-IV. Proteasomes cleave peptides through threonine-dependent nucleophilic attack and transition state inhibitors have been designed to inhibit this process. The boron containing TSAI of proteasomes need higher lipophilicity (clogP 0.8−1.3) to effectively bind in the active site of the proteasome. Bortezomib is a potent boronic acid inhibitor of the chymotryptic-like site of the 20S proteasome (Ki 0.62 nM) and establishes a slowly reversible (half-life ∼20 min) tetrahedral intermediate with the active site *N*-terminal threonine residue.[131]

Table 9.6 Chemical structure, physicochemical properties and clearance, bioavailability and absorption estimates of boronic acid containing TSAI.

Compound	*Structure*	*Target*	*MW*	*clogP*	*TPSA* *HBA* *HBD*	*pKa*	*Vss (L/kg)*	*PPB (%)*	*CL (ml/min/kg)*	*F%*	*% absorbed*
Dutogliptin		DPP-IV	241	-2.2	85 3 4	Zwitterion (9.7 A, 10.5 B)					
Flovagatran Ref 298		Thrombin	524	2.2	140 4 3	Weak acid (9.7)	0.6 h			Dosed IV	
Tri-50b Ref 134		Thrombin	608	5.0	115 4 2	Neutral				24 r	
DuP-714		Thrombin	460	−2.4	183 5 8	Weak acid (9.6)					

S-18326 Ref 299		Thrombin	488	−1.3	181 6 8	Weak acid (9.6)				27 dfa 6 dfe	
Bortezomib (approved) Ref. 136–138, 247		Proteasome	384	0.8	124 4 4	Weak acid (9.7)	~3–13 h Vz ~ 24–7 h	93 r 94 d 83 h	14 mo 13–27 hsd ~3.5–8.5 hmd	11 mo	
CEP-18770		Proteasome	413	1.6	132 4 5	Weak acid (9.7)	10 mo 18 r	99.9 r 99.9 d 99.8 h	23 mo 3.2 r	39 mo 54 r	~80[a]

dfa = dog fasted, dfe = dog fed, hmd = human multiple dose, hsd human single dose, r = rat.
[a]Assuming blood : plasma = 1.
mo = mouse.

In general the boronic acid compounds discussed have reasonable PSA values for oral absorption (six out of seven compounds have PSA in the range 85–140 $Å^2$). One reason for this is that the boronic acid compounds can be considered as prodrugs as they are converted to the final, more polar tetrahedral transition state analogue inhibitor in the body.

Polar boronic acids are poorly absorbed transcellularly as would be expected from their physicochemistry; for instance DUP-714, a thrombin inhibitor containing both a guanidine and boronic acid group (clogP −2.4, PSA 183 $Å^2$), has low permeability through rat jejunum (Papp 1.5×10^{-6} cm sec^{-1}, pH 7.4). Some analogues of DUP-714 had significantly higher permeability (Papp 18×10^{-6} cm sec^{-1}), which was reduced in the presence of known inhibitors of the oligopeptide transporter,[132] suggesting that while passive permeability was not high this could be overcome if the compounds cross the membrane by active influx mechanisms. Dutogliptin is a small (MW 241), basic, polar (clogP −2.2) DPP IV inhibitor. It is orally bioavailable (CL/F 10–30 ml min^{-1} kg^{-1}) in humans and Tmax was 3–5 h. Given the physicochemical properties of dutogliptin, it is likely that this compound is absorbed *via* the paracellular route.

The more lipophilic protease inhibitor CEP-18770 (clogP 1.6) has reached phase I trials. In pre-clinical species it has high absorption and oral bioavailability, low clearance (although the protein binding and hence CL_u are high) and a long half-life.[133] The data with CEP-18770 demonstrate that the presence of the boronic acid moiety *per se* does not preclude good transcellular absorption.

In an effort to increase the absorption of poorly absorbed boronic acid TSAIs, prodrug strategies using boronic esters (which decrease the number of HBD by two) have been employed. TRI-50b is a pinanediol ester of flovagatran which showed modest bioavailability in the rat and is currently in clinical trials—phase I (oral as prodrug) and phase II (iv as flovagatran).[12,134,135]

The distribution of boronic acid compounds *in vivo* can be influenced by their ability to form reversible covalent bonds. For example, bortezomib in addition to having moderate binding to human plasma proteins, binds slowly to the proteasome in red blood cells. This binding has been shown to complicate the bioanalysis of bortezomib in blood with differential recoveries between incurred and spiked standard samples being noted.[251] Bortezomib has marked multiphasic pharmacokinetics after intravenous dosing with a long terminal elimination phase (half-life ~9–15 h) and a large terminal volume of distribution (>10 L kg^{-1}).[136–137,247] Some studies have reported a change in volume on multiple dosing,[136] whilst others have shown it to be unchanged.[138] Extensive distribution of bortezomib related radioactivity into tissues (apart from the brain) was also seen in monkeys dosed with radiolabelled bortezomib.

The elimination and clearance pathways of boronic acid compounds vary from compound to compound. After intravenous dosing of flovagatran, 14% of the dose was excreted unchanged in the urine; in contrast after intravenous administration bortezomib is extensively metabolised with little excretion of

unchanged bortezomib in the urine and none in the faeces of humans dosed with the compound.[139,252] The initial route of metabolism of bortezomib is oxidative deboronation (none of the thirteen metabolites in human plasma contain the boronic acid group), which removes the functional group mimicking the transition state producing metabolites inactive against the proteosome.[139] Extensive deboronation is likely to be a major metabolic pathway for any boronic acid compound lipophilic enough to undergo CYP metabolism and is consistent with the finding that boronate compounds in general are susceptible to undergoing Baeyer–Villiger-type oxidative deboronation reactions.[139–142]

In human liver, CYP 3A4 is the predominant isozyme metabolising bortezomib with quantitatively important contributions also from CYP 2C19 and CYP 1A2.[143] *In vivo* drug–drug interaction (DDI) studies showed that clearance of bortezomib was decreased; AUC increased by a mean value of 35% ($CI_{90\%}$ 1.03 to 1.8) in the presence of ketoconazole (a CYP 3A4 inhibitor) but not in the presence of omeprazole (a potent CYP 2C19 inhibitor).[247] As well as being metabolised by CYP 2C19, bortezomib is also a weak inhibitor of the isozyme ($IC_{50} = 18\,\mu M$),[144] although the inhibition is related to the scaffold of the compound rather than to the presence of the boronic acid group *per se*. Mechanistic studies[145] suggest that human P450 can deboronate bortezomib *via* multiple mechanisms including CYP mediated formation of reactive oxygen species (*e.g.* superoxide) in addition to direct oxidation by the hypervalent oxo-iron species [Fe(V) = O].

Bortezomib and CEP-18770 can also theoretically cause CYP-mediated DDI by inhibiting the degradation of CYP isozymes by the 20S proteasome (their pharmacological target). In rats, bortezomib inhibits the degradation of CYP 2E1 following induction of the isozyme by ethanol but paradoxically decreased the levels of CYP 3A1/2 and total CYP content.[144] In humans, bortezomib administration caused no changes in results from the erythromycin breath test (an *in vivo* measure of CYP 3A4 activity).

The physical form and stability of boronic acid TSAIs can also pose a problem in their development as therapeutics. For example in solid form bortezomib exists in its cyclic anhydride form as a trimeric boroxine. To overcome this, the bortezomib drug product is a mannitol ester which, when reconstituted, exists in equilibrium with the monomeric boronic acid form (solubility ~ 3 mg ml^{-1}).[146] Bortezomib is also chemically unstable in a number of matrices and acidification was needed to stabilise bortezomib during protein precipitation extractions.[139]

9.3.1.5 Phosphonic Acid Containing TSAI of Proteases

Angiotensin converting enzyme (ACE) is a Zn^{2+} metalloproteinase that converts angiotensin I (ANG I; 10AA) to angiotensin II (ANG II; 8AA) a major regulator of fluid and sodium balance and haemodynamics, but also of cellular growth and cardiovascular remodelling.[253] The tetrahedral transition state in the conversion of ANGI to II by ACE is shown in Figure 9.12.[254]

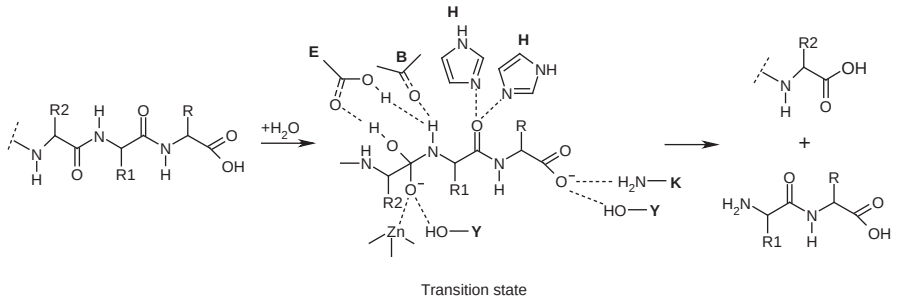


Figure 9.12 Proposed tetrahedral intermediate in the conversion of Angiotensin I to Angiotensin II by Angiotensin converting enzyme.

Three different structural classes of ACE inhibitors have been approved as drugs:

- sulfhydryl (*e.g.* captopril);
- dicarboxylate (*e.g.* enalaprilat and ramiprilat);
- phosphonic (fosinoprilat).

Although potent inhibitors of ACE, the sulfhydryl (Zn^{2+} chelation) and carboxylate compounds are not TSAI (Figure 9.13). The phosphonic acid compounds such as fosinoprilat, however, mimic the tetrahedral nature of the transition state.

Due to the highly polar nature of fosinoprilat it has limited membrane permeability and oral bioavailability is low. To improve bioavailability, fosinoprilat is administered as a phosphinic ester prodrug, fosinopril. Although fosinopril has physicochemical properties more typical of an oral drug (log D 2.63 and PSA 70 $Å^2$), it is predominantly in its ionised state in the gastrointestinal tract and this contributes to its incomplete absorption ($\sim$35%). As well as passive transcellular permeability, the influx transporter PEPT-1 may play a role in absorption of fosinopril.[255,256] The polar, acidic nature of fosinoprilat limits its distribution outside the extravascular space in humans (Vss 0.14 L kg^{-1}). Clearance of fosinoprilat is low (0.56 ml min^{-1} kg^{-1}) with a high renal component (0.24 ml min^{-1} kg^{-1}). After intravenous dosing of fosinoprilat, excretion is only as unchanged drug. After oral administration of the more lipophilic fosinopril, additional metabolites of fosinoprilat (including glucuronide and oxidised metabolites) are observed. This may arise from metabolism in the intestine or because the more lipophilic prodrug is able to undergo metabolism in the liver prior to cleavage and liberation of fosinoprilat metabolites.[147,148] Studies in the dog showed that there was significant hydrolysis of fosinopril in both the intestine and liver (extraction ratios of 0.6–0.9), but because the intestine sees drug first, it is responsible for metabolising $>$75% of an oral dose of fosinopril. In addition it was shown that following systemic administration of fosinopril, extrahepatic sites

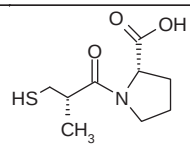
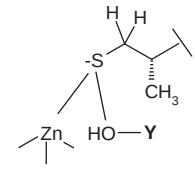
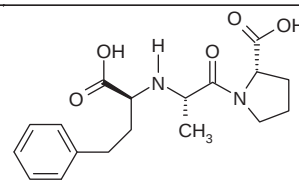
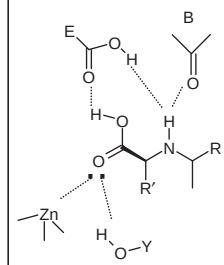
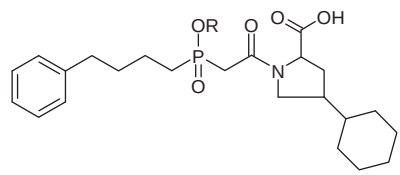
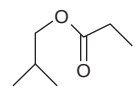
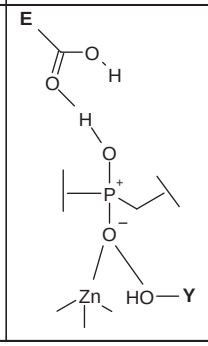
Chemical group	Example ACE inhibitors	Key interactions with the active site Zn^{2+}
Sulfhydryl	 Captopril	
Carboxylic acid	 Enalaprilat	
Phosphonic acid	 R= H Fosinoprilat R= Fosinopril 	

Figure 9.13 Example of sulfhydryl, carboxylic and phosphonic acid ACE inhibitors.

(including the kidney) were responsible for about 50% of the systemic clearance.[147]

9.3.2 Neuraminidase TSAI

The neuraminidase enzyme of the influenza virus plays a key role in viral infectivity and has emerged as a useful therapeutic target.[149] The substrate of viral neuraminidase is the extremely polar terminal sialic acid residue of glycoproteins.[150] The neuraminidase catalytic mechanism is thought to involve two chemical steps—cleavage of the C2 glycosidic bond and subsequent hydroxylation of a planar oxocarbonium ion intermediate.[150] To bind efficiently in the polar active site of viral neuraminidase and to mimic the oxocarbonium intermediate, the neuraminidase TSAI also need to be very polar[151]

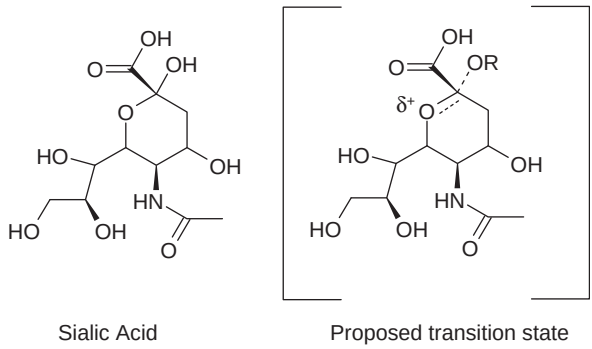


Figure 9.14 Structure of sialic acid and the proposed transition state of sialic acid within viral neuraminidase.

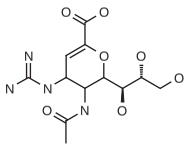
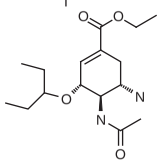
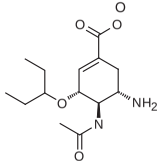
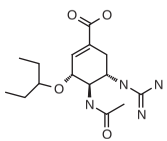
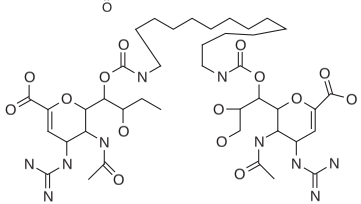
(Figure 9.14, Table 9.7). Although, the interactions of sialic acid, zanamivir and oseltamivir acid with viral neuraminidase are similar, the TSAI bind to the enzyme $0.5–1.5\times10^{6}$ times more tightly than sialic acid and show time-dependent and slowly reversible inhibition kinetics.[150,152]

Zanamivir is a highly polar zwitterionic compound and as a result has poor membrane permeability, limited absorption and low bioavailability in rat and human (Table 9.7). Zanamivir has higher F% when administered *via* the intranasal ($\sim$10%) or inhaled routes ($\sim$15–20%) in humans.[153] Inhaled administration of zanamivir Tc^{99m} (10 mg) in healthy volunteers showed that 4–17% of the dose was deposited in the lungs. After dry powder inhalation administration, the Tmax of zanamivir in the systemic circulation was 1–2 h; the systemic half-life of zanamivir was longer than after intravenous administration (3.6–5 *vs.* 1.7 h) suggesting that absorption through the lung is slow enough to become the rate limiting step in the elimination of zanamivir (flip-flop kinetics).

Oseltamivir acid is 4 log units more lipophilic than zanamivir and has a PSA more typical of an oral drug ($<140\,A^2$). However, oseltamivir acid is still quite hydrophilic clogP -1.2 and *in vitro* has limited ability to cross cell monolayers (Papp $<2\times10^{-6}\,cm\,s^{-1}$). When administered orally to rats or humans, oseltamivir acid has low bioavailability, primarily due to low absorption.[154] To overcome this limitation, the ethyl ester prodrug (oseltamivir) of oseltamivir acid was prepared. Oseltamivir is about 3 log units more lipophilic than oseltamivir acid and has high aqueous solubility ($>500\,mg\,ml^{-1}$) and a moderate ability to cross cell monolayers *in vitro* (Papp $6–12\times10^{-6}\,cm\,s^{-1}$ in Caco-2 and LLC-PK1 cell lines).[155,156] Despite its relatively small size, oseltamivir is a substrate for human P-gp.

In pre-clinical pharmacokinetic studies, the bioavailability of oseltamivir acid following oral dosing of oseltamivir was 11–73% (Table 9.7). The lowest F% was seen in the ferret which had a relative inability to cleave oseltamivir to the acid metabolite suggesting that, even in this species, absorption of oseltamivir was good.[157] The improvement in F% (<5 to $\sim$75–80%)[154] of

Table 9.7 Chemical structure, physicochemical properties and clearance, bioavailability and absorption estimates of neuraminidase TSAI.

Compound	*Structure*	*MW*	*clogP*	*TPSA*	*pKa*	*HBA*	*HBD*	*CL (ml/min/kg)*	*Oral F%*
Zanamivir Ref 153		332	−5.6	198	3.8 A 11.3 B	10	9	1.4–32 r 1.6 h	3–4 r 2 h
Oseltamivir Ref 154		312	2.1	91	8.8 B	4	3		
Oseltamivir acid Ref 154		284	−1.2	102	2.2 A 9.7 B	5	4	25 r 5–6 h	4.3 r < 5 h 11 f[a] 30–73* m, r, d, ma 75–80 h[a]
GS–4116 Ref 157		326	−1.7	138	4.3 A 12.8 B	7	6	22	4 r 2 r[a]
Dimeric zanamivir Ref 172		943	−5.5	412		19	17	0.7	< 1

d = dog, f = ferret, h = human, m = mouse, ma = marmoset, r = rat. [a]Following dosing of ethyl ester prodrug.

oseltamivir acid seen in humans when dosed as oseltamivir is one of the highest seen with an ester prodrug [158] and it is unusual to get such a big increase in F% with such a simple modification. In a study to look at regional absorption of oseltamivir in humans, the prodrug was well absorbed from the stomach, jejunum and ileum.[156] Oseltamivir is absorbed at least in part transcellularly, as slow absorption was also seen from the colon (a region where tight junctions have low porosity and paracellular absorption is minimal).

Although oseltamivir was a successful prodrug, attempts to 'prodrug' a more polar analogue (GS-4116: clogP −1.7, PSA 138 $Å^2$) were unsuccessful (Table 9.7). The ethyl ester prodrug of GS-4116 showed no improvement in oral bioavailability (F% ~2) in the rat compared with oral administration of the acid compound itself.[157] As illustrated by this example, prodrugging of compounds is unlikely to be successful if the PSA of the parent molecule is too far from ideal oral drug space.[159]

The distribution of zanamivir and oseltamivir acid within the body is heavily influenced by their polar physicochemical characteristics. Plasma protein binding of zanamivir is low and that of oseltamivir negligible, whilst the more lipophilic oseltamivir has moderate plasma protein binding in humans (~42%). Distribution of zanamivir into red blood cells is also negligible (BP = 0.4–0.6).[160,161] The steady-state volume of distribution (Vss) in humans of both zanamivir and oseltamivir acid is low (0.2–0.4 $L\,kg^{-1}$) and similar to the volume of extracellular fluid. The volume of distribution in dog was similar to that in human (0.3 $L\,kg^{-1}$) but higher volumes of distribution (1, 3 and 9 $L\,kg^{-1}$) were seen in rat, mouse and ferret, respectively.[157]

Some concerns about neuropsychiatric side effects of oseltamivir arising in Japan[162] have led to a number of studies looking at concentrations of oseltamivir and oseltamivir acid in the CNS. In rats following oral dosing of oseltamivir, the brain: plasma AUC ratio was 0.15 for oseltamivir and 0.01 for oseltamivir acid. *In vitro* studies showed that rat and human S9 brain homogenates had little ability to convert oseltamivir to oseltamivir acid.[162] The limited brain penetration of oseltamivir is in part due to transporter-mediated efflux. Oseltamivir is a substrate for both human and murine P-gp and the brain to plasma ratio of oseltamivir was increased (~5-fold) in P-gp knockout mice or in wild-type mice dosed with the P-gp inhibitor GF-120918. The brain penetration of oseltamivir is also lower in older rats that are known to have increased P-gp expression in the blood–brain barrier than younger animals. The limited brain penetration of oseltamivir acid is largely due to the poor membrane permeability of the compound as the brain to plasma ratio of oseltamivir acid was unaffected by the P-gp inhibitor and was similar in P-gp knockout and wild-type mice,[155,163] although other transporters may have a role in limiting oseltamivir acid brain penetration. The penetration of oseltamivir and oseltamivir acid into the CNS of humans was also low (CSF: free plasma concentration ratio was <0.05) and CSF Cmax was delayed relative to that in the plasma suggesting a barrier to free permeability of the compounds in the CNS.[164]

Zanamivir and oseltamivir acid are highly polar and are almost exclusively (87–90%) excreted into urine as unchanged drug following intravenous

dosing.[160] The renal clearance of zanamivir was similar to the glomerular filtration rate (GFR) in humans suggesting minimal reabsorption of zanamivir (a highly polar; water soluble compound) occurs in the kidney presumably due to its poor membrane permeability. Renal clearance of oseltamivir acid ($\sim$4.5 ml min^{-1} kg^{-1}) in humans is greater than GFR suggesting active secretion into the urine occurs. *In vitro* studies have shown that oseltamivir acid is a substrate and weak inhibitor (Ki 45 mM) of human renal transporter hOAT1[165] and renal Cl in humans *in vivo* is inhibited by probenecid (a known hOAT inhibitor).[166] In the presence of probenecid, the renal Cl of oseltamivir acid is roughly equal to GFR suggesting that the active component of oseltamivir acid is completely inhibited by probenecid. Oseltamivir itself is also actively secreted in to human urine (mean renal Cl 360–480 ml min^{-1}, $\sim$6 ml min^{-1} kg^{-1}), although the responsible transporter has not been identified.[167]

Oseltamivir acid is the only metabolite of oseltamivir identified in humans. In rats, however, some additional minor metabolites have been identified of which the *R*-omega (ω) carboxylic acid metabolite was the most abundant (metabolism occurs on the terminal carbon of the pentoxy group). Rat liver microsomes in the presence of NADPH metabolise oseltamivir to the ω-hydroxy metabolite—a first step on the pathway to the acid.[168]

As with other ester prodrugs,[158] the tissues hydrolysing oseltamivir to oseltamivir acid differ across species. The hydrolysis of oseltamivir is rapid in rat plasma but relatively slow in ferret, dog and human plasma.[157,169] Oseltamivir is also hydrolysed to oseltamivir acid in human liver. There are two major carboxylesterase isozymes with differing substrate specificity in human liver, HCE-1 and 2.[170] Hydrolysis of oseltamivir in human liver correlates with HCE-1 activity and the Km in human liver microsomes (187 μM) and recombinant HCE-1(177 μM) were similar. The rate of hydrolysis of oseltamivir was shown to vary when some naturally occurring variants of HCE1 were assessed for catalytic activity. HCE1S58N slightly increased the hydrolysis ($\sim$25%) whereas variants HCE1C70F and HCE1R128H markedly decreased the hydrolysis.[171] To date the impact of these mutations on oseltamivir human pharmacokinetics has not been reported.

Once formed from orally administered oseltamivir, the elimination half-life of oseltamivir acid in humans (6–10 h) is considerably longer than that seen following intravenous administration of oseltamivir acid itself (2 h);[154] a similar phenomenon is seen in pre-clinical species.[154] As oseltamivir itself is rapidly absorbed, the longer plasma half-life of oseltamivir acid seen after oral administration of oseltamivir probably reflects the rate at which the acid metabolite is formed by hydrolysis and crosses the sinusoidal membrane of the liver to re-enter the blood. Given the polarity of oseltamivir acid and its poor membrane permeability, it is likely that transporters play a role in the re-entry of the compound in to the circulation from the liver.

Attempts have been made to increase the half-life and retention of zanamivir in the lung using polymers of di-, tri- and tetrameric zanamivir molecules linked by lipophilic spacer chains, and by making a dextran linked polymer of zanamivir. These modifications have resulted in molecules with greater potency and

a longer residence time in *in vivo* models of influenza infection.[172] The zanamivir dimer shown in Table 9.7 is 100 times more potent than zanamivir in a mouse influenza model, and levels in rat lung one week after dosing are 100-fold higher than monomeric zanamivir.[172] In rat studies where the pharmacokinetics of zanamivir and the dimer were directly compared, the dimer had a two-fold lower Cl than zanamivir and a larger although still small volume (0.2 *vs.* 0.08 $L\,kg^{-1}$) and has high F% (67) after intratracheal dosing.

9.3.3 *N*-Ribosyltransferase TSAI

N-Ribosyltransferase enzymes catalyse the transfer of ribose or deoxyribose moieties from nucleosides and nucleotides.[173] Purine nucleoside phosphorylase (PNP) (E.C. 2.4.2.1) is a key enzyme in the purine salvage pathway; it catalyses the reversible cleavage of purine nucleosides to the corresponding purine free base and ribo- or deoxyribose-1-phosphate.[174–177] Inherited PNP deficiency in children leads to selective depletion of their T-lymphocytes making PNP inhibition an attractive target for T-cell proliferative disorders (including organ transplant rejection and T-cell lymphoma and leukaemia).[178–185]

The catalytic mechanism of PNP is summarised in Figure 9.15. The doubly ionised phosphate, which is stabilised by His86, attacks the anomeric centre of the ribose. The ribose–purine bond is weakened by charge separation, resulting in oxonium cation formation enabling facile attack by the phosphate dianion,[174,186] The transition states of human, bovine and *Plasmodium falciparum* PNP have been studied in great detail with active site mutagenesis and kinetic isotope experiments used to design a series of more potent TSAI of PNP with drug-like attributes.[179] The key features incorporated into the TSAI were the oxocarbenium ion character of the ribosyl group and a C1 substitution without the potential to become a leaving group.[186] Some of the compounds arising from this initiative are shown in Figure 9.16.[187,188]

Forodesine is a potent inhibitor of bovine (23 pM) and human (66 pM) PNP[175,188] due to favourable hydrogen bonding interactions in the active site and effective mimicry of the developing positive charge of the early transition state.[186,188] The human transition state of PNP was found to have a larger C1′–N distance and increased cationic character compared to the bovine enzyme; this explained the potency drop for forodesine in the human enzyme. To effectively act as inhibitors of human PNP, a series of 4-deaza-1-aza-2-deoxy-1,9- methylene immucillin compounds were designed where the cationic charge was moved to the 1′- position of the iminoribitol ring and a methylene was added between the ribosyl ring and the nucleoside base.

Of these analogues, BCX-4208 was a potent (Kd 16 pM) inhibitor of human PNP. BCX-4208 is orally available in mice and a single oral dose causes inhibition of murine red blood cell PNP. Once inhibited by BCX-4208, the return of PNP activity to pre-dose levels principally reflects red blood cell replacement (half-life for recovery of PNP activity 11.5 days),[191,192]

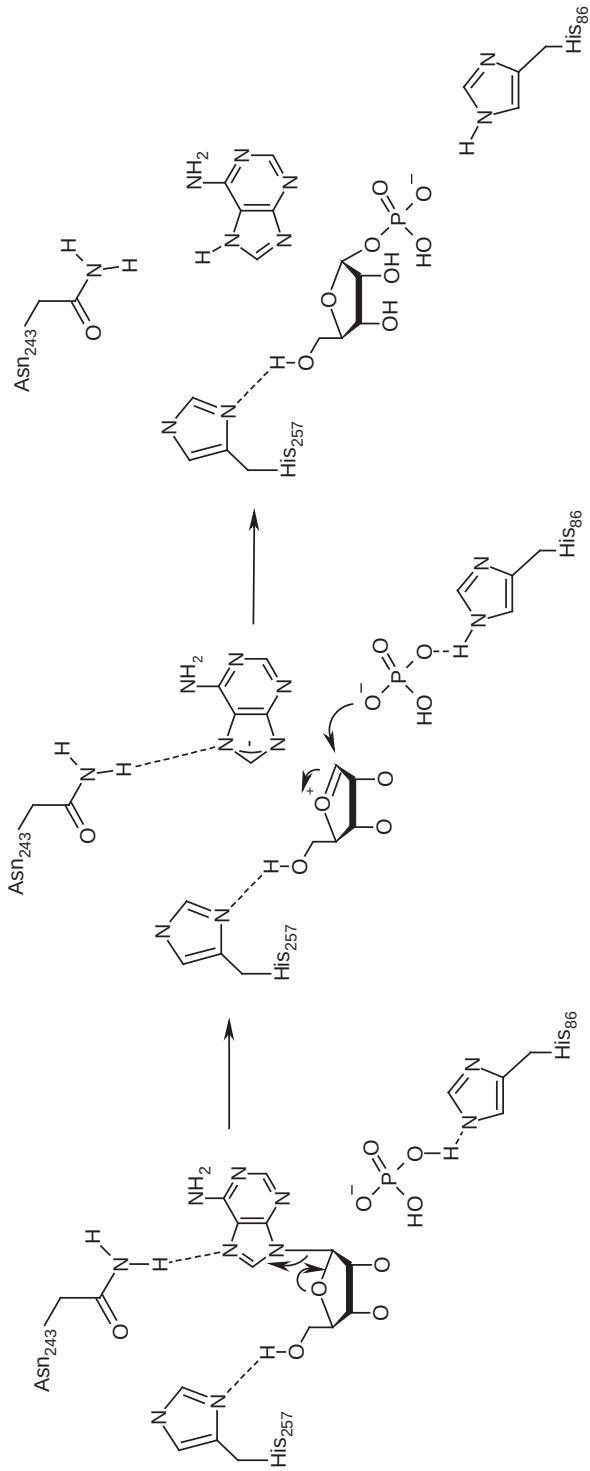


Figure 9.15 Proposed mechanism for PNP-catalysed phosphorolysis.

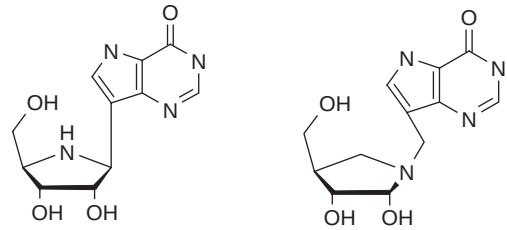


Figure 9.16 TSAIs of PNP.[174,188–190]

Table 9.8 Pharmacokinetics of forodesine.[183,193,195,196]

Species	*CL (ml/min/kg)*	*Vss (L/kg)*	*F%*	*Fa*
Mouse		a	63	
Rat	~7	~0.6 (Vz)	7–22	
Dog			100	
Monkey	5–10		3–13	
Human	~1.5	~1.3	30–50	32–53

a = terminal volume.

Forodesine (BCX-1777, immucillin-H) is the most advanced of the PNP TSAI and has been studied in phase I/II clinical trials for T- and B-cell mediated disorders. Forodesine is polar (clogP -2.4, PSA 134 $Å^2$) and inhibits the proliferation of activated human T-cells ($IC_{50} = 0.05\,\mu M$).[181–183] The pharmacokinetics of forodesine are summarised in Table 9.8.

Absorption of forodesine was high in mice and dog after oral administration, although flip-flop kinetics were observed with the oral half-life (2.2 h) being longer than the intravenous half-life (0.8 h). Given the physicochemistry of forodesine, it is likely that the compound is absorbed either paracellularly or *via* an active transporter-mediated transcellular pathway.[183] Oral F% was lower in the rat and monkey[193] and absorption was slow (Tmax 4–11 h) and incomplete in both species and saturated as the dose of compound was increased. Changing the formulation [5 mg kg^{-1} in citrate buffered (pH 6.7) sodium lauryl sulfate] of forodesine failed to improve the oral absorption and bioavailability in the monkey.[193]

Despite the high polarity of forodesine, the compound was also well absorbed in humans. In clinical trials in cancer patients, the bioavailability of forodesine is 30–50% with some evidence of saturation of bioavailability at higher doses of the drug. In addition to oral exposure, pharmacodynamic effects of forodesine were elicited after oral dosing with all patients demonstrating elevated levels of the dGuo biomarker.[194]

The clearance of forodesine was low in rats and humans, moderate in mouse and high in monkey. In keeping with the physicochemical properties of forodesine, *in vitro* studies using liver microsomes from mice, rats, primates and

humans did not produce measurable metabolites suggesting that CYP metabolism will have a minor role in clearance of the compound. In humans, 40–70% of an intravenous forodesine dose was recovered in the urine unchanged in the first 24 h after dosing suggesting that this is the major route of clearance of the compound.[83,193,195,196]

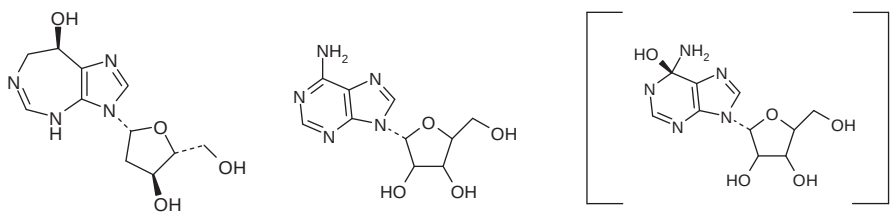
The volume of distribution of forodesine was $\geq$ body water in rat, humans and mouse suggesting that, despite its small polar nature, forodesine can distribute into the tissues of the body. The half-life of forodesine was short in all pre-clinical species (0.8–3 h) but reasonably long in humans (median half-life of 11 h) (Table 9.8 and references cited therein).

9.3.4 Nucleoside Deaminase TSAI

9.3.4.1 Purine Deaminase Inhibitors

Pentostatin (2′-deoxycoformycin) (Figure 9.17) is a very potent (2.5 pM against human erythrocyte enzyme) TSAI of adenosine deaminase (ADA) and is approved for the treatment of hairy cell leukaemia. In addition pentostatin has been used to boost the pharmacokinetics of other cytotoxic drugs that are metabolised by ADA (*e.g.* vidaribine).[197]

Pentostatin undergoes rapid acid catalysed glycosidic cleavage ($T_{1/2} < 6$ min at pH 1), which makes oral administration challenging due to the low pH in the stomach.[197] As would be expected for a small, polar compound, the Vss of pentostatin following intravenous dosing in humans is low ($\sim 0.6\,L\,kg^{-1}$) and close to the volume of total body water. Pentostatin is believed to enter cells *via* nucleoside transporters and shows extensive distribution into red blood cells (blood to plasma ratio is 2–4).[259] Pentostatin is essentially free in human plasma (PPB 3.8%) (PPB = plasma protein binding) [198] and, not surprisingly for a polar compound, CSF penetration of pentostatin is low (10–25% of serum levels). [197] However, studies in rats show that 90–95% inhibition of ADA in the brain can be achieved following intraperitoneal injection of pentostatin.[199] Clearance of pentostatin is also low (~ 1.4–$1.9\,ml\,min^{-1}\,kg^{-1}$) and the human half-life is about 6 h in patients with normal renal function. Clearance (total and renal) is



pentostatin adenosine ADA transition state intermediate

Figure 9.17 Chemical structure of pentostatin, the substrate of ADA (adenosine) and the proposed transition state intermediate of ADA.

lower and half-life longer in patients with impaired renal function[198] and a large proportion of pentostatin is excreted unchanged in the urine in humans (estimates vary from 20–66, 32–48, 50–73 up to 90% in different studies) and animals (mice 91–100%, rats 81–86%, dogs 54–83%).[198,199,197,259]

9.3.4.2 *Pyrimidine Deaminase Inhibitors*

Important anticancer agents which are cytidine analogues (including cytosine arabinoside (Ara-C), gemcitabine, capecitabine, decitabine and 5-azacytidine)[200] are converted to inactive or toxic metabolites by cytidine deaminase (CDA) resulting in short *in vivo* half-lives and poor bioavailabilities of these drugs.[201,202] As a result, inhibition of CDA has emerged as a target to enhance the efficacy and reduce the toxicity of chemotherapeutic cytidine analogues.[203,204]

Crystallographic studies of CDA–ligand complexes have shown that there are four key features of the enzymatic mechanism of CDA:[205]

(i) The zinc atom in the active site activates water to OH^-, which subsequently attacks the C4 position of substrate.
(ii) The resulting tetrahedral transition state is stabilised by a strong interaction with the carboxylate group of the enzyme present in the vicinity.
(iii) Intramolecular proton transfer is mediated entirely by the carboxylate group.
(iv) Stabilisation of the leaving NH_3 by hydrogen bonds to the protein backbone carbonyl oxygen of T127.[206]

A schematic of the transition state intermediate of cytidine deaminase is shown in Figure 9.18.

Zebularine and 3,4,5,6-terahydrouridine (THU) are rationally designed inhibitors of cytidine deaminase that share structural features with the proposed transition state. Replacing the NH_3 group of cytidine with a proton (zebularine) produces a compound that reacts with water to produce a transition state analog inhibitor of CDA in which the conversion of the tetrahedral transition state into product is prevented. The 4-OH compound binds to the enzyme 10^7-fold more potently than zebularine itself.[207] It is also likely that zebularine can form a stable alkoxide-like transition state analogue inhibitor in the active site of CDA following nucleophilic attack by the Zn^{2+}-hydroxide group of the enzyme with two concerted proton transfers.[208] As well as acting as an inhibitor of CDA, it has also been shown that zebularine can also form a covalent complex with DNA methyltransferases and has gene silencing activity.[209,210]

The amine group of cytidine is replaced by a hydroxyl group in THU which mimics the transition state of CDA yielding a potent, competitive inhibitor [*K*i 28–240 nM (7–60 ng ml^{-1})].[211,212] Complete inhibition of CDA *in vitro* is achieved with THU concentrations of 1 μg ml^{-1}.[213]

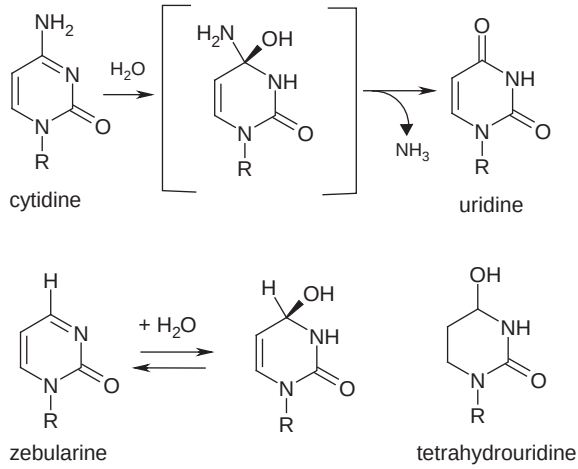


Figure 9.18 Proposed scheme of hydrolytic deamination of cytidine deaminase and structures of zebularine, 4-hydroxyzebularine and tetrahydrouridine (R = ribose).[206]

Like the transition state of CDA that they mimic, zebularine (MW 228, clogP −2.1) and THU (MW 248, −1.90) are small polar compounds. Both compounds have low oral F% due to a combination of extensive first-pass metabolism and limited passive permeability and poor absorption.[201,214–216] In contrast to the low oral bioavailability, subcutaneous bioavailability of THU was high (F% 84) in humans.[201,216] The pharmacokinetics of zebularine and THU are summarised in Table 9.9.

Despite the compounds having similar physicochemistry, their clearance mechanisms are quite different. THU is excreted in the urine predominantly as unchanged drug (80–90%) after intravenous dosing in humans, rats, dogs and monkeys.[216,218] In the mouse renal excretion of unchanged drug is lower (55%) and renal clearance (5.0–6.2 ml min^{-1} kg^{-1}) is less than GFR in this species ($\sim$10 ml min^{-1} kg^{-1})[219] suggesting that there is some renal reabsorption of this polar compound in the mouse following glomerular filtration.[217] The clearance of THU in mice is low compared to other pyrimidine analogues because the compound is not a substrate for the enzymes (thymidine phosphorylase or uridine phosphorylase) that typically cleave the ribose moiety of pyrimidine analogues.[217] In humans, no metabolites of THU were seen after intravenous or subcutaneous dosing, but after oral dosing of ^{14}C THU, the radiochemical and biochemical assays did not give the same results suggesting that a significant part of the dose was metabolised to inactive species. This may be due to metabolism by intestinal microflora as well as to first-pass intestinal metabolism.[216]

In contrast to THU, zebularine is extensively metabolised. Enzymatic degradation of zebularine occurs in both rat and human plasma, although the

Table 9.9 Pharmacokinetics of zebularine and THU.[214–218]

Compound	*CL (ml/min/kg)*	*PPB (%)*	*Volume (L/kg)*	*Oral F%*	*Fa (%)*	*Half-life (hours)*
Zebularine	63 m 14 m (100 mpk) 4 r 4–11 mk	43 m[b] 73 r[b] 34 d 42 mk 57 h	1.1 m 0.2–0.3 r 0.4-0.7 mk	7 m ~3–6 r <1 mk		0.7 m 6 r 1.2–2.5 mk
THU	9 m ~1.6 h	~0 m, h	0.9 m ~0.18 h	15–23 m 10-14 h	18–29 m[a] ~14 h[a]	1.2 m 1–1.5 r 1.1 d 1.2 mk 7.2 h

h = human, m = mouse, mk = monkey, r = rat.
[a]based on urinary recovery of radioactivity
[b]@ 1 μg ml^{-1}
mpk = mg per kg

Table 9.10 Metabolism of zebularine by aldehyde oxidase in hepatic cytosol from different species.[214,257]

Species	*Sex*	*Km (μM)*	*Vmax (nmol/min/mg protein)*	*Clint (μl/min/mg protein)*	*In vivo CL (ml/min/kg)*	*F%*
Mouse	M	102	11	108	14	7
	F	27	0.2	7		
Rat	M	11	0.9	81	4	3–6
	F	11	0.9	81		
Dog	M		No metabolic activity			
Monkey	M	15	4.6	307	6–10	<1
Human	M	7.3	2.1	288		
	F	8.4	2.7	321		

responsible enzymes were not identified.[214] The 2-hydroxypyrimidine base of zebularine is also a substrate for aldehyde oxidase (AO).[220,221]

The ability of liver cytosol from different species to metabolise zebularine has been studied *in vitro* (Table 9.10).[220] The intrinsic clearance of zebularine in liver cytosol was highest in monkey and human. Reasonably high levels of metabolism was also seen in the rat and male mouse liver cytosol with low levels of metabolism seen in the female mouse liver cytosol and no observable metabolism seen in the dog liver cytosol. AO is also known to be expressed in the intestine[222] and as such may play a role in intestinal first-pass metabolism (Fg) of zebularine limiting it's oral bioavailability.[214]

Quantitatively, AO was shown to convert 38% of an intravenous dose of [^{14}C]-zebularine in the mouse to uracil with another 30% of the dose excreted as unchanged drug in the urine *via* an active process ($CL_r > GFR$),[215,220] although the transporters involved have not been identified. Once zebularine is oxidised by AO, the radioactive material is further metabolised by endogenous metabolic pathways and a significant proportion of the dose is ultimately lost as expired $^{14}CO_2$.

The metabolism of zebularine *in vivo* proceeds as shown in Figure 9.19 with uridine, uracil and dihydrouracil identified as the primary metabolites of zebularine in mice.[215] There was no evidence of 2-pyrimidinone as a metabolite in either plasma or urine suggesting that, like cytidine, zebularine is not a substrate for uridine phosphorylase and must first be converted to uridine before the *N*-glycosidic bond can be cleaved.

Zebularine and THU have low to moderate volumes of distribution (Table 9.9)[214,216] suggesting the compounds do not distribute widely into tissues. Studies in mice with radiolabelled zebularine also showed a modest distribution of zebularine to tissues.[215] In keeping with the polar nature of zebularine, the compound showed minimal distribution into red blood cells[223] and fat cells, and the compound had low penetration of the blood–brain and the blood–testes barrier.[215]

One use of zebularine and THU is to prolong the activity of anticancer drugs that are substrates for CDA (*e.g.* Ara-C) and this aspect has been studied in

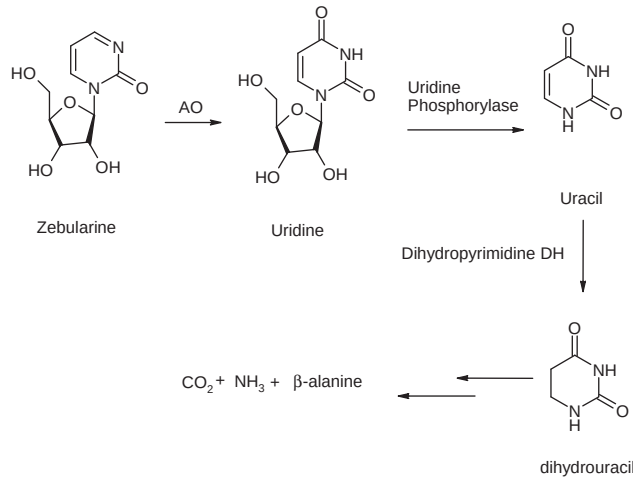


Figure 9.19 Metabolic conversion of zebularine to uridine by aldehyde oxidases.[220]

animals and humans. Zebularine increases (two-fold) the steady state plasma levels of 5-aza-2′-deoxycytidine when the compounds are co-infused in mice, whilst concomitant administration of THU markedly increases the myelosuppressive potency of Ara-C in monkeys.[224] In humans, simultaneous oral administration of THU (10–50 mg kg^{-1}) and Ara-C resulted in a two-fold increase of blood levels of Ara-C at all time points over a four hour period and a slight increase in the half-life of Ara-C. The inhibitory effect of THU on CDA metabolism of Ara-C was also reflected in a considerably increased ratio of THU/uracil arabinoside excretion in the urine.[225] Although there is a theoretical risk of toxicity from zebularine administration due to the large amount of uridine formed, in reality this does not seem to be an issue.[226–230]

9.3.5 Inosine 5-monophosphate Dehydrogenase TSAI

Inosine 5-monophosphate dehydrogenase (IMPDH) catalyses the conversion of inosine monophosphate to xanthine monophosphate with reduction of NAD; this is the rate limiting step in *de novo* guanine nucleotide biosynthesis. As cell proliferation is strongly linked to the size of the guanine nucleotide pool, IMPDH inhibition has emerged as an attractive therapeutic target.[231]

Mizoribine (formerly known as bredinin) is an imidazole nucleoside antibiotic (Figure 9.20) that is phosphorylated to produce mizoribine 5′-monophosphate, a transition state analogue inhibitor of IMPDH.[232] Mizoribine has been increasingly used in Japan since 1978 as a substitute to azathioprine for clinical renal transplantations.[231,233–236]

Mizoribine is a polar nucleoside analogue (clogP −1.4, TPSA 151 Å^2) and as such is expected to have limited passive membrane permeability. However,

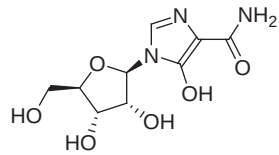


Figure 9.20 Chemical structure of mizoribine.

when used as a therapeutic in humans, mizoribine is administered orally and is almost completely absorbed.[237] In the presence of inosine, the absorption rate of mizoribine is significantly decreased[238] suggesting a role for the nucleoside transporter CNT_2/N1 (model substrates are inosine, guanosine and formycin-B) in the active absorption of mizoribine.[239]

As expected for a polar compound, renal clearance (21 mL min^{-1}) accounts for 56% of total clearance of mizoribine in the dog. Infusion of mizoribine into the renal artery showed that renal clearance in the dog decreases with increasing dose, indicating that the renal elimination pathway was a saturable transporter-mediated pathway. The half-life was longer in renal-transplanted animals than in healthy dogs, reflecting the better renal function in healthy animals.[240]

The pharmacokinetics of mizoribine has also been determined in healthy humans and in renal transplant patients.[237] Following oral doses (3–12 mg kg^{-1}) to healthy volunteers, the half-life was $\sim$3 h and CL/F was $\sim$3.2 ml min^{-1} kg^{-1}. Renal excretion of unchanged drug in to the urine accounted for 65–100% of the dose in humans. In renal transplant patients, there was significant variation in the terminal half-life and trough concentrations of mizoribine that were dependent on the degree of renal function of the individual.[241,242]

Mizoribine has a moderate volume of distribution in dogs (0.5 l kg^{-1}) and humans (0.8 l kg^{-1}) in keeping with its polar nature.[240,243] An oral study with ^{14}C-mizoribine (radioactivity Tmax = 1.5 h) in rats also showed moderate tissue distribution of radioactivity with high levels of radioactivity observed in the gastrointestinal tract, kidneys, liver, spleen and thymus.[258]

9.3.6 Aspartate Carbamyl Transferase TSAI

Phosphonoacetyl-L-acetate (PALA) inhibits aspartate carbamyl transferase (ACT) and blocks *de novo* synthesis of pyrimidines *in vitro* and *in vivo*.[244] Although cytotoxic to tumour cells *in vitro*, it was shown to be devoid of antitumour effect *in vivo* and more recently has been assessed for its ability to enhance the antitumour activity of 5-fluorouracil. PALA binds about 1000 times more potently to the enzyme (Ki 27 nM) than carbamyl phosphate for which it is a competitive inhibitor. However, in PALA the phosphate group is replaced by a C-linked phosphonate which cannot act as a leaving group (Figure 9.21) and PALA may act as a multi-substrate analogue rather than as a true TSAI.[245]

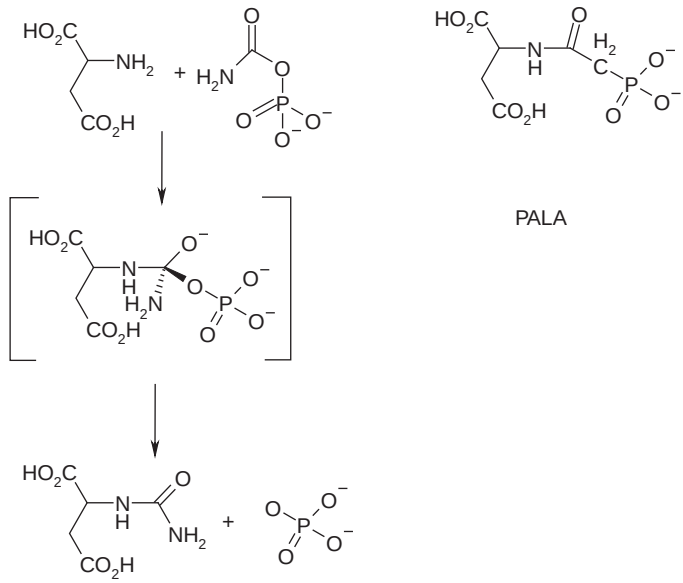


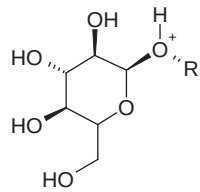
Figure 9.21 Reaction catalysed by aspartate carbamyl transferase, the proposed transition state and the structure of PALA.

As would be expected for such a polar compound (clogP −2.2, clogD −8.9), the pharmacokinetics in animals and humans are characterised by poor oral bioavailability, elimination unchanged by the kidney, little metabolism and distribution into extracellular water with little tissue affinity.[244] In humans, clearance was $\sim 1.6\,\mathrm{ml\,min^{-1}\,kg^{-1}}$ and renal excretion of unchanged drug was 80–85%. The renal CL of PALA approximates to the glomerular filtration rate, suggesting that CL may be by passive filtration with no reabsorption (though equivalent rates of active renal secretion and reabsorption can not be ruled out). The volume of distribution was $\sim 0.3\,\mathrm{L\,kg^{-1}}$ reflecting the limited distribution of PALA into tissues and the half-life in man was 5–12 h.

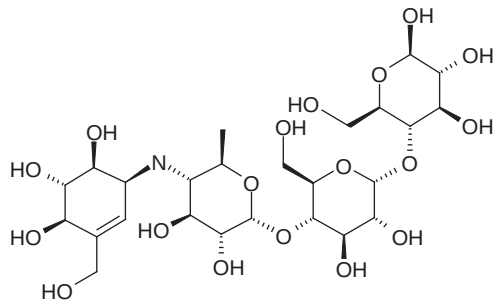
9.3.7 Glycosidase Inhibitor TSAI

Acarbose is a polar (clog D −4.2), high molecular weight (646) potent inhibitor of α-glycosidase inhibitor. Structurally, acarbose has an amine group in the position of the protonated exocyclic oxygen in the transition state (Figure 9.22). As expected from its complex polysaccharide structure, acarbose is a very polar compound (PSA 329 $\mathrm{Å}^2$) with high solubility. Acarbose has 19 hydrogen bond acceptors and 14 hydrogen bond donor groups.

The main site of action of acarbose is in the luminal side of the intestine and therefore absorption and systemic exposure of acarbose is not needed for its efficacy, which is just as well as the reported bioavailability in humans for



Putative transition state with
protonated exocyclic oxygen



Acarbose

Figure 9.22 Chemical structure of acarbose and the putative transition state of the glycosidase enzyme.

acarbose is 0.5–1.7% and across a number of pre-clinical species absorption ranged from 1–4%.

Following intravenous administration to humans, Vss is $0.32\,L\,kg^{-1}$ and a similar Vss is seen in other species. This suggests that acarbose has limited distribution beyond the extracellular fluid volume. As would be expected for such a polar compound, protein binding of acarbose is negligible.

Acarbose undergoes significant degradation in the gut due to hydrolysis of the glucosidic linkages. It is likely that the gut microflora plays a role in this metabolism. When dosed intravenously, acarbose is predominantly excreted unchanged in the urine (79% rat, 95% dog and 94% human). The clearance of acarbose is about $2\,mL\,min^{-1}\,kg^{-1}$ in humans, which is similar to GFR.[21–23,246,4]

9.4 Conclusions

In general, the group of transition state analogues discussed in this chapter have a relatively high PSA, particularly when this value is compared with the PSA value of the top 200 selling drugs. This high polar surface area imparts a number of pharmacokinetic properties to this group of compounds.

Compounds with low clog P and high TPSA typically had poor membrane permeability and low transcellular oral absorption. This has led to a number of

compounds either being dosed as prodrugs or being given by other routes such as intravenous, subcutaneous or inhaled administration. Even when this type of transition state analogue compound shows good absorption and high bioavailability, this can often be attributed to paracellular or transport-mediated absorption rather than to good transcellular permeability.

One strategy to improve oral absorption of polar transition state analogues is to increase clogP significantly and there are examples such as the HIV protease inhibitors where the compounds have both high clogP and high TPSA yet still show good absorption. The downside is that the extra lipophilicity added to increase absorption also makes this class of compounds susceptible to rapid metabolic clearance and frequently F% is low because the higher absorption is counteracted by higher Cl and first-pass extraction.

Other properties of this group of compounds such as clearance and volume of distribution are as would be expected from their physicochemical properties. Polar compounds tend to have a low volume of distribution and are often excreted unchanged into the urine. More lipophilic compounds have relatively higher volumes of distribution and tend to be rapidly cleared by metabolism. In some cases (*e.g.* boronic acid and activated ketones), the metabolism involves the functional groups mimicking the transition states whilst in many other compounds the metabolism occurs on the molecular scaffold.

The high PSA of these compounds also suggests that attempting to inhibit central (behind blood–brain barrier) targets with TSAI will be challenging. The affinity of many of the compounds to P-glycoprotein will exacerbate the challenge of inhibiting central targets.

Striking a balance between absorption and clearance is the main challenge for scientists looking to develop transition state analogues as therapeutic agents. However, the high potency (and often slow offset from the target) of these compounds means that, if the balance between absorption and clearance can be achieved, useful therapeutic agents can be discovered.

9.5 Abbreviations

ACE	angiotensin converting enzyme
ACT	aspartate carbamyl transferase
ADA	adenosine deaminase
ADME	absorption, distribution, metabolism and excretion
ANG I	angiotensin I
ANG II	angiotensin II
AO	aldehyde oxidase
Ara-C	cytosine arabinoside
BP	blood : plasma ratio
Cav_{0-t}	average concentration from 0 to time t
CDA	cytidine deaminase
CL_h	hepatic blood clearance
CNS	central nervous system
CSF	cerebrospinal fluid

CYP	cytochrome P450
DDI	drug–drug interaction
DPP-IV	dipeptidyl peptidase IV
F	bioavailability
Fa	fraction absorbed
Fg	fraction escaping first-pass metabolism in the gastrointestinal tract
Fh	fraction escaping first-pass clearance in the liver
GFR	glomerular filtration rate
HBA	hydrogen bond acceptor
HBD	hydrogen bond donor
HCV	Hepatitis C virus
HIV	human immunodeficiency virus
IMPDH	inosine 5-monophosphate dehydrogenase
MTAP	methylthioadenosine phosphorylase
OATP	organic anion polypeptide
P-gp	P-glycoprotein
PALA	phosphonoacetyl-L-acetate
PNP	purine nucleoside phosphorylase
Q	hepatic blood flow
THU	3,4,5,6-terahydrouridine
TPSA	topological polar absorption area
TSAI(s)	transition state analogue inhibitors(s)
V_{ss}	steady state volume of distribution

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CHAPTER 10

Alcohols and Phenols: Absorption, Distribution, Metabolism and Excretion

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10.1 Physicochemical Properties of Alcohols and Phenols and their Prevalence in Drugs

Many drugs contain a hydroxyl group (aliphatic alcohols and phenols). While contributing to the polarity and hydrophilicity of a molecule, the addition of a hydroxyl group (-OH) does not alter (in almost all cases) the charge of a molecule at physiological pH. The pKa of simple aliphatic alcohols is greater than 14 and for phenols is typically in the range of 9–12. Thus, in drug design, an alcohol group can offer a moderate increase in hydrophilicity without altering the charge. For phenols of rings that possess highly electron deficient substituents (*e.g.* nitro), the pKa of the hydroxyl group can be in the range of physiological pH, thus causing a significant fraction of the drug to be anionic in the body. And, in some cases, if the deprotonated anionic form can be resonance stabilised (*e.g.* warfarin, meloxicam, *etc.*), the hydroxyl group can be nearly as acidic as a carboxylic acid.

The hydroxyl group possesses simultaneously a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), which can provide binding energy to macromolecule targets and increase affinity as well as increase water

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solubility. Also, by decreasing lipophilicity, the addition of a hydroxyl group will generally decrease tissue partitioning. The increase in hydrophilicity and hydrogen-bonding capacity can also decrease membrane penetration. If not oriented toward direct interaction with substituents on the macromolecule target, a hydroxyl group can reside in solvent-exposed space. With these diverse capabilities, the hydroxyl group offers the medicinal chemist a structural modification that can substantially alter the behaviour of a molecule at the target and in the body (Table 10.1).

The presence of the hydroxyl group in drugs is high, with 40 of the 150 most frequently prescribed medications containing an alcohol or phenol function, most of which are taken orally (Table 10.2). These drugs span a range of indications including those which require penetration into the central nervous system (CNS), supporting the notion that a hydroxyl group does not preclude penetration across membranes or the blood–brain barrier.

Table 10.1 Average physicochemical properties for a set of fifteen drugs and their hydroxy analogies.

Property	*Without -OH*	*With -OH*
log P	2.7	1.9
log $D_{7.4}$	1.3	0.6
PSA ($Å^2$)	39	60
Molecular weight	237	253
HBD + HBA	4	6

Table 10.2 Prevalence of drugs containing an OH group among the top 150 most frequently prescribed drugs.

Drug	*Indication*	*Drug*	*Indication*
Acetaminophen	Analgesic/Antipyretic	Lorazepam	Anxiolytic
Albuterol	Anti-asthmatic	Losartan	Antihypertensive
Alendronate	Anti-osteoperotic	Lovastatin	Anti-atherosclerotic
Amoxicillin	Antibacterial	Meloxicam	Anti-inflammatory
Aspirin	Analgesic/antipyretic	Methylprednisolone	Anti-inflammatory
Atenolol	Antihypertensive	Metoprolol	Antihypertensive
Atorvastatin	Anti-atherosclerotic	Metronidazole	Antifungal
Azithromycin	Antibacterial	Montelukast	Anti-asthmatic
Carvedilol	Antihypertensive	Morphine	Narcotic analgesic
Clarithromycin	Antibacterial	Olmesartan	Antihypertensive
Clindamycin	Antibacterial	Oxycodone	Narcotic analgesic
Codeine	Narcotic analgesic	Prednisone	Anti-inflammatory
Digoxin	Anti-arrythmic	Propranolol	Antihypertensive
Doxycycline	Antibacterial	Quetiapine	Antipsychotic
Ethinyl estradiol	Oral contraceptive	Rosuvastatin	Anti-atherosclerotic
Ezetimibe	Anti-atherosclerotic	Salmeterol	Anti-asthmatic
Fexofenadine	Antihistamine	Simvastatin	Anti-atherosclerotic
Fluticasone	Anti-inflammatory	Tramadol	Narcotic analgesic
Hydroxyzine	Antihistamine	Venlafaxine	Antidepressant
Levothyroxine	Hypothroidism	Warfarin	Anticoagulant

Introduction of a hydroxyl group by oxidative metabolism is extremely common and, in many cases, the hydroxyl metabolite can possess pharmacological activity similar to the parent drug—albeit that in many cases the intrinsic potency is altered. In such cases, the absorption, distribution, metabolism and excretion (ADME) properties of the metabolites have been explored to varying degrees and compared to that of the parent drug, either to ensure an understanding of the contribution of the metabolite to drug effect or to explore the possibility that the metabolite itself could be pharmacologically active. There are, therefore, a number of reports in the scientific literature in which the ADME properties of a structure with and without a hydroxyl group have been directly compared. From these reports, the general impact of hydroxyl on ADME properties can be derived. These cases are included in this chapter for illustration purposes.

10.2 Comparative Pharmacokinetics of Alcohols, Phenols and their Counterparts Lacking the Hydroxy Group

The pharmacokinetic parameters, clearance (CL) and volume of distribution (VD), are the most important ones that help define the dosing regimen of a drug. A comparison of these parameters for analogues lacking or possessing a hydroxyl group is given in Table 10.3.

The addition of a hydroxyl group can either increase or decrease total CL; in the examples listed, the split between an increase and a decrease is 50/50. Clearance is a function of intrinsic clearance (*i.e.* the rate at which drug metabolising enzymes and transporters can act upon the drug) and the free fraction.

Addition of hydroxyl group can cause the compound to be a substrate for conjugating enzymes [*e.g.* UDP-glucuronosyltransferase (UGT), sulfotransferase (SULT)] or other oxidative enzymes (*e.g.* alcohol dehydrogenase, peroxidases); they may also impart greater substrate properties for some drug transport proteins. However, it is important to note that the addition of hydroxyl can cause the free fraction in plasma to increase—in some cases substantially. Thus, free clearance for hydroxyl analogues is lower than their corresponding analogues (Table 10.4). Since it is unbound exposure that is important for drug effect, the addition of a hydroxyl group is likely to lead to an improvement, even if the total CL appears to increase (provided that intrinsic target potency is retained as well as the ability of the hydroxyl containing analogue to penetrate the target tissue).

Addition of a hydroxyl group can yield substantial changes in the volume of distribution. In many cases, it can decrease markedly (*e.g.* imipramine *vs.* 2-hydroxyimipramine, maprotiline *vs.* oxaprotiline; Table 10.3). VD is a function of the relative partitioning of compounds between the central compartment (*i.e.* plasma) and the peripheral tissues. This partitioning is a function of the relative non-specific binding capacity of macromolecular entities in tissues compared with proteins in plasma that bind drugs (*e.g.* albumin, alpha-1-acid glycoprotein). In general, compounds that are lipophilic tend to partition more extensively into tissues and have relatively higher VD values.[1] The addition of a

Table 10.3 Comparison of the parenteral pharmacokinetics of compounds possessing or lacking a hydroxy group.

Compound lacking -OH	*Compound possessing -OH*	*Species*	*CL (mL/min/kg body weight)*		*VD (L/kg body weight)*		*Half-life (h)*	
			–OH	*+OH*	*–OH*	*+OH*	*–OH*	*+OH*
Buspirone	6-Hydroxybuspirone	Rat	69	47	2.5	2.6	0.9	1.2
Dantrolene	5-Hydroxydantrolene	Dog	14	17	1.2	0.9	1.1	0.6
Glimiperide	Hydroxglimepiride	Human	0.5	1.1	0.19	NF	10	1.2
Maprotiline	Oxiprotiline	Human	14	13	45	19	51	19
Diazepam	Temazepam	Rat	82	45	4.5	4.6	1.1	4.0
Ezlopitant	Hydroxyezlopitant	Rat	65	53	22	8.9	7.7	1.8
Ezlopitant	Hydroxyezlopitant	Dog	27	24	5.6	3.2	2.5	1.7
Cotinine	3′-Hydroxycotinine	Human	0.89	1.8	1.1	0.85	17	5.9
Imipramine	2-Hydroximipramine	Human	13	9.2	12	6.6	16	10
Nordiazepam	Oxazepam	Human	0.12	1.1	0.45	0.59	46	6.7
Chloroquine	Hydroxchloroquine	Human	4.1	11	140	700	570	850
Daunorubicin	Adriamycin	Dog	210	51	26	7.2	22	26
Tetracycline	Oxytetracycline	Human	1.5	2.0	1.2	1.7	9.4	10
Amphetamine	Phenylpropanolamine	Dog	8.0	9.4	2.7	4.3	4.5	6.0
Amphetamine	Phenylpropanolamine	Rat	58	25	4.1	5.5	1.1	2.2
Amphetamine	*p*-Hydroxyamphetamine	Rat	58	88	4.1	3.8	1.1	0.66
Limonene	Perillyl alcohol	Rat	50	22	12	1.7	4.7	1.4

Table 10.4 Comparison of the parenteral pharmacokinetic parameters corrected for plasma protein binding of compounds possessing or lacking a hydroxy group.

		CL_u (mL/min/kg body weight)		VD_u (L/kg body weight)	
Drug pair	*Species*	*–OH*	*+OH*	*–OH*	*+OH*
Maprotiline/Oxaprotiline	Human	140	76	450	110
Ezlopitant/Hydroxyezlopitant	Rat	3000	230	1000	39
Ezlopitant/Hydroxyezlopitant	Dog	3000	130	620	17
Imipramine/2-Hydroxyimipramine	Human	170	26	160	18
Nordiazepam/Oxazepam	Human	4.2	28	15	15
Chloroquine/Hydroxychloroquine	Human	10	19	330	1200
Tetracycline/Oxytetracycline	Human	1.9	2.2	1.5	1.9
Amphetamine/Phenylpropanolamine	Rat	97	42	6.8	9.2

CL_u = free clearance; VD_u = free volume distribution.

hydroxyl group will decrease lipophilicity and increase PSA (Table 10.1), and thus it is expected that tissue distribution will decrease and plasma protein binding will also decrease. Hence, the impact on VD will depend on the relative effect that addition of the hydroxyl will have on these two opposing properties. After correcting for differences in plasma protein binding for drugs and their hydroxyl containing analogues, the free VD values for the hydroxyl containing set are generally lower than their non-hydroxyl counterparts. This reflects the notion that addition of hydroxyl will decrease tissue partitioning (Table 10.4).

CL and VD determine the half-life ($t_{1/2}$). Since the addition of a hydroxyl group can impact both these properties, one may not be able to necessarily predict the impact on half-life when a hydroxyl group is added during drug design. The addition of the hydroxyl group could direct a compound to drug metabolising enzymes that ordinarily would not act upon the parent, while at the same time decreasing the rate of metabolism by P450 enzymes. For any new chemical scaffold being used in drug design efforts, this determination needs to be made experimentally, although there are increasingly improving computational approaches to predicting VD and plasma protein binding, as well as interactions with drug-metabolising enzymes. The impact of a hydroxyl group on $t_{1/2}$ for a number of drugs is listed in Table 10.3. Comparisons of CL, VD and $t_{1/2}$ for hydroxyl containing compounds and their counterparts are shown in Figure 10.1. It should be noted that, while the impact on CL and VD is varied, the addition of a hydroxyl more often leads to a decrease in $t_{1/2}$.

10.3 Biochemical Determinants of ADME Characteristics of Drugs Possessing Hydroxyl Groups

The thousands of interactions that occur between a small molecule drug and macromolecular structures within the body are what determine the

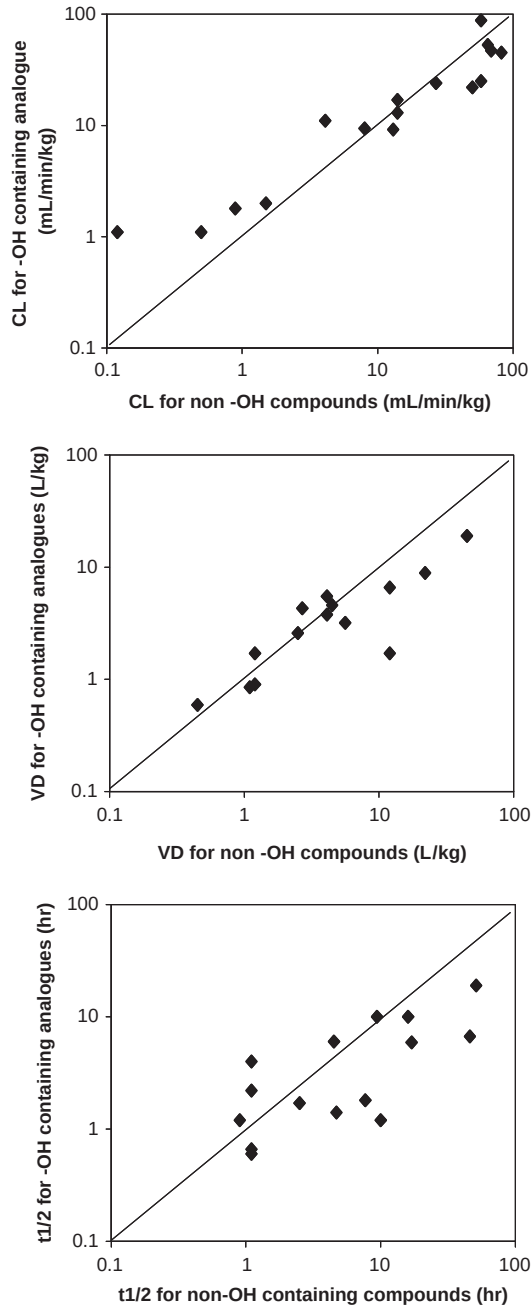


Figure 10.1 Comparison of CL, VD, and $t_{1/.2}$ for compounds possessing and lacking a hydroxyl group. The median CL for the OH-containing is approximately the same as for the non-OH counterparts, albeit there may be a trend for high CL *vs*. low CL compound pairs. For VD, the median value for the OH-containing compounds is 85% of the value for the non-OH compounds. For half-life, the median value for the OH-containing compounds is 60% of that of the non-OH counterparts.

pharmacokinetic characteristics of that compound and, together with the specifics regarding the interaction of that drug and its intended target, will determine its overall efficacy and usefulness for treating disease. Thus, during the drug design process, consideration of the potential impact of the addition or removal of a hydroxyl group on these interactions is important. The questions that need to be asked include:

- Will the addition/removal of the hydroxyl result in increased or decreased potency at the target? (This is a case-by-case consideration for each pharmacological target and is beyond the scope of this chapter.)
- Will addition/removal of the hydroxyl group alter the free fraction?
- Will the addition/removal of the hydroxyl group increase or decrease non-specific binding in tissues?
- Will the addition/removal of the hydroxyl group increase or decrease the ability of the molecule to penetrate membranes?
- Will the addition/removal of the hydroxyl group alter interaction(s) with membrane transporters?
- What impact will the addition/removal of the hydroxyl group have on interactions with various drug-metabolising enzymes, and will this affect the rate and routes of metabolic clearance?

Based on our level of understanding, these questions can be addressed to varying degrees of satisfaction.

10.3.1 Plasma Protein Binding and Tissue Distribution

In general, designing drugs with the intent of altering plasma protein binding alone is not a fruitful venture, as increases in free fraction will not necessarily yield higher free concentrations since clearance will also increase. However, knowledge of the plasma protein binding value is critical to understanding *in vivo* disposition and linking that to *in vitro* data. (For example, relating *in vivo* efficacious concentrations to receptor binding affinity requires knowledge of the free fraction. In addition, understanding whether a drug can freely distribute to target tissues or whether there is a drug transporter altering this equilibrium requires knowledge of the free fraction in plasma.) Decreases in plasma protein binding can also lead to lower dose requirements, provided target affinity is unchanged.

Comparisons of plasma protein binding of compounds and their analogues possessing a single additional hydroxyl group are listed in Table 10.5. For this set of ten drugs, it can be readily seen that free fraction either remains the same or increases (considerably in some cases) when an hydroxyl is present in the molecule. Typical increases in free fraction are around two-fold. For the highly bound compounds, two example sets (both benzodiazepines) did not show a difference in protein binding between the hydroxyl containing and hydroxyl lacking analogues; however, for two others (imipramine and ezlopitant) the

Table 10.5 Comparison of the plasma protein binding of compounds possessing or lacking a hydroxy group.

Drug pair	*Species*	*Free fraction*	
		–OH	*+OH*
Tacrine/Velnacrine	Human	0.25	0.50
Diazepam/Temazepam	Human	0.02	0.02
Maprotiline/Oxaprotiline	Human	0.10	0.17
Imipramine/2-Hydroxyimipramine	Human	0.075	0.36
Nordiazepam/Oxazepam	Human	0.03	0.04
Chloroquine/Hydroxychloroquine	Human	0.43	0.57
Tetracycline/Oxytetracycline	Human	0.78	0.90
Ezlopitant/Hydroxyezlopitant	Rat	0.022	0.23
	Dog	0.009	0.19
Risperidone/Paliperidone	Human	0.10	0.23
	Dog	0.08	0.20
	Rat	0.12	0.25
Amphetamine/Phenylpropanolamine	Rat	0.60	0.60
	Human	0.84	0.45

addition of an hydroxyl yielded marked changes in free fraction. Obviously, for compounds that are not highly bound, the hydroxyl containing and hydroxyl lacking analogues will show little difference. The list of examples contains drugs bound to albumin and alpha-1-acid glycoprotein.

Studies on tissue distribution of hydroxyl and non-hydroxy analogues are rare, but an excellent demonstration of the role hydroxyl can play in tissue partitioning is exemplified for a series of progesterone analogues. Using lung tissue, the partitioning of progesterone, 11β-hydroxy, 11β,21-dihydroxy, and 11β,17α,21-trihydroxy was measured and shown to be directly related to the number of hydroxyl groups, with partition coefficients decreasing from 22, 3.7, 1.7, and 0.86, respectively.[2] Since these analogues are unionised at physiological pH, the change in tissue partitioning can be concluded to be driven solely by the lipophilicity decreases that each successive hydroxyl group imparts to the structure.

10.3.2 Interactions of Hydroxyl Group Containing Drugs with Drug Transporters and Impact on Absorption, Distribution and Excretion

One of the most compelling examples of a comparison of two compounds that differ by a single hydroxyl group is risperidone *vs.* its metabolite 9-hydroxyrisperidone (paliperidone) as a result to the desire to better understand the relative contributions of these two entities to the effects of risperidone. Tissue distribution of these two compounds was compared in the rat after administration of risperidone.[3,4] The total brain exposures were similar between the two compounds, but the total plasma exposure to the hydroxyl metabolite was over four-fold greater (hence the free plasma exposure is over eight-fold greater), indicating that the brain–plasma ratio was

greater for the non-hydroxyl parent compound. Distribution within specific brain regions also differed.[5] Other tissue–plasma ratios did not differ as much. It was shown that, while both risperidone and 9-hydroxyrisperidone are substrates for P-glycoprotein (an important efflux transporter at the blood–brain barrier), the 9-hydroxy metabolite was a better substrate and had a greater impact.[6–8] Similarly, for ezlopitant metabolites, it was shown that the cerebrospinal fluid (CSF) to free plasma ratio in subarachnoid catheterised dogs was much greater for a non-hydroxyl metabolite *vs*. a benzyl alcohol metabolite despite the fact that the benzyl alcohol metabolite was present in much greater concentrations in plasma.[9] The 7-hydroxy metabolite of methotrexate was considerably less distributed to tissues in the rat relative to the parent drug.[10]

10.3.3 Metabolism and Interaction with Drug-metabolising Enzymes

The metabolism of hydroxy groups in drugs includes both oxidative and conjugative reactions. Primary alcohols are oxidised to aldehydes, which are usually further oxidised to carboxylic acids. Secondary alcohols are oxidised to ketones. In addition to these oxidative pathways, alcohols also can undergo conjugative metabolism. The enzymes catalysing the reaction from alcohol to aldehyde or ketone metabolites include alcohol dehydrogenases, cytochrome P450s (*e.g*. CYP2E1), and prostaglandin H synthease. Phenols undergo conjugative metabolism including glucuronidation, sulfation and methylation. In general, glucuronidation and sulfation have greater impacts on the pharmacokinetics and bioavailability of hydroxyl containing drugs than oxidative metabolism or methylation due to the facile elimination and enterohepatic circulation of conjugates.

Both oxidative and conjugative metabolism of the hydroxy group has the potential to generate reactive metabolites. The oxidation of alcohols yields aldehydes which can covalently bond to macromolecules and potentially cause toxicity. Many phenol drugs and catechols can form quinoid structures that can react with glutathione (GSH) (a detoxication pathway) or nucleophilic groups on proteins and nucleic acids. Sulfation of benzylic and allylic alcohols has been documented as a mechanism of bioactivation of alcohol drugs. These are discussed in greater detail below.

10.3.3.1 Enzymes of Oxidative Metabolism

10.3.3.1.1 Alcohol Dehydrogenase (ADH). Alcohol dehydrogenases are a family of zinc-containing isozymes that catalyse the reversible oxidation of alcohols to their corresponding ketones or aldehydes using NAD^+ or $NADP^+$ as cofactors. (The reverse reaction uses the reduced cofactors.) Mammalian ADHs are located almost exclusively in the cytoplasm of cells. They have been categorised into six classes, of which the first five have been identified in humans;[11,12]

ADH-1 is the best known among the classes and is the enzyme mainly responsible the metabolism of ethanol. ADH-1 is widely distributed in human tissues, with the greatest abundance in liver. Class II ADH activity has been found mainly in liver and shows a high K_M value for ethanol. Class III ADH is a poor ethanol dehydrogenase, but has been proposed to play a significant role in cytoprotection by metabolism of formaldehyde. Class IV is extra-hepatically expressed with high ethanol metabolising activity in stomach mucosa.[13] Class V ADH transcripts were found to be at significantly higher levels in fetal liver compared with adult liver, suggesting that human class V is a predominant fetal alcohol dehydrogenase.[13] Several polymorphisms in genes coding for alcohol dehydrogenases have been well characterised. Although ADHs are important in the metabolism of ethanol, susceptibility to alcoholism and alcoholic liver disease, they are generally not of great importance in the metabolism of commonly used drugs.[14] ADHs have broad substrate specificities; however, the class I isozymes are mostly responsible for the oxidation of ethanol and other small, aliphatic alcohols. Class II and Class III enzymes preferentially oxidise aromatic or medium to long chain alkyl chain aliphatic alcohols.

Endogenous compounds metabolised by ADH include steroids and retinol. A number of drugs are metabolised by ADH. The primary alcohols are oxidised to aldehydes which usually are further oxidised to corresponding carboxylic acids. The subsequent oxidation of aldehyde to carboxylic acid is facile and often involves other enzymes such as aldehyde dehydrogenase; in some cases, CYPs are also involved. Therefore, it is difficult to establish the identity of the particular alcohol-oxidising enzyme *in vivo*. ADHs appear to be involved in humans in the metabolism of ethambutol and celecoxib.[15,16] In both cases, the end products are carboxylic acids.[17] In the metabolism of the reverse transcriptase inhibitor abacavir,[18] ADH1A is able to carry out not only the step from abacavir (an alcohol) to the corresponding aldehyde, but also the second step from the aldehyde to the acid (Figure 10.2). Although secondary alcohols are susceptible to oxidation to their corresponding ketones, this reaction is not often as important in drug metabolism because the reverse reaction occurs readily and also because they can form glucuronides that are rapidly eliminated.

10.3.3.1.2 Cytochrome P450. Cytochrome P450s (CYPs) also play a role in the metabolism of hydroxy groups in drugs as well as ethanol. The microsomal oxidation of ethanol has been the subject of extensive research since its discovery in the 1970s.[19] Recently, it has been demonstrated that most CYP isoforms (except CYP2A6 and CYP2C18) are capable of oxidising ethanol to acetaldehyde with K_M value around 10 mM.[20] Cytochrome P4502E1 (CYP2E1), which is induced by chronic ethanol consumption, was found to have a prevailing role in this oxidation. P450s are also involved in the metabolism of many hydroxy containing drugs. At the normal therapeutic doses, acetaminophen is safe; its major metabolic pathways include glucuronidation and sulfation, and only small amounts of the reactive intermediate, a quinonimine, are formed by the CYP enzymes (see Chapter 6,

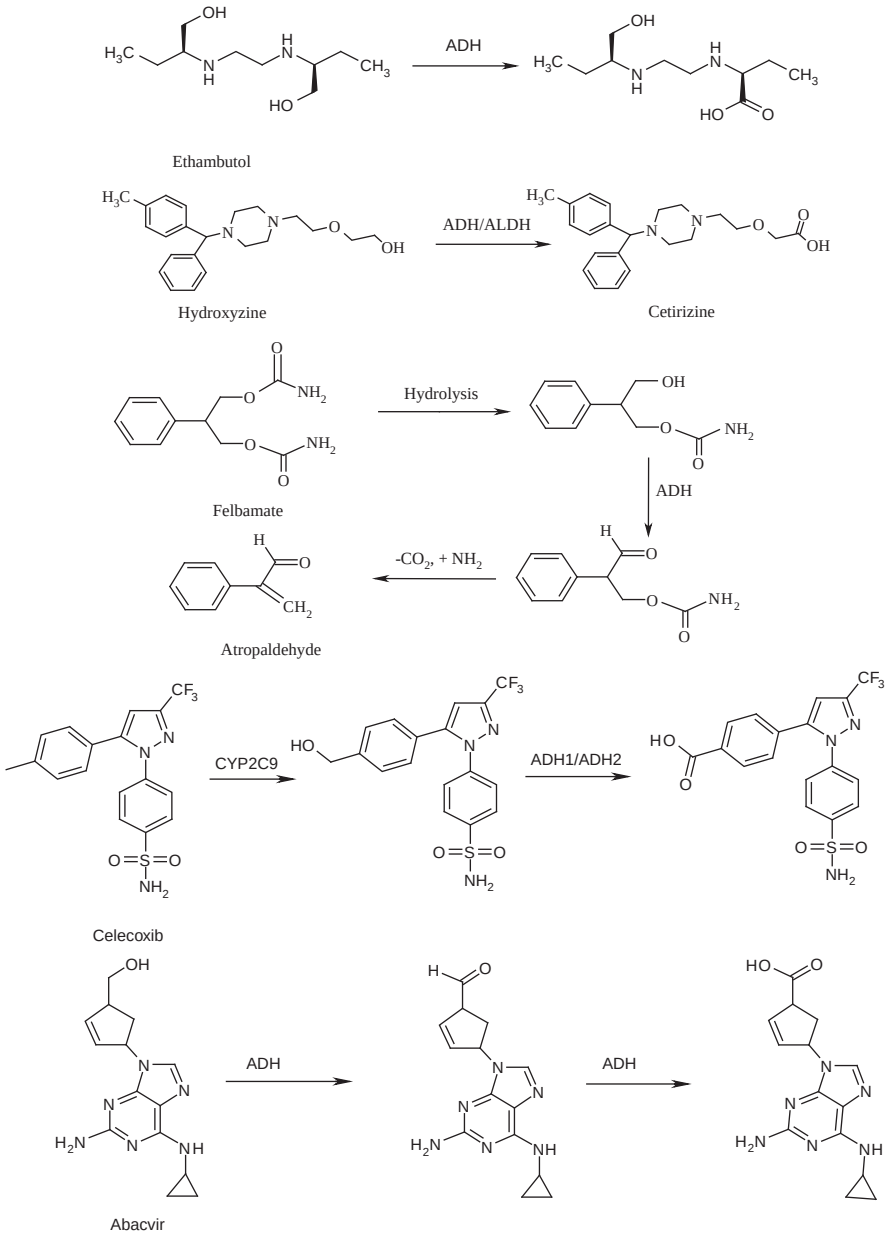


Figure 10.2 Hydroxy containing drugs metabolised by ADH.

section 2). Among several CYP isoforms including CYP3A4, CYP1A2 and CYP2E1, CYP2E1 was found to play a major role in the bioactivation of acetaminophen causing hepatotoxicity when large doses are ingested.[21,22]

P450 enzymes are also frequently involved in the further metabolism of the intermediary hydroxyl metabolites also generated by these enzymes. For example, the antihistamine, terfenadine, is sequentially oxidised by CYP3A4 to an alcohol metabolite that is converted into the circulating active metabolite, fexofenadine, which is a carboxylic acid. Losartan, an angiotensin II antagonist possessing a hydroxymethyl group, is metabolised by CYP2C9 to the corresponding aldehyde and carboxylic acid metabolites (the latter is referred to as E-3174),[23,24] with the acid metabolite being largely responsible for efficacy.[25] These findings have been verified *in vivo*, with marked differences in the urinary ratios of losartan/E-3174 across populations of different CYP2C9 genotypes.[26–28] Possession of one *3 allele results in 2–3 times lower plasma exposure to E-3174 and *3 homozygous individuals have very markedly lower exposure to E-3174, consistent with the diminished activity of CYP2C9*3 to convert losartan to E-3174, but not as much of a difference in losartan exposure. It is likely that the carboxylic acid metabolite contributes to the activity since the blood pressure lowering ability of losartan is diminished in patients possessing a *3 allele.[29,30] From the examples of terfenadine and losartan, it is clear that alcohol containing drugs could yield pharmacologically active carboxylic acid metabolites.

10.3.3.1.3 Prostaglandin H Synthetase. PGHS is a haem-containing protein with two enzymatic activities: a cyclooxygenase (COX) and a peroxidase. To date, two structurally related isoforms of PGHS have been identified, COX-1 and -2. Both isoforms participate in the synthesis of important biological mediators called prostanoids (including prostaglandins, prostacyclin and thromboxane) [PHS-1]. During the synthesis of prostaglandin H2 (PGH2), the precursor of the series-2 prostanoids, COX catalyses bioxygenation of free fatty acid substrates and converts arachidonate to PGG2 (endoperoxide–hydroperoxide); and the peroxidase activity reduces this intermediate to the corresponding alcohol (PGH2) in the presence of reducing co-substrates. Since phenol and hydroquinone are good reducing co-substrates as electron donors for the peroxidase of PGHS,[31] these phenolic compounds undergo one electron oxygenation to form reactive species which can bind to macromolecules (PGHS-2).

The PGHS-catalysed oxidation of paracetamol is an example. The one-electron oxidation product *N*-acetyl-*p*-benzo-semiquinone imine may be further oxidised to *N*-acetyl-*p*-quinoneimine (NAPQI) or dimerise; it can also undergo disproportionation, forming paracetamol and NAPQI. PGHS can catalyse both one- and two-electron oxidations of paracetamol. In contrast, CYP metabolism occurs by two-electron oxidation and did not produce oligomers, but instead NAPQI (Figure 10.3).

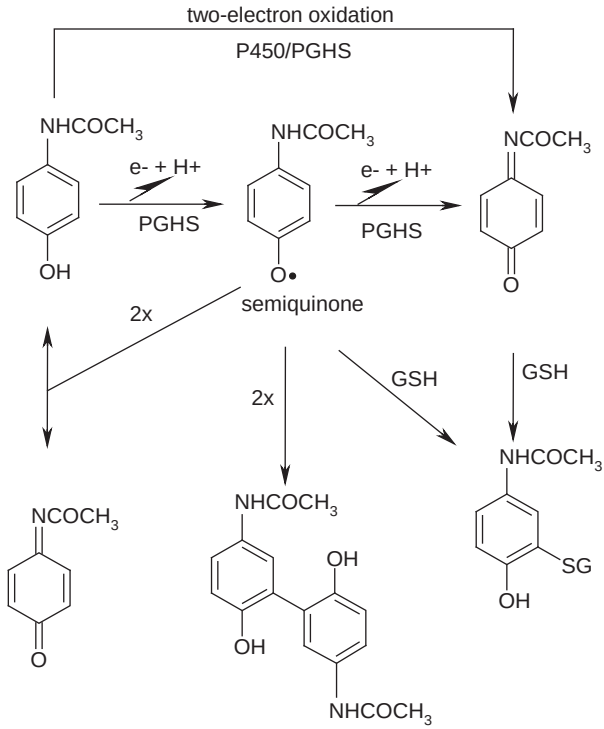


Figure 10.3 Bioactivation of acetaminophen by PGHS.

10.3.3.2 Conjugative Metabolism of Hydroxy Containing Drugs

Compared with oxidative reactions, conjugative metabolic reactions such as glucuronidation and sulfation have a greater prevalence in the metabolism of alcohol containing drugs. Furthermore, the impact on the pharmacological activity and ADME properties of the drug are substantially altered following conjugation. In most cases, the products of phase II metabolism are not pharmacologically active and are readily eliminated due to their greatly increased polarity.

Many hydroxy containing drugs have excellent oral absorption, but due to high 'first-pass' effects largely attributable to phase II metabolism, their oral bioavailability can be poor. For example, the analgesic drug meptazinol (Figure 10.4) is rapidly and completely absorbed after oral and intramuscular administration.[32] However, the absolute bioavailability of the drug following oral dosage is low (4.5–8.7%), whereas the drug is totally systemic available after intramuscular (i.m.) dosage. The elimination of the drug proceeds rapidly ($t_{1/2} = \sim 2h$), largely due to glucuronidation and sulfation of the phenolic function in the molecule. Following oral administration of ^{3}H-labelled drug, 90% of the radioactivity was excreted through urine where no parent drug was detected.[33] The highly efficient phase II metabolism of meptazinol can be

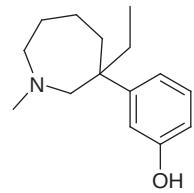


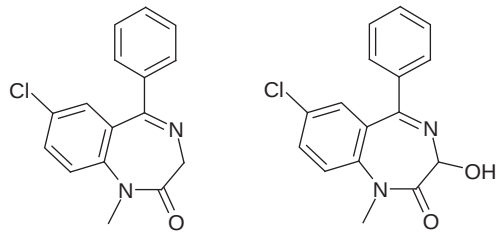
Figure 10.4 Structure of meptazinol.

blocked by making benzoyl esters as prodrugs to improve oral bioavailability.[34] Many dietary flavonoids and other polyphenols also exhibit poor oral bioavailability, mainly due to highly efficient glucuronic acid and sulfate conjugation of these mono- or polyhydroxylated agents. Methyl capping of all free hydroxyl groups of flavones results in dramatically increased metabolic stability, as the metabolism is shifted to less efficient CYP-mediated oxidation.[35] When hydroxy containing drugs undergo extensive glucuronidation and the glucuronic acid conjugates are biliarily excreted, enterohepatic circulation can occur and cause prolonged drug exposure and multiple peaks in plasma concentration *vs*. time profiles.

10.3.3.2.1 Uridine 5′-diphospho-glucuronosyltransferase (UGT). UGTs are one of the most important drug metabolising enzyme families. They catalyse the addition of glucuronic acid from UDP-glucuronic acid (UDPGA) to a multitude of endobiotic and xenobiotic compounds.[36] The products of glucuronidation are generally more polar, less toxic and more easily excreted from the body through bile or urine. UGTs have a wide tissue distribution with the liver possessing the highest level of activity. UGT enzymes are subdivided into two families designated as UGT1A and UGT2A/UGT2B.[37] To date, more than 60 different UGT enzymes have been isolated in several mammalian species.[38] These transferases exhibit distinct but overlapping substrate specificities and are known to catalyse the glucuronidation of a variety of alcoholic and phenolic drugs.

Glucuronidation has a profound impact on the ADME properties of hydroxy containing drugs. This can be illustrated with diazepam and temazepam (Figure 10.5). They both have common metabolic pathways such as N-demethylation, *p*-hydroxylation, and glucuronidation of *p*-hydroxy metabolites. The major difference is that temazepam has a hydroxy group which undergoes direct glucuronidation extensively.

In humans, temazepam is eliminated mainly through urine accounting for 80% of dose, of which 88% was the direct glucuronide.[39] While in rats, the urinary excretion only accounted for 15% of dose; the majority of dose was eliminated though biliary excretion ($>85\%$).[39,40] When temazepam glucuronide is excreted through bile, it undergoes enterohepatic recirculation which gives rise to the prolonged duration of drug in the body. Therefore in rats, temazepam has a longer half-life and lower CL than its non-hydroxy counterpart, diazepam



Diazepam Temazepam

Figure 10.5 Structures of diazepam and temazepam.

(*i.e.* $T_{1/2}$ and CL are 4.0 h and 45 mL min^{-1} kg^{-1} for temazepam, and 1.1 h and 80 mL min^{-1} kg^{-1} for diazepam, respectively).[41,42] In humans, although the major metabolite of temazepam is also direct glucuronide, the excretion of the glucuronide is mainly through urine. The enterohepatic circulation is minimal, so the half-life is 8–10 h for temazepam and 32–33 h for diazepam.[41,43]

A single hydroxy group in the molecule makes significant difference in the pharmacokinetics and pharmacodynamics of temazepam and diazepam. The former is categorised as a short-acting benzodiazepine used mainly as a hypnotic, and the latter is the long-acting one used for the treatment of anxiety.

10.3.3.2.2 Sulfotransferases. Hydroxy containing drugs also undergo sulfate conjugation. The reaction is catalysed by sulfotransferase (SULT) using 3′-phosphoadenosine-5′-phosphosulfate (PAPS) as cofactor. In mammals, SULTs are classified into five families (SULT 1–5), with SULT1 and SULT2 as the most important for xenobiotic metabolism. Conjugation of xenobiotics with sulfate mainly occurs with phenols and occasionally with alcohols, aromatic amines and hydroxylamines.

Sulfate conjugation of phenolic compounds is less prevalent than glucuronidation. When a phenolic compound is the substrate for both glucuronidation and sulfation, these two metabolic pathways compete with each other. Paracetamol is metabolised by both UGT and SULT, and at normal doses in adults the *O*-glucuronide is the major urinary metabolite and the *O*-sulfate the minor one. At low doses, the major metabolite is sulfate conjugate. As dose increases, the proportion of the acetaminophen conjugated with sulfate decreases, whereas the proportion of glucuronic acid conjugate increases. This is due to the different affinity and capacity that UGT and SULT possess; UGT has low affinity but high capacity, with the SULT being the opposite. The low capacity of sulfation is limited by the co-substrate PAPS availability due to limited supply of inorganic sulfate.[44] Interestingly, in infants and children, the predominant urinary metabolite of paracetamol is the sulfate conjugate, since in neonates and young children, glucuronidation capacity is reduced due to the undeveloped glucuronosyltransferase.[45,46] In general, alcohols have less prevalence of being conjugated with sulfuric acid than phenols; however, sulfate

conjugates of benzylic alcohols, allylic alcohols and aromatic hydroxylamines are known to be toxic and can covalently bind to cellular macromolecules, DNA and RNA.[47,48] These are discussed later.

10.3.3.2.3 Methyltransferases. Catechols encompass a wide range of physiological, biologically and medically importance substances such as catecholamines, catecholestrogens, flavonoids and drugs used for the treatment of Parkinson's disease.

Phenolic drugs also undergo oxidative metabolism to form catechols. The metabolic fate of catechols is frequently *via* O-methylation catalysed by catechol-*O*-methyltransferase, which transfers a methyl group from *S*-adenosylmethionine (SAM) to the *m*-hydroxyl group of catechol compounds. In mammals, there is only one gene located on chromosome 22q11 for COMT; this single gene encodes both the soluble COMT (*S*-COMT) and the membrane-bound COMT (*MB*-COMT).[49] COMT is widely distributed in mammalian tissues with the highest level in liver, followed by kidney and gastrointestinal tract.[50–53] COMT is also presented in spleen, pancreas, lung, heart, eye and spinal membrane.[54–57] The soluble form (*S*-COMT) are the dominant form in most rat or human tissues, and only a small fraction is present in the membrane-bound form (*MB*-COMT). The exception is human brain where 70% of the total COMT proteins were found to be *MB*-COMT and 30% *S*-COMT.

COMT plays a major role in the metabolic inactivation of catecholic neurotransmitters such as dopamine, norepinephrine and epinephrine. It also is responsible for the inactivation of catecholestrogens such as 2-hydroxyestrone and 4-hydroxyestrone. In addition to inactivating endogenous catecholamines and catechol estrogens (Figure 10.6), COMT also catalyses the *O*-methylation metabolism of a large number of catechol containing xenobiotics. Many dietary bioflavonoids such as quercetin, fisetin and tea polyphenols are exceptionally good substrates for *O*-methylation by cytosolic COMT from porcine liver or hamster kidney.[58] The rates of the COMT-mediated *O*-methylation metabolism of these bioflavonoids were up to several orders of magnitude higher than those for the endogenous catechols. Many phenolic drugs undergo P450-mediated oxidation to form catechols and are subsequently methylated by COMT. For example, some neuroactive drugs possessing a catechol structure [*e.g.* L-3,4-dihydroxyphenylalanine (L-Dopa)] are also *O*-methylated by COMT (Figure 10.7).[59] Traxoprodil (TRX), a selective *N*-methyl-*d*-aspartate receptor antagonist, is a phenolic drug and it first undergoes P450-mediated oxidation to form a catechol, and is then further metabolised by COMT.[60]

10.3.3.3 Bioactivation of Hydroxy Containing Compounds to Reactive Metabolites

Primary aliphatic alcohols can be oxidised to aldehydes, which are hard electrophiles capable of covalently binding to macromolecules; however, the

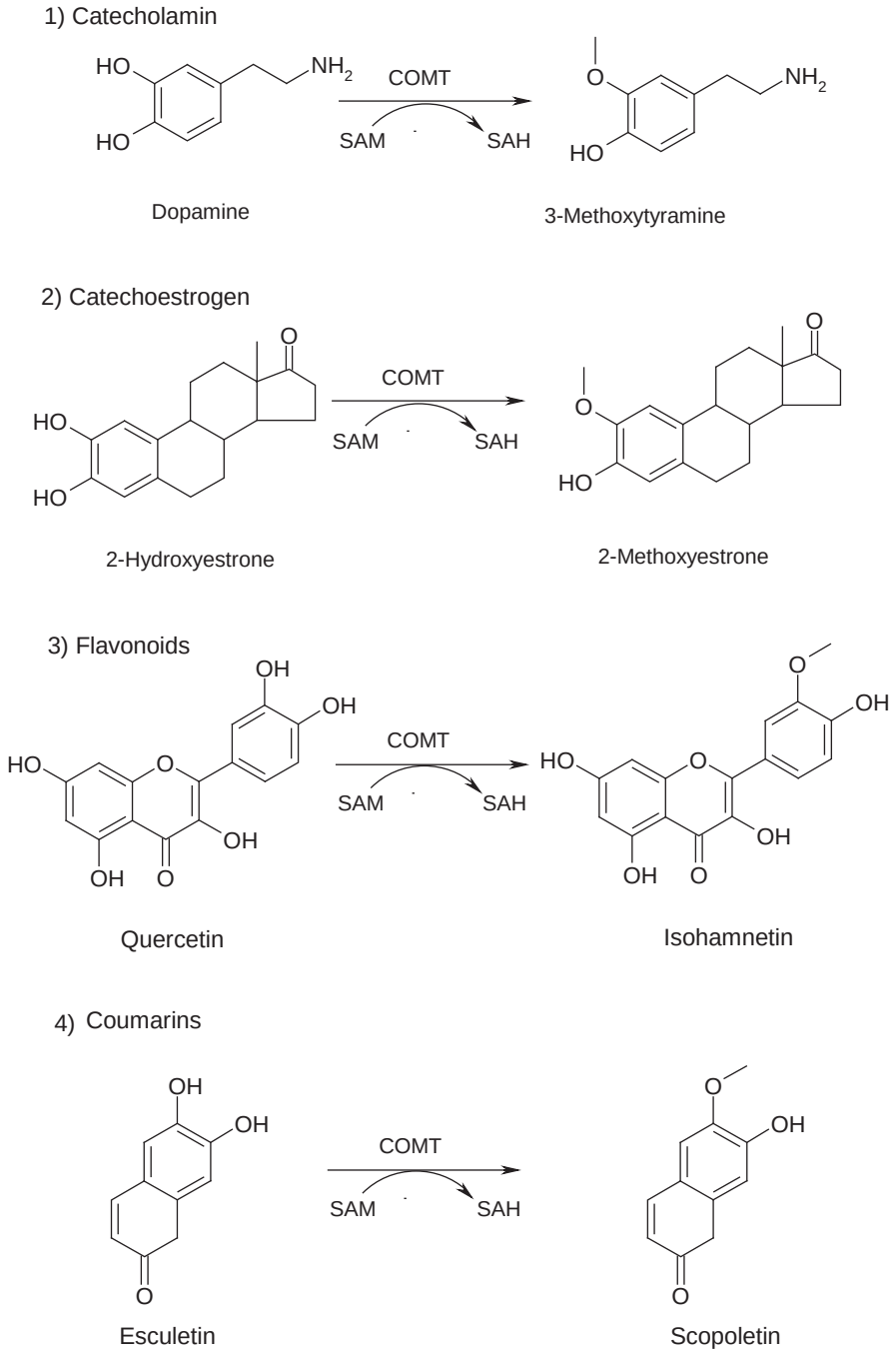


Figure 10.6 COMT mediated biotransformation of neurotransmitters, catecholestrogens, dietary bioflavonoids and coumarins.

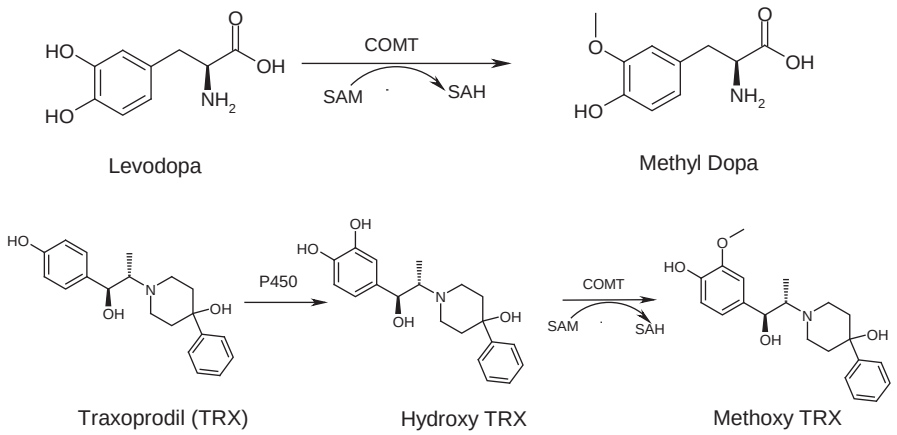


Figure 10.7 COMT-mediated methylation biotransformation of drugs.

capacity of aldehyde dehydrogenase is typically high such that toxic aldehydes do not accumulate in general. In cases of excess ethanol consumption, acetaldehyde can build up and cause toxicity. Allyl alcohol and crotyl alcohol can be bioactivated by alcohol dehydrogenases to aldehydes that can react with proteins and cause toxicity.[61] Longer chain polyfluorinated alcohols are environmental contaminants shown to be bioactivated to toxic aldehydes by P450 enzymes.[62] Abacavir is a reverse transcriptase inhibitor marketed for the treatment of HIV-1 infection.

A small percentage of patients experience a hypersensitivity reaction indicating immune system involvement and bioactivation. Abacavir is metabolised to an aldehyde by ADH, although the exact mechanism causing hypersensitivity remain unclear. The formation of aldehyde and its covalent binding to proteins was thoroughly studied by the incubation of ^{14}C labelled abacavir in human cytosol or human isoforms of alcohol dehydrogenase (ADH) in the presence of human albumin[63] (Figure 10.8).

Phenols can undergo oxidative bioactivation to quinones, quinonemethides or quinonimines depending on the structure of the drug. Such intermediates are good Michael acceptors and stable adduct formation is driven by the re-aromatisation of the adduct. The bioactivation of paracetamol has been well characterised and serves as a quintessential example of drug bioactivation causing hepatic toxicity.[64] Overdoses of paracetamol are common and represent an important healthcare concern. Ordinarily, conjugative pathways handle the clearance of this drug, but when this capacity is exceeded or if the drug is taken with ethanol which induces CYP2E1, substantial oxidative metabolism can occur in which the phenol is oxidised to a chemically reactive quinoneimine (Figure 10.9).

Remoxipride itself does not contain a phenol, but it is metabolised to two phenol intermediates, one *via* *O*-demethylation and one *via* hydroxylation, which are subsequently oxidised to a *p*-quinone. This pathway has been

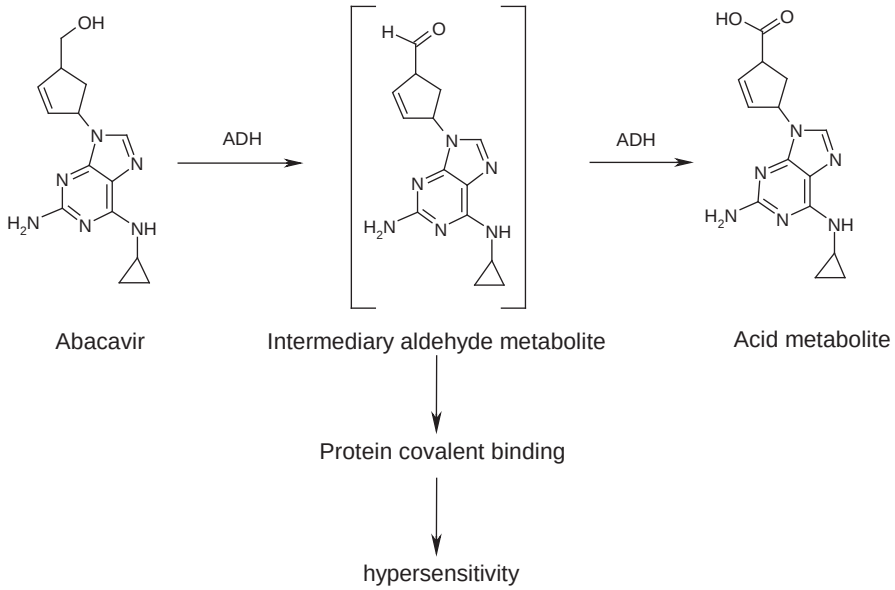


Figure 10.8 Bioactivation of abacavir.

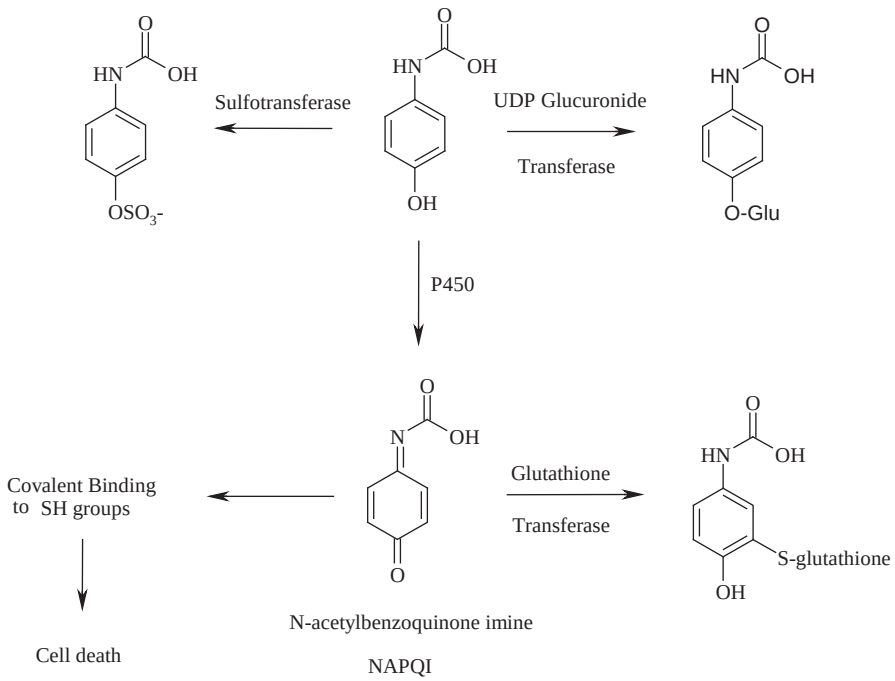


Figure 10.9 Metabolism and bioactivation of acetaminophen (paracetamol).

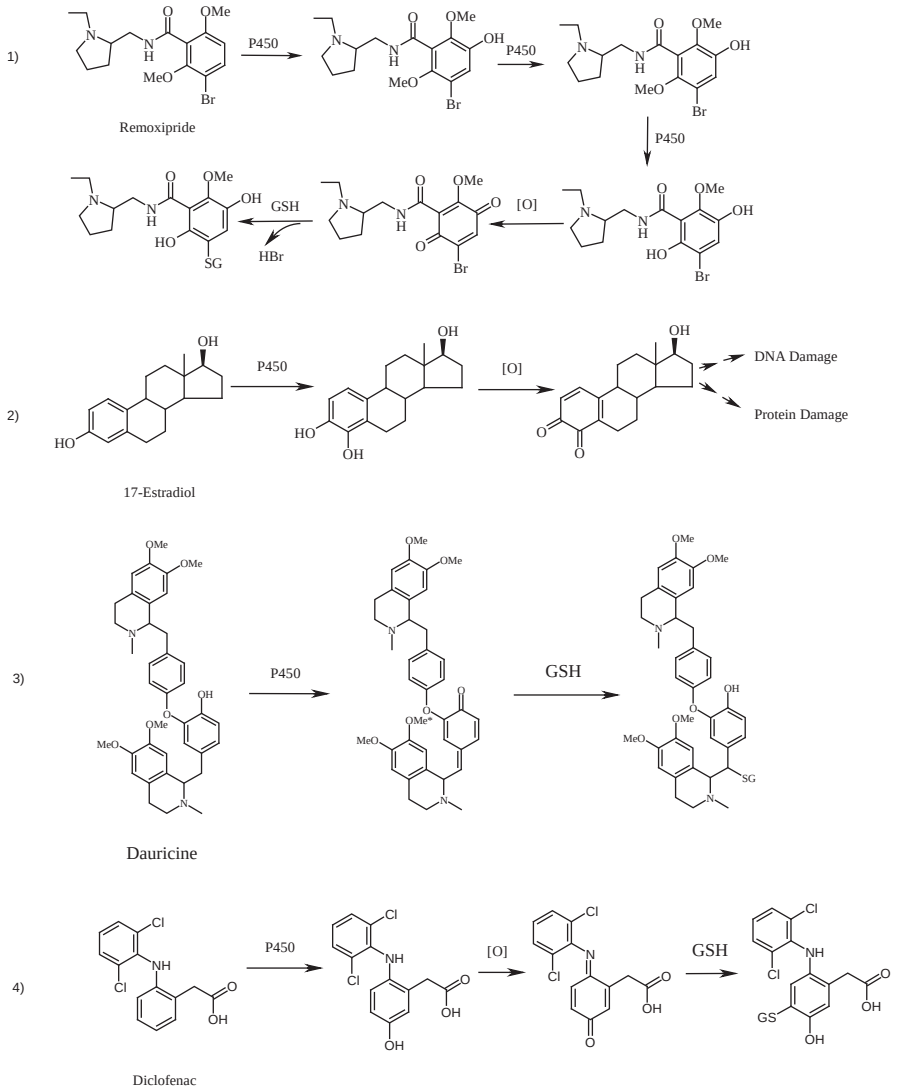


Figure 10.10 Examples of phenol containing drugs that are bioactivated.

implicated as responsible for the idiosyncratic toxicity that led to the withdrawal of this agent from clinical use[65] (Figure 10.10). Other examples of drugs and endobiotics that undergo bioactivation to quinoid structures include the estrogens, dauricine and diclofenac.[66–68] In these cases, the phenols are oxidised to highly conjugated quinonemethides. Diclofenac is oxidised to 5-hydroxydiclofenac by CYP3A; this *p*-aminophenol metabolite can then undergo a second oxidation to a reactive quinoneimine, which may be involved in the hepatotoxicity of this drug.

Conjugative metabolism can also bioactivate alcohols to electrophiles. Sulfation of benzylic and allylic alcohols is a well-established mechanism of bioactivation. The sulfate ester is reactive by virtue of the excellent leaving group properties of the sulfate group and reactions occur with tissue nucleophiles (*e.g.* DNA) *via* SN2 reactions. For example, tamoxifen is well-established as a hepatic carcinogen and clinical data have shown a link between tamoxifen therapy and an increased incidence of endometrial cancer.[69] The major metabolic pathway is *N*-demethylation. Tamoxifen also forms two hydroxy metabolites; one metabolite, α-hydroxy-tamoxifen has an allylic hydroxyl group that undergoes sulfation. The resulting sulfate is hypothesised to be responsible for the formation of DNA adducts[48,70] (Figure 10.11).

10.3.4 Fluorine as an Isostere of Hydroxy Groups

When a lead compound is identified during drug discovery, a common chemical strategy is to use bioisosteric substitution to synthesise new compounds that have less undesirable characteristics (*e.g.* low bioavailability, inadequate half-life and potential to form reactive metabolites).

One of the most common classical bioisosteric substitution is the incorporation of fluorine into a compound in replacement of a hydroxyl group.[71] The rationale for such replacement is based on the fact that the size of the fluorine atom is intermediate between that of hydrogen and oxygen, and that the substitution of the hydroxy group with fluorine is particularly favoured when the presence of an electronegative atom is necessary for the interaction of the ligand with the target protein.[72,73] The van der Waals radius of fluorine (1.35 Å) is intermediate between that of hydrogen (1.2 Å) and oxygen (1.40 Å). The C–F bond length (1.39 Å) is closest to the C–O bond (1.43 Å) compared with the C–H bond (1.09 Å) and other halogens.[73] Fluorine has the strongest electronegativity in the periodic table, and the next strongest is oxygen. Both fluorine and a hydroxy group can form hydrogen bonds, though the difference between the fluorine atom and hydroxy oxygen is that the former can only act as the hydrogen bond acceptor and cannot replace the hydrogen bonding donor role as it is devoid of the acidic hydrogen of the hydroxy group.

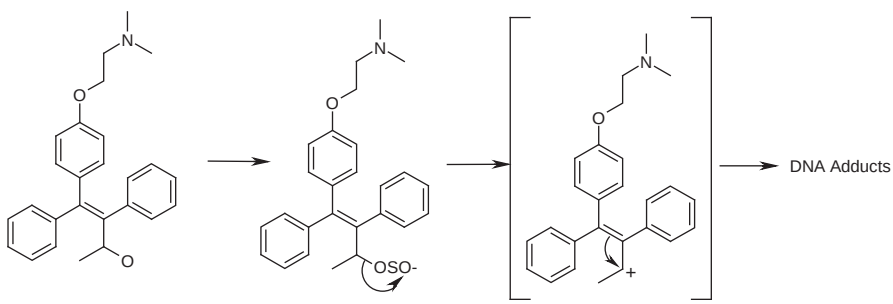


Figure 10.11 Bioactivation of tamoxifen via an allylic sulfate.

The substitution of a hydroxy group with fluorine, in theory, will have profound effect on the rate and extent of metabolism for those compounds which would undergo predominantly phase II metabolism on the substituted hydroxy group. Such phase II metabolism includes methylation, acetylation, sulfation and glucuronidation. Many phenolic hydroxyl compounds have the potential for bioactivation when they are present either *ortho*- or *para*- to a primary or secondary aryl amine group, or a methylene group. Subsequent oxidation can generate *ortho*- or *para*-quinone imine and quinone methide, groups which are associated with toxicity *in vivo*. There are numerous examples in the literature citing the elimination of such a bioactivation through the substitution with fluorine.

Amodiaquine (AQ) (Figure 10.12) is a phenolic 4-aminoquinoline antimalarial effective against chloroquine-sensitive and resistant strains of *Plasmodium falciparum*.[74] However, idiosyncratic adverse drug reactions have severely restricted its use. Both direct cytotoxic and indirect immunogenic causal mechanisms have been proposed to explain the toxicity observed.[75] AQ is known to undergo extensive bioactivation to a chemically reactive quinone imine (AQQI) *in vivo* in the rat.[76] Fluorine substitution at the C-4 position of

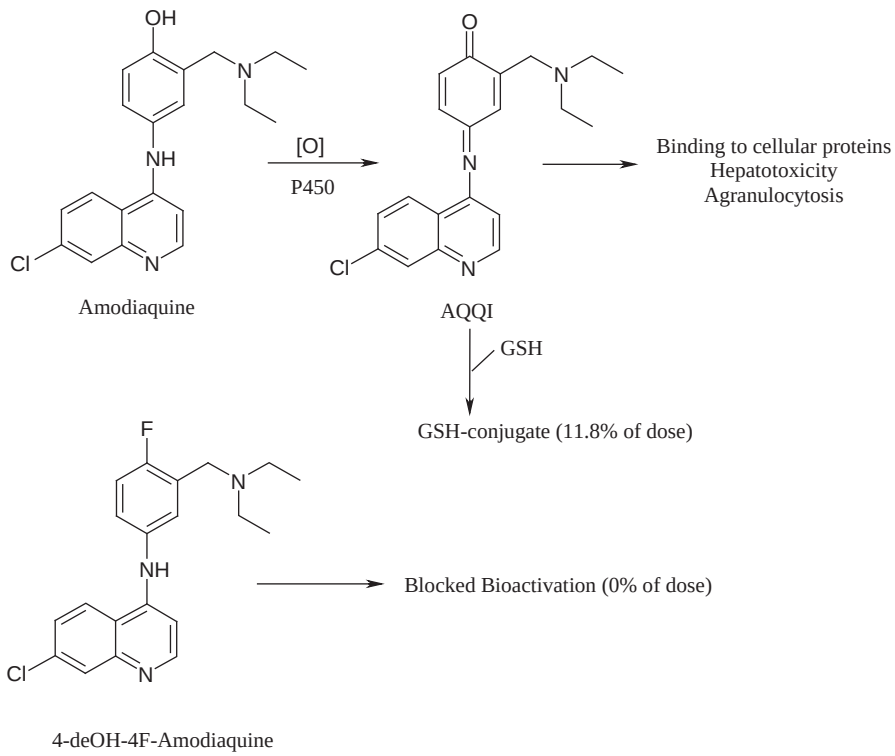


Figure 10.12 Fluorine substitution of hydroxy group in amodiaquine blocked the bioactivation in CD1 mice.

AQ completely blocked bioactivation, since none of the dose resulted in thioether conjugates. This is in contrast to 10.9% of a dose of AQ excreted as thioether conjugate in CD1 mice[77] (Figure 10.12).

10.4 Conclusions

The hydroxyl group represents an important substituent in drug design. Its specific hydrogen-bonding capability can enhance or disrupt interactions with macromolecule targets. The physicochemical properties that it imparts to drug molecules can have a large impact on dispositional behaviour and result in alterations in the pharmacokinetics relative to analogues lacking this group. Furthermore, inclusion of a hydroxyl group in a molecule offers a new handle which can be acted upon by drug-metabolising enzymes and metabolism can be shifted from cytochrome P450 enzymes to conjugative enzymes such as UGTs.

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CHAPTER 11

Future Targets and Chemistry and ADME Needs

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11.1 The Human Genome

Modern molecular biology techniques have enabled cloning and sequencing which have revolutionised approaches to identify new targets through whole genome sequencing, with the human genome being the most notable example. The human genome project[1] (HGP) was started in 1990 with a $3 billion funding from the US National Institutes of Health (NIH) and the US Department of Energy. It was expected to take 15 years to sequence the entire human genome, which is 3.2 gigabases in length and 25 times bigger than any previously mapped genome, and therefore a significant undertaking. The project actually became a consortium with 20 sequencing centres from several different countries including the USA, China, France, Japan, Germany and the UK. An initial incomplete draft[2,3] of the genome was announced in 2001, which was generated by a hierarchical mapping and sequencing strategy. The completed genome[1] was published in April 2004.

Alongside the NIH-backed HGP, another sequencing campaign was run in parallel by the American researcher J. Craig Venter through the company Celera. This effort started in 1998 and was expected to cost a fraction ($300 million) of the NIH effort due to the use of a faster more high risk technique known as

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the 'whole-genome random shotgun' approach. The aim of this effort was to sequence the human genome in three years and make the information available as a commercial product. In 2000, Celera scientists publicly announced their first draft and, in 2001, it was published.[4]

At this stage, ~83% of the human genome had been sequenced, but it was not until 2003 and 2005 that a fuller picture emerged, with >99% of genome sequenced. Estimates at the start of both approaches suggested that the genome may contain up to two million genes, but on completion, the general consensus appears to be much less at approximately 30000.

This landmark achievement in science created a huge opportunity for the pharmaceutical industry, academia and governmental agencies to convert this wealth of information into drugs to treat disease.

11.2 Drug Targets within the Genome

This chapter confines discussion to the discovery and development of small molecule drugs derived from the human genome and does not cover other approaches such as biologics, vaccines or non-human targets. This chapter describes drug-like properties as absorption, distribution, metabolism and excretion (ADME) space which concentrates on lipophilicity and hydrogen bonding rather than molecular weight (MW).

With estimates of the human genome comprising 30 000 genes, two important questions emerge for drug discoverers. Firstly, how many of these are disease-related genes and secondly how many of the 30 000+ proteins derived from these genes are druggable with small molecules. Of course, it is the intersection of these two datasets where the hunt for small molecule drugs should focus.

To address the first question, Drews[5,6] estimated the number of disease-related genes of interest at 5000–10 000. This was based on the assumptions that:

- there are 100–150 multifactorial diseases in the developed world;
- each disease is caused by ten genes;
- each of these genes is 'linked' to 5–10 other genes which offer an opportunity for therapeutic intervention.

The second question then relates to how many of the 30 000 gene products may be seen as 'druggable'. Hopkins and Groom took the gene family approach and focussed on proteins as the most significant druggable biomolecule compared with the other major classes of biomolecules—DNA, polysaccharides and lipids.[7] They first examined the chemical substrate used to target current gene families and assumed that the binding site architecture was conserved across a gene family. Future chemical substrates were then assumed to also be similar. An assessment of the druggability[8] of the small molecule substrate within each gene family was then defined using the 'rule of five' (Ro5). It emerged from this analysis that, of the 30 000 genes in the human genome, approximately 3000

genes encode proteins with binding sites suitable for binding molecules which are likely to have acceptable ADME properties.

The estimate of 5000–10 000 disease-related genes and 3000 genes which encode proteins that are druggable prompts the question 'how many disease targets reside at the intersection'. In reality, the answer is that we do not have the tools to give a precise estimate. An estimate by Hopkins and Groom was put at 600–1500, and was based on extrapolation from the number of anti-fungal targets in the yeast genome.[7]

It is this intersection of druggable proteins with disease-modifying proteins that the majority of drugs in the current pharmacopeia occupy. An analysis by Drews in 2000[6] showed that the modern pharmacopoeia modulates 483 targets. A different analysis by Hopkins and Groom[7] in 2002 put the number of proteins that the pharmacopeia modulates at only 120 drugs with properties commensurate for acceptable pharmacokinetic properties. These estimates suggest that as researchers we have only scratched the surface of druggable and disease-modifying proteins which could lead to important new drugs.

11.3 The Genome Gap

What are universities, government institutes and industry currently doing to address this gap? In 2006, Zheng and co-workers[9] analysed the distribution of therapeutic targets against disease classes. What was evident from this analysis is that there is a significant increase in the level of exploration within each disease class, suggesting that the sequenced human genome is impacting the number of new targets that are being explored (Figure 11.1.). According to the therapeutics target database, there were around 500 research targets being pursued in 1996, with 1535 targets in 2006.

The analysis by Hopkins and Groom[7] in 2002 not only gives estimates of the number of druggable protein targets but also provides a breakdown of the human genome in terms of gene family (Figure 11.2). This analysis suggests that G-protein coupled receptors (GPCRs) and protein kinases are two of the largest

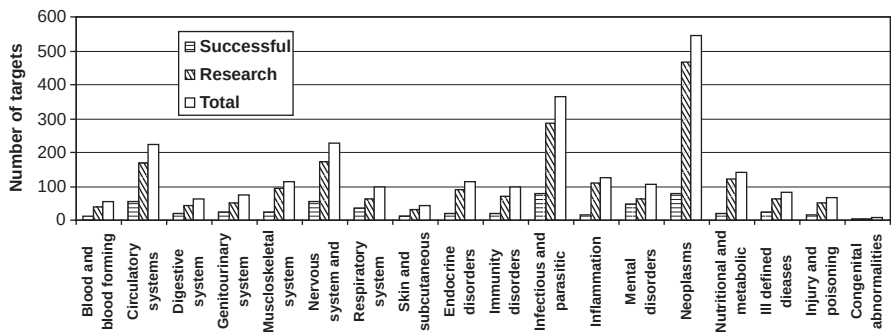


Figure 11.1 Therapeutic targets and disease: successful marketed drugs, drugs in development and total.

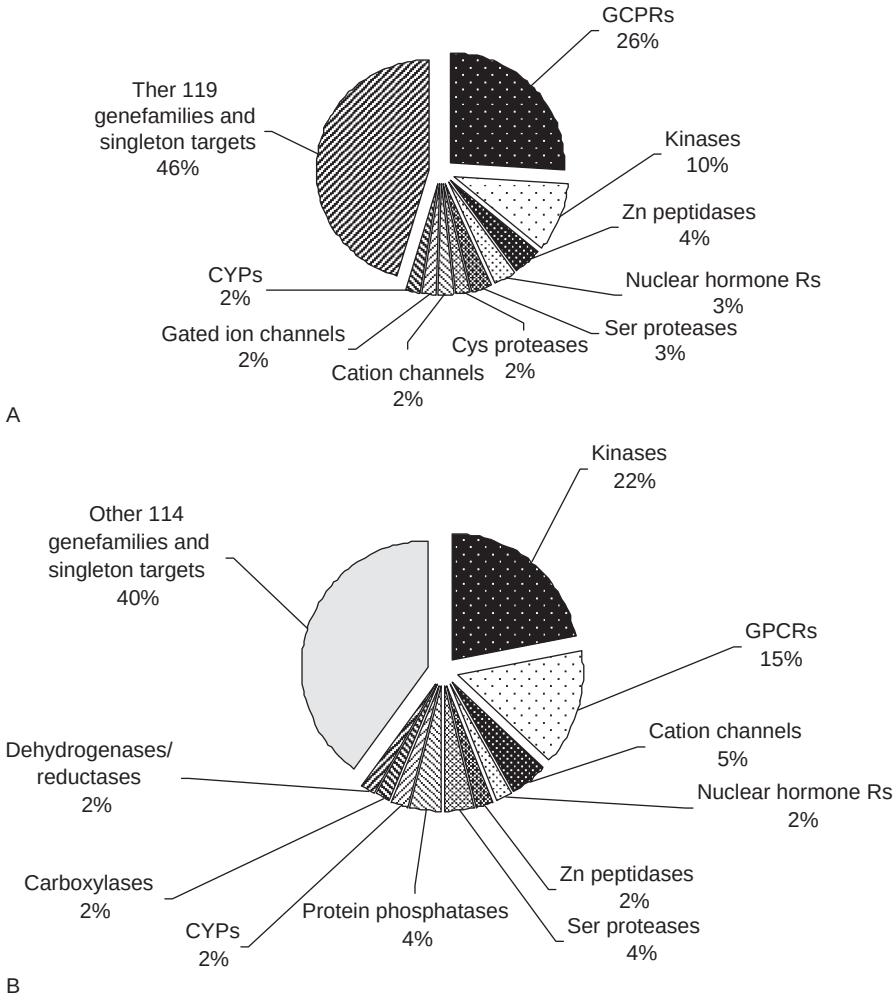


Figure 11.2 Drug target families shown as A—the molecular targets of experimental and marketed drugs with physicochemical properties compliant with druggable and B—the druggable genome.

druggable gene families within the human genome, although smaller than previously expected. GPCRs have previously been rich hunting grounds for drug discovery[9,10] and protein kinases have begun to yield some success stories. Alongside these important classes, the total of zinc metallopeptidases, serine proteases, phosphodiesterases and nuclear hormone receptors make up nearly half of the gene families within the druggable human genome. In contrast to what was initially expected, proteases in particular have emerged as a significant gene family.

A conservative estimate by Russ and Lampel made in 2005 puts the number of druggable proteases at ~230 proteins.[11] Together with kinases, GPCRs and other families, the number of druggable targets reached in this analysis is just over 3000, which is remarkably similar to that estimated by Hopkins and Groom.[7]

11.3.1 New Mechanisms and the Druggable Genome

There are other approaches which have not been extensively investigated such as epigenetics[12] and inhibition of protein–protein interactions,[13,14] for which the small molecule substrate required is only just beginning to emerge.

Protein trafficking[15–19] is another potential mechanism for which small molecule intervention might be appropriate. Evidence is emerging that the expression of cell surface proteins can be influenced by small molecules, both in terms of increased expression and decreased expression.

A number of diseases are caused by genetic polymorphisms which code for proteins that misfold and do not reach the cell surface. An example of this is long QT syndrome, which is associated with low cell surface expression of the HERG channel. Antagonists or blockers of the HERG channel can decrease the expression of the wild-type HERG channel, but also can raise the expression of the mutated form. One hypothesis suggests that the compounds bind to the same or similar residues (in the active site) in the nascent folding channel (at the endoplasmic reticulum) as they do when the channel is expressed at the cell surface.

These growing insights suggest that compounds closely related to existing agonists and antagonists may be active in the control of cell surface expression. Critical here for indications such as long QT is that the structure–activity relationships (SARs) for antagonism and aiding protein folding are separate. Importantly, the chemical space of such molecules is unlikely to be substantially different from existing agents which have good physicochemical and resultant ADME properties.

One example of such an approach is a treatment for cystic fibrosis. Cystic fibrosis (CF) is a fatal genetic disease caused by mutations in *cftr*, a gene encoding a protein kinase A (PKA) regulated chloride channel. The most common mutation results in a deletion of phenylalanine at position 508 (**D**F508-CFTR); this impairs protein folding, trafficking and channel gating in epithelial cells. In the airway, these defects alter salt and fluid transport, leading to chronic infection, inflammation and loss of lung function.

Two classes of novel, potent small molecules[19] have been identified from screening compound libraries which restore the function of **D**F508-CFTR in both recombinant cells and cultures of human bronchial epithelia isolated from CF patients. The first class partially corrects the trafficking defect by facilitating exit from the endoplasmic reticulum and restores **D**F508-CFTR-mediated chloride transport to more than 10% of that observed in non-CF human bronchial epithelial cultures; this level is one expected to result in a clinical

benefit in CF patients. The second class of compounds potentiates cyclic adenosine monophosphate (cAMP) mediated gating of **D**F508-CFTR and achieves single-channel activity similar to wild-type CFTR.

The CFTR-activating effects of the two mechanisms are additive and support the rationale of a drug discovery strategy based on rescue of the basic genetic defect responsible for CF.

11.4 The Need for New ADME tools

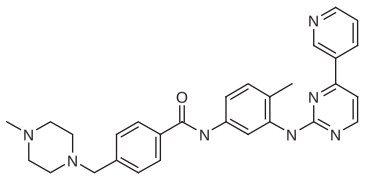
Analysis of the human genome suggests that no large gene families remain undiscovered and that therefore future drug discovery efforts will reside largely within gene families with which we are already familiar and with which the industry as a whole has some experience in discovering and developing new drugs.

Looking at the current pharmacopoeia and breaking that down into gene families gives an idea of the properties of the small molecule chemical matter that is required to modulate these targets. For example, in the area of kinases, several drugs have made it to market since the turn of the century,[20] with imatinib being the first in 2001. Many of these drugs have similar physicochemical properties, which although not unique to kinase inhibitors, are indicative of what future chemical substrates will look like if adenosine triphosphate (ATP) binding site inhibitors are targeted. Most of these drugs are lipophilic, with many containing weakly basic centres (Figure 11.3). Therefore future campaigns targeting ATP-binding site inhibitors will likely be in similar physicochemical space.

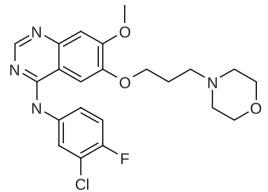
Similar trends emerge if we look more widely at other gene families that will probably be targets of the future. The work of Paolini *et al.*[21] was instrumental in observing these gene family trends with respect to their chemical matter. The shift from an average molecular weight of the aminergic GPCR modulators of 376 Daltons (Da) to other gene families such as peptide GPCR modulators (average MW 477 Da), metalloproteases (average MW 429 Da) and serine proteases (average MW 463 Da) has meant that, over the last two decades, the average molecular weight of approved drugs has increased by $>20\%$. This is of course at odds with the Ro5 and pushes leads within these gene families closer to the edge of currently perceived successful oral drug space. But is molecular weight a key determinant of oral absorption? The relevance of molecular weight with oral absorption is a topic discussed later in this chapter.

Looking at other physicochemical properties of chemical matter related to future and current gene family targets reveals more worrying trends. Much of the chemical matter required to modulate gene families of the future also contain more hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs), including many of the targets within the protease gene family. Similarly, the clogP value of many of these compounds is also moving closer to 5.

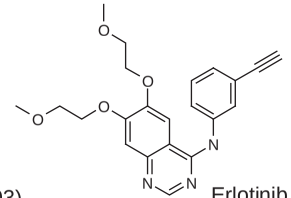
These upward trends in physicochemical properties considered important for oral bioavailability suggest that medicinal chemists will be working closer to the edge of oral drug space more often. Therefore, there is a growing need to



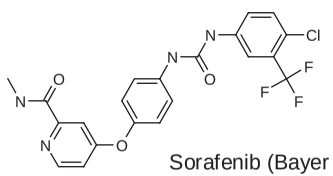
Imatinib (Novartis - 2001)
MW 494
clogP 4.5
TPSA 86Å^2



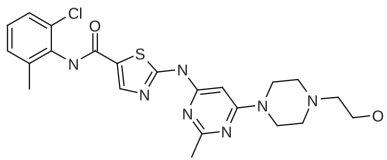
Gefitinib (AstraZeneca - 2003)
MW 447
clogP 5.6
TPSA 69Å^2



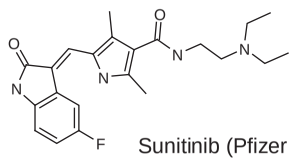
Erlotinib (OSI, Roche - 2004)
MW 393
clogP 4.3
TPSA 75Å^2



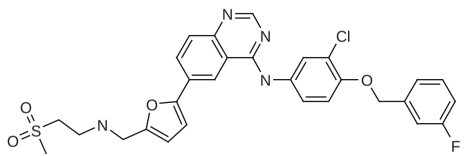
Sorafenib (Bayer - 2005)
MW 465
clogP 5.5
TPSA 92Å^2



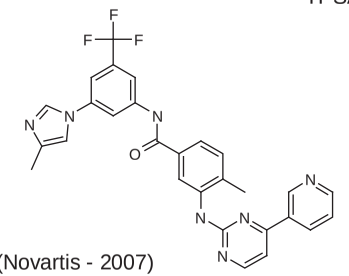
Dasatinib (BMS - 2006)
MW 488
clogP 2.5
TPSA 135Å^2



Sunitinib (Pfizer - 2006)
MW 494
clogP 4.5
TPSA 86Å^2

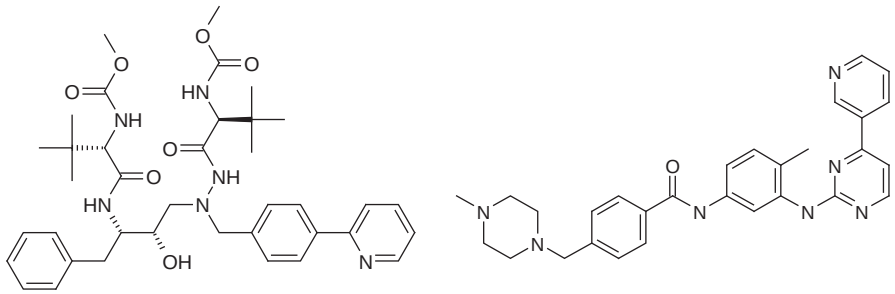


Lapatinib (GSK - 2007)
MW 581
clogP 6
TPSA 115Å^2



Nilotinib (Novartis - 2007)
MW 530
clogP 5.8
TPSA 98Å^2

Figure 11.3 Structures of marketed kinase inhibitors (TPSA=topological polar surface area).



Atazanavir (BMS - 2003)
MW 705
clogP 5.9
TPSA 171Å^2

Imatinib (Novartis - 2001)
MW 494
clogP 4.5
TPSA 86Å^2

Figure 11.4 Structures of atazanavir and imatinib—compounds with pharmacokinetics not predicted by calculation of their physicochemical properties.

develop new tools to better understand the properties of molecules within this challenging physicochemical space and ultimately to increase confidence preclinically that the required ADME profiles will be achieved.

Alongside this, a deeper and more rational understanding of the physical processes that occur during oral absorption is required. These tools should include improved *in silico* methods for prospective, property based design of orally bioavailable compounds, as well as more relevant *in vitro* methods for investigating membrane permeability (both gut and blood–brain barrier penetration).

Analysis of the HIV protease inhibitor, atazanavir, and the first kinase inhibitor, imatinib, illustrates these points well (Figure 11.4). Both are high molecular weight, relatively lipophilic compounds. Atazanavir moves further out of desirable ADME space due to a high hydrogen bond donor count and high polar surface area (PSA), yet displays remarkably good human oral pharmacokinetics.[22,23] Similarly, imatinib[24] is highly orally bioavailable despite its high molecular weight and lipophilic character. If property-based design principles[25] were applied to these molecules, both would be at the high end of predicted risk for poor oral pharmacokinetics. In reality, both are highly successful oral agents.

11.5 The Chemistry Gap

Accessing desirable chemical space for current and future drug discovery efforts is an ongoing challenge in medicinal chemistry. The best theoretical drug design in the world is no use if you cannot synthesise the 'right' molecule. In this section we briefly highlight several areas where synthetic chemistry could potentially impact future drug discovery efforts. We focus on the synthesis and commercial availability of aryl building blocks, the importance of fluorination

in drug discovery and finally exemplify areas where improved methods in asymmetric synthesis are required.

A common feature of small molecule drug-like compounds is the presence of heteroaromatic rings that act as core systems for further elaboration and diversification. Many of these cores are linked to other heteroaromatics or functionalised phenyl rings through biaryl like carbon–carbon bond connections as well as anilinic and aryl ether like connections. This raises an interesting philosophical question with regard to the chemical structure of 'drug-like molecules'. Are aromatic ring systems abundant in drug molecules because they are required to bind to the target protein, or is this merely a consequence of synthetic ease?

With respect to the synthesis of heteroaromatic rings a recent report[26] suggested, through calculation, that ~25 000 small heteroaromatic ring systems are theoretically possible. However, a search of the literature suggested that only ~7% of these heteroaromatic rings have ever been made and published. Moreover, this report found that only 5–10 novel heteroaromatic ring systems are appearing in the literature every year. With heteroaromatic chemical space continuing to be patented year on year, as well as the ability of heteroaromatics to offer potentially modified ADME properties, the synthesis of novel systems is becoming increasingly important in drug discovery. This example perhaps highlights the fact that, although synthetic chemistry may be widely considered as a 'mature' science, there is still a huge challenge open to the organic chemistry community.

A good example of how synthetic chemistry can impact in the discovery of a new drug is the case of the phosphodiesterase-5 inhibitor (PDE5), vardenafil. The first PDE5 inhibitor to reach the market was sildenafil (Viagra™), which was later followed by a structural analogue, vardenafil (Figure 11.5). Vardenafil differs from sildenafil by the position of a nitrogen atom in the heterocyclic ring system as well as a one carbon homologation of the piperazine substituent, leading to increased potency for the PDE5 enzyme and a lower dose. Clearly the ability to synthesise new heterocyclic ring systems, with different properties, had an impact on the discovery of this drug.

Allied to bespoke syntheses of polyfunctionalised heteroaromatic ring systems, accessing commercially available functionalised phenyl and heterocyclic

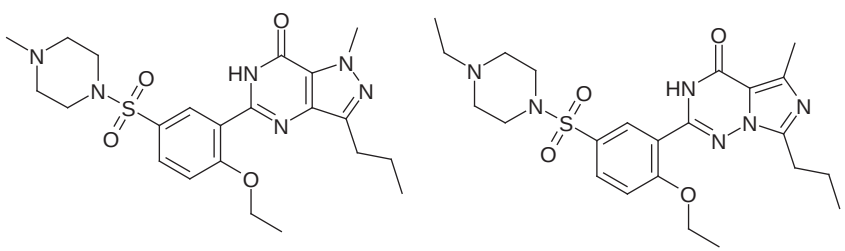


Figure 11.5 Chemical structures of the PDE5 inhibitors, sildenafil and vardenafil.

building blocks for singleton and library syntheses is critical to the rapid advance of drug discovery programmes. An analysis of commercially available tri- and tetra-substituted phenyl rings reveals that these molecular building blocks are under-represented in supplier catalogues relative to the numbers of combinations and permutations theoretically possible with such highly substituted ring systems. A similar scenario exists for tri-substituted pyridine ring systems (Table 11.1). This is presumably due to synthetic complexity and expense, but again highlights where synthetic chemistry could add value to advancing medicinal chemistry programmes.

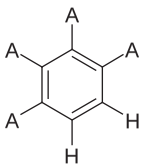
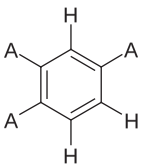
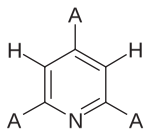
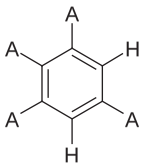
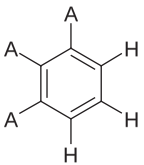
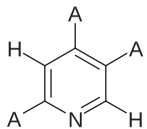
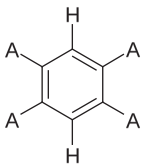
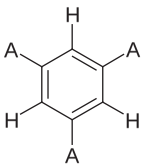
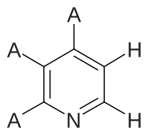
Moving beyond the paradigm of chemical space exploration, that is largely driven by commercial availability of suitable building blocks, is a must. To reiterate a point made above, the design and synthesis of the 'right' molecule is key even if this involves significant investment in synthesis.

The synthetic methodology required to covalently join functionalised phenyl and heteroaromatic ring systems and derivatise them has advanced significantly in the last 10–15 years. A comprehensive review of this synthetic methodology is beyond the scope of this chapter, but it has been reviewed extensively elsewhere.[27] Many of these chemical reactions are now sufficiently robust to be carried out by parallel synthesis protocols. Moreover, advances in catalysts have enabled a greater variety in coupling partners which has broadened the utility of many of the commercially available compounds analysed in Table 11.1. But to reiterate the point made previously, the major limitation in accessing this chemical space remains with the availability of diverse and highly substituted compatible building blocks.

With many drug targets favouring lipophilic chemotypes such as the kinase drugs described in Figure 11.3, P450-mediated oxidative metabolism is often an inevitable consequence. This can then lead to poor oral bioavailability and undesirable pharmacokinetic half-lives. Fluorination as a means to block P450-mediated metabolism, as well as offering the potential to increase potency, is emerging as a favoured tactic.[28,29] Fluorination is also a useful strategy to modulate the pKa of basic drugs, which is often important for designing compounds with reduced affinity for the HERG cardiac potassium channel.

The DPP-4 inhibitor, sitagliptin, is an example of a highly fluorinated drug that benefits from improved potency and oral bioavailability by judicious use of its fluorine substituents. Again, fluorination strategies in the NK1 antagonist, aprepitant led to an improved duration of action, potency and central nervous system (CNS) penetration (Figure 11.6). The role of fluorine as an isostere is discussed at length in Chapter 10 section 3.4. With fluorine substitution capable of imparting such stark pharmacokinetic and pharmacodynamic advantages for such a small molecular change (often H to F), synthetic methods to introduce this atom into molecules of interest is therefore high on the wish list of most medicinal chemists. Advances in synthetic methodology to introduce fluorine have emerged in recent years, yet fluorine scans still represent an enormous synthetic challenge and therefore improved methods and/or more commercially available fluorinated building blocks would have a huge impact.

Table 11.1 Commercially available tri- and tetra-substituted benzenes

Tetra-substitution[a]	*Commercially available compounds (MW ≤ 200 Da)*	*Tri-substitution*[a]	*Commercially available compounds (MW ≤ 180 Da)*	*Pyridines*[a]	*Commercially available compounds (MW ≤ 180 Da)*
	1006		4967		273
	1728		1574		278
	897		595		305

[a]A = non-hydrogen.

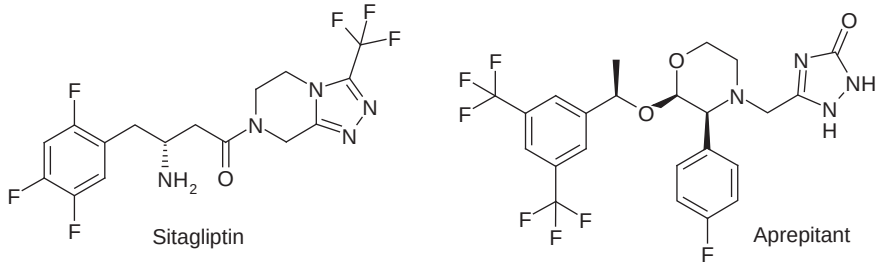


Figure 11.6 Fluorinated drugs, sitagliptin and aprepitant.

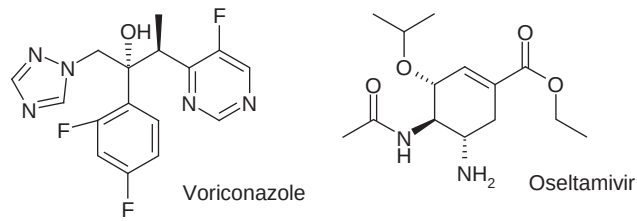


Figure 11.7 Voriconazole and oseltamivir.

As the endogenous substrates of many proteins are chiral (*e.g.* peptides), it is important for medicinal chemists to access three-dimensional space through introduction of chirality into molecules. Moreover, introduction of chirality can often lead to very different pharmacological selectivity and/or pharmacokinetic profiles between stereoisomers. This tactic of course often costs little or results in no increase in the molecular weight of the molecule. A recent report has also suggested that compound complexity, as measured by the presence of chiral centres is directly related to the likelihood of clinical progression of drug candidates.[30] A comprehensive review of the literature concerning asymmetric synthesis is also beyond the scope of this chapter but the reader is directed towards several excellent reviews.[31,32]

Advances in asymmetric synthesis have continued to emerge in recent years, though often in the context of natural product synthesis. Application to 'drug-like' compounds often necessitates different synthetic strategies and therefore huge challenges still exist for synthetic chemistry in the drug discovery arena. A noteworthy example of this point is the antifungal drug, voriconazole (Figure 11.7). This drug contains two chiral centres, with the active enantiomer being 500 times more potent than the opposite antipode. Current synthetic methods rely on a chiral resolution towards the end of the synthetic route, leading to an inefficient and costly process. Clearly an improved method for installing this chirality is required.

Another illustrative example is the currently important antiviral drug, oseltamivir (Figure 11.7). The biological target, the neuraminidase enzyme, is

involved in the hydrolysis of silaic acid (a carbohydrate derived molecule) from newly formed virus particles. Therefore to inhibit this enzyme, a carbohydrate like molecule with three contiguous asymmetric centres is required. This complex molecular structure led to an extremely challenging and costly synthesis by its makers, Hoffmann La Roche. In fact, the naturally sourced starting material (shikimic acid) required for the synthesis of oseltamivir contains all three asymmetric centres, with current synthetic methods unable to produce this molecule in a reliable way. This example and the others highlighted above again suggest that the seemingly mature science of synthetic organic chemistry still does not have all the answers and that significant challenges exist.

11.6 The Knowledge Gap in Drug Design

In the preceding sections we have referred to druggability, Ro5 and ADME space as descriptors of a set of physicochemical properties that define good delivery of a small drug molecule from an oral dose form to its target. We have also given examples where the compounds behave very differently with what we would have expected based on those properties. In the following section we review some of the current concepts and also identify areas where our knowledge and scholarship could be improved.

11.7 Permeability of Membranes: A Pivotal Role in Drug Disposition

Permeability of membranes is obviously central to the absorption and distribution of drugs. Drugs can cross the bilayer of the membrane of cells or traffic through organs or tissues by the tight junctions (aqueous channels) that are formed at the junctions between cells. The tight junctions allow limited permeability to small drugs through the gastrointestinal tract and also into interstitial water through the capillary walls. This aqueous route has been exploited with aminergic GPCR agonists and antagonists with drugs such as ranitidine, cimetidine, atenolol and sumatriptan. Passage out of the capillaries of the brain is much more restricted. The blood–brain barrier is formed by 'tighter' tight junctions between the brain microvessel endothelial cells than comparative junctions elsewhere. In addition, anthracitic end-feet ensheath the vessel wall and maintain the barrier properties of the brain capillary endothelial cells. Because of the tight junctions, the aqueous pore pathway is of very limited availability and only lipophilic compounds can cross by passive diffusion. Most drugs are absorbed and reach their intended target by crossing the lipid bilayer of the gastrointestinal tract. When a target is intracellular, the lipid bilayer generally has to be crossed to access the target.

Transfer through the lipid bilayer necessitates the removal of the molecule from its solution in water. Drugs are a hybrid of lipophilic and hydrophilic functionality. Lipophilic functionality has a positive solvation free energy

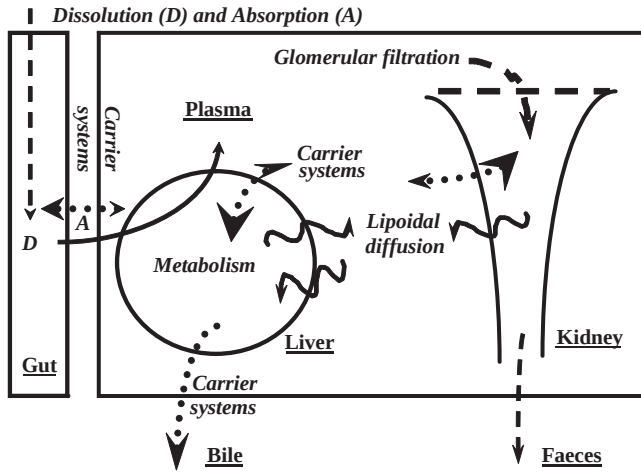


Figure 11.8 Simplified schematic for the processes of drug absorption and clearance. The figure illustrates the role of passive and active transport, and enzymatic processes.

which favours the move from an aqueous environment to the lipophilic environment of the core of the membrane bilayer. Conversely, the desolvation of hydrophilic functionality is energetically unfavourable and hinders transfer of the drug through the lipophilic core of the membrane bilayer.

It is now realised that not only absorption but clearance of drug are governed by drug permeability. Figure 11.8 provides a simplified schematic for the processes of drug absorption and clearance. Passive processes include lipoidal diffusion, aqueous channel diffusion and glomerular filtration. Active transport processes—P-glycoprotein (P-gp) and multidrug resistance proteins, organic anion and organic cation transporting polypeptides, *etc.*—are involved potentially both ways in the influx and efflux of drugs across the intestine, the secretion and reabsorption of drugs in the kidney, and the uptake and trafficking of the drug into the bile or back into the circulation. Enzymatic processes occur with metabolism principally in the liver and, with some drugs, the gut. In addition, transport proteins are expressed at brain capillary endothelial cells and astrocytic end-feet and efflux drugs further creating the 'blood–brain barrier'. Not all these processes occur with many drugs. Most transport processes and drug-metabolising enzymes have binding sites that depend largely on the removal of the groups at the binding interface from water. The loss of solvent is a major contributor to the binding free energy. Lipophilic groups have a positive solvation free energy; this together with favourable direct van der Waals interactions and their incorporation makes the drug a more likely substrate for transporters or drug-metabolising enzymes.

Many existing drug classes have their disposition mainly characterised by passive diffusion. The small hydrophilic aminergic GPCR agonists (low permeability) and antagonists referred to above have low protein binding and are

cleared renally at a rate close to glomerular filtration rate (GFR). More lipophilic (high permeability) aminergic GPCR agonists and antagonists may also undergo some degrees of filtration, but any drug excreted this way is rapidly reabsorbed as the urine is concentrated on its passage through the kidney tubules. These drugs are cleared by metabolism. Permeability across membranes is high—the rate equal to or exceeding the rate that drug transporters can transport drugs across membranes. Such a high rate of diffusion renders the influence of transporters as negligible, even if the drug is a substrate. These definitions are not absolute. As drugs increase in lipophilicity, but not sufficient for substantial membrane bilayer permeability, they can show evidence of affinity for active transport systems in the liver and the kidney.

Many of the post-genomic drug classes do not fall easily into a hydrophilic or lipophilic/low permeability or high permeability description. The drugs are often of higher molecular weight and contain large amounts of both hydrophilic and lipophilic functionality. Because of the large areas of hydrophilicity (PSA), these drugs are of low or at best moderate permeability and their passage across membranes can be attenuated or aided by active transport systems. Their absorption across the intestine and penetration into the brain is adversely affected by efflux transporters (P-gp), whilst their passage into the liver may be facilitated by further active transport. This step may be the actual systemic clearance mechanism or it could be a combination of this, metabolism and even efflux into the bile.

The differentiation of drug disposition has been rationalised as ADME space (which is comparable to target space)—a physicochemical space that encloses the properties most likely to be associated with permeable drugs. The dimensions of such a space and its boundaries are illustrated as Figure 11.9. The formula 'Log P=MW – PSA' is conceptual but links the hydrophilic (PSA) and lipophilic (log P) functionality referred to above with the size of the molecule.

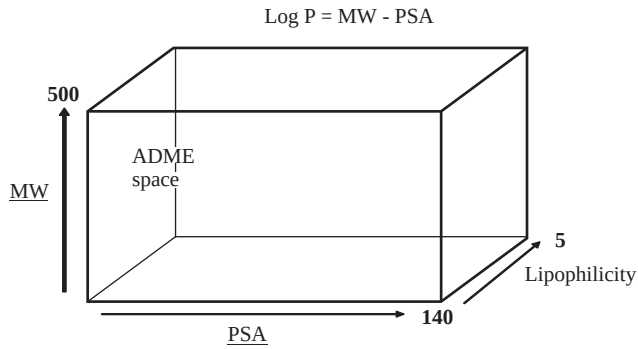


Figure 11.9 ADME space bounded by the interconnected physicochemical properties of molecular weight, polar surface area and lipophilicity. Drugs with desirable pharmacokinetic properties such as absorption are much more likely to occupy the space. The cut-off values of PSA=140 $Å^2$and log P–5 have been determined from the literature.

PSA can be viewed as the energy cost in desolvation for the molecule to enter the membrane; so, as higher values of PSA are reached, membrane permeability is energetically unfavourable. Lipophilicity aids drug permeability but decreases drug solubility to the point where dissolution of the drug is too low to allow any absorption The interconnection with molecular weight has spawned a belief in that particular property being important *per se*. Most likely it is the fact that drug molecules are made from carbon (lipophilicity), oxygen and nitrogen (PSA) and that as, molecular weight approaches 500 Da, the chances of too high a lipophilicity or too great a PSA increase.

An elegant study[33] examined P-gp substrates with the MDRI–MDCKII cell monolayer as a measure of permeability. The ratio of B to A transport over A to B transport increased initially with intrinsic absorptive permeability, but later reduced with compounds with high intrinsic absorptive permeability. This is explained initially by the substrate needing to penetrate the membrane to be a substrate of P-gp in this study would be penetration and permeability are such that the rapid bi-directional flux negates any transport. Most of the drugs shown to be significantly influenced by P-gp were found in the top right-hand corner of ADME space (Figure 11.9), with molecular weight around or exceeding 500 Da and PSAs $>75\,\text{Å}^2$.

To illustrate this region of ADME space, several aminergic GPCR receptor antagonist and agonist drugs, and their physicochemical properties. are listed in Table 11.2.

Table 11.2 illustrates how all the compounds sit well within the boundaries of ADME space and largely divide into high permeability (mainly metabolic clearance) and low permeability (renal clearance) purely on the measurement of lipophilicity (log D). Fraction absorbed is a useful index of permeability. Sumatriptan is unusual in that the drug is metabolised by monoamine oxidase, an enzyme that has natural hydrophilic substrates. Ranitidine and cimetidine show evidence for active tubular secretion with renal clearance values above GFR.

In contrast, a series of drugs against the HIV protease are shown in Table 11.3. All of the molecules are on the boundary or outside ADME space

Table 11.2 Physicochemical properties of agonists and antagonists of aminergic GCPRs.

	MW	*PSA* (*Å*^{2})	*Log P*	*Log D*	*Fraction absorbed*	*Renal clearance (ml/min/kg)*	*Hepatic clearance (ml/min/kg)*
Atenolol	266	85	0.1	−2.0	0.56	2.0	0
Nadolol	309	82	1.3	−0.83	0.34	2.6	1.0
Betaxolol	307	51	2.7	0.7	1.00	1.5	8.9
Propranolol	259	42	3.1	1.0	1.00	<0.5	122
Sumatriptan	295	74	0.7	−1.73	0.3	2.7	16.3 *
Cimetidine	252	114	0.1	−0.45	0.8	6.3	3.7
Ranitidine	314	112	1.2	−0.1	0.52	8.5	3.7

Table 11.3 Physicochemical properties of HIV protease inhibitors contrasted with propranolol.

	MW	*PSA* (Å^2)	*Log P*	*Log D*	*A-B* (*nm/s*)	*B-A* (*nm/s*)	*Fraction absorbed*
Ritonavir	721	202	5.3	5.3	16	852	0.7
Saquinavir	671	167	4.4	3.9	2	395	0.4
Lopinavir	629	120	6.3	6.3			
Indinavir	614	118	2.9	2.9			0.6
Nelfinavir	568	127	7.0	6.0	16	852	0.8
Amprenavir	506	140	4.2	4.2	5	30	0.7
Propranolol[a]	259	42	3.1	1.0	450	700	1.0

[a]Propranolol is a high permeability drug (see Table 11.2).

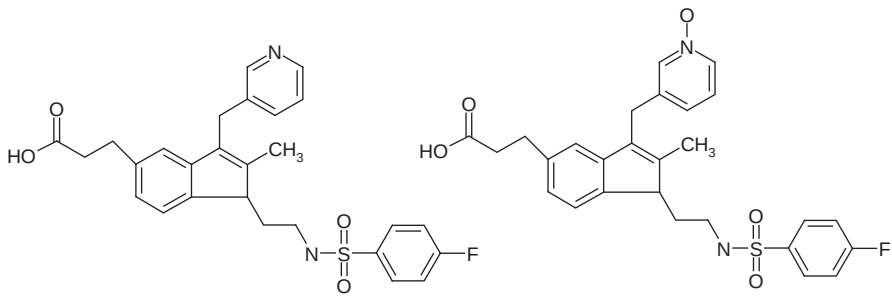


Figure 11.10 Structures of UK-102 333, a combined thromboxane receptor antagonist/synthetase inhibitor, and its *N*-oxide metabolite.

with molecular weight exceeding 500 Da and combining high lipophilicity and high PSA (118–202 Å^2). These drugs have much lower permeabilities with highly polarised flux due to transporters. Although low flux is evident, reasonable bioavailabilities can be achieved mainly due to the high local concentrations of drug present in the gastrointestinal tract after oral administration and resultant saturation of the transporters.

The properties of the protease inhibitors in Table 11.3 (which are discussed in detail in Chapter 10) move the drugs outside the boundaries of ADME space (high PSA and high lipophilicities) and the drugs have all low permeability; their disposition will be highly influenced by transporters as illustrated by the flux across Caco-2 cells.

The low permeability also manifests itself in clearance since most transporter systems work to influx the drug into the hepatocyte or kidney tubule (poor permeability essentially making it a one-way process) and then efflux it into the urine or bile. With poor permeability, the process of metabolism may be secondary to the process of systemic clearance.

Figure 11.10 shows the structure of a combined thromboxane receptor antagonist/synthetase inhibitor and its major *N*-oxide metabolite. The physicochemical properties of the molecule are a log P of 4.3 and a PSA of 106 Å^2.

The compound is therefore likely to be of lower permeability. As an acid with lipophilic regions, it is likely also to be a substrate for organic acid transport protein (OATP) transporters. The biliary excretion and clearance of the compound was monitored in a series of experiments in the isolated perfused rat liver.[34] In the first series of experiments in the untreated rat liver, systemic plasma clearance was around 15 ml min^{-1} kg^{-1} and the drug was excreted alongside its *N*-oxide, which represented the majority portion of the excretion. In a second series of experiments, clearance remained unchanged following ketoconazole pretreatment (a CYP450 inhibitor), but the *N*-oxide metabolite was absent and only the parent was detected in bile. This compound was therefore cleared by hepatic uptake (influx) and metabolism was subsequent to the event (post-clearance metabolism).

Further examples of the interplay between uptake and metabolism with low permeability compounds are provided by studies with HIV protease inhibitors. The intrinsic metabolic clearance of saquinavir, nelfinavir and ritonavir[35] was determined in both rat liver microsomes and fresh isolated rat hepatocytes in suspension. In the absence of metabolism (achieved by pretreating hepatocytes with a mechanism-based inhibitor of cytochrome P450), the protease inhibitors were actively and rapidly taken up into hepatocytes; intracellular unbound drug concentrations were 5- and 12-fold higher than extracellular unbound concentrations. Comparison of the rate of uptake into hepatocytes with the rate of metabolism in hepatocytes and microsomes indicates that the former is the rate-limiting step at low concentrations. These findings explain the rapid clearance of saquinavir, nelfinavir and ritonavir and also indicate that the rate of uptake limits the metabolic clearance of these three drugs. Further studies (using an hepatic cell model, Hep G2 and Xenopus laevis oocytes over-expressing human OATP-A) strongly indicate that basolaterally located OATP-A (influx transporter) in human hepatocytes is the influx transporter and is acting in concert with apically located P-gp and/or MRP2 (efflux transporters) for the vectorial transport and excretion of saquinavir into bile.[36]

11.8 Future CNS Targets and ADME Space

Targeting the CNS has been a rewarding area for drug discovery although the precise nature of the modulations of signalling required, the families of receptors and transporters direct the medicinal chemist to small molecules of simple structure—mainly antagonists of aminergic 7TM receptors or inhibitors of the transporters of the natural agonists of these receptors. In recent years, there has been a pronounced shift from disease areas such as depression and schizophrenia to neurodegeneration to try to meet the 'epidemic' of such diseases as Alzheimer's brought on by an ageing population. The targets for these are ones that, if previously tackled for a particular disease, did not reside in such protected areas as the CNS.

The presence of transport proteins at the 'blood–brain barrier' is of greater significance than in the gastrointestinal tract. Most drugs when given by the

oral route achieve concentrations in the gastrointestinal tract that saturate the efflux transporters. The Km value for transporters such as P-gp is usually 10–100 μM, a concentration that is exceeded by dissolution of the drug in the nominal 250 ml of gastrointestinal fluid. This saturation leads to a higher flux across the gastrointestinal tract. In contrast, unbound drug concentrations in the systemic circulation will rarely achieve concentrations sufficient to saturate the transporters, with most drugs requiring nanomolar plasma concentrations to achieve target efficacy. To gain access to the CNS, drugs have to be sufficiently permeable to have a passive diffusion that is not greatly attenuated by any efflux transport.

This difference largely explains why ADME space for CNS drugs is more restricted, possibly having an upper limit of PSA around 75 $Å^2$ to ensure sufficient permeability. This has not been a problem for many CNS drugs which target aminergic GPCRs, monoamine transporters or enzymes such as acetylcholinesterase.

Table 11.4 lists the physicochemical characteristics and blood–brain barrier penetration of a number of CNS drugs against these targets. It is becoming a bigger issue as drug targets for degenerative diseases of the brain are identified, including proteases, δ and β secretases, *etc*. Table 11.4 includes examples of some of these drugs and highlights the fact that inhibitors of these targets are on the edge of ADME space and will have low relative permeability.

One technical problem with CNS penetration is that the existing datasets are misleading, with many being based on total brain versus total blood concentration. This suggests a much larger dynamic scale. Most of the range is measuring partitioning of the drug into brain lipid rather than the drug that is available to receptors or enzymes. Ideally, unbound drug in the plasma should be compared with brain extracellular fluid concentration. Practically, the most realistic measure is unbound concentration in plasma versus brain cerebrospinal fluid. Figure 11.11 depicts the data in Table 11.4.

Table 11.4 Physicochemtcical parameters of selected drugs and their permeability as measured by cerebrospinal fluid (CSF) to unbound plasma concentration ratio.

	MW	*PSA* ($Å^2$)	*Log P*	*Log D*	*Concentration CSF/Cp free*
Erlotinib	393	75	2.4	2.4	0.3
Nelfinavir	568	127	7.0	6.0	0.09
Indinavir	614	118	2.9	2.9	0.015
Nevirapine	266	58	1.8	1.8	1.5
CP-615 003	373	83	1.8	−0.44	0.14
CP-141 938	403.5	79	−1.11	−1.2	0.11
Fluoxetine	309	21	4.1	1.4	1.1
Propranolol	259	42	3.1	1.2	1.3
Caffeine	194	53	−0.13	−0.13	1.0
Diazepam	284	33	3.0	3.0	1.0

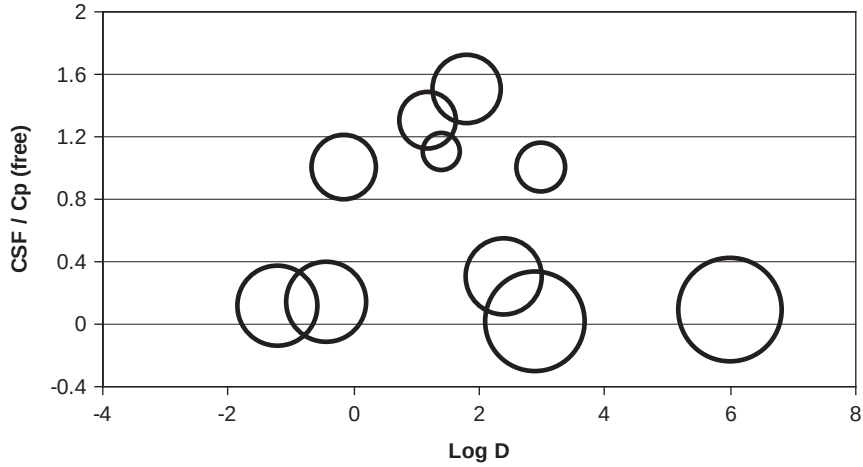
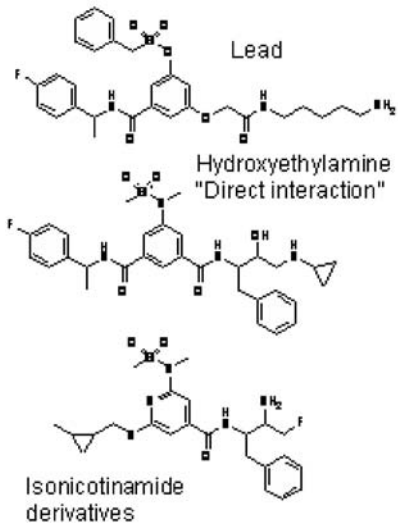


Figure 11.11 Penetration across the blood–brain barrier for the drugs shown in Table 11.3. The graph plots penetration expressed as CSF to unbound drug in plasma ratio against lipophilicity (log P) with the size of point indicative PSA. Note that high molecular weight drugs have penetration decreased by poor permeability due to polar surface area, despite high apparent lipophilicities. These trends are illustrative of the concepts depicted in Figure 11.2.

To access the brain and interact with many of the new drug targets is a major challenge. Figure 11.12 illustrates the properties of a series of β-secretase inhibitors. Aβ peptide is associated with the damage caused during Alzheimer's disease and is formed by processing amyloid precursor protein. β-secretase (BACE-1) is the first enzyme in the cascade; it is an aspartyl protease (a target that is already challenging), made even more difficult by being present inside the CNS. Other aspartyl protease inhibitors such as renin and HIV protease have traditionally started with transition state isosteres, but in this approach new templates were screened for with selectivity to the enzyme.[37] Considerable synthetic diversity and large increases in potency were achieved from this lead. The initial direction was to incorporate a hydroxyethylamine transition state isostere in the molecule.[38] This dramatically improves potency but also increases PSA. Despite good cellular activity of 10 nM, brain penetration was negligible. Reduction of PSA[39] was attempted by truncation of the hydroxyethylamine isostere, initially to a primary alcohol and then to an amine (Figure 11.12). Despite impressive potency, poor pharmacokinetics (including limited transfer across the blood–brain barrier) hamper the series.

The fundamental problem of such targets is the need to maintain considerable hydrogen bonding functionality to achieve selectivity and potency. The resultant high PSA (and consequent permeability issues) attenuates absorption and access to the target, which may be due to high clearance through efflux.



Potency nM	PSA	Log P	MW
1400	117	2.8	484
11	136	2.2	596
2	120	1.4	477

Figure 11.12 β-Secretase (BACE-1) inhibitors, structure and resultant ADME properties which exist on the edge or outside ADME space.

11.9 Penetration into the Cell—Intracellular Drug Targets

In parallel to the above discussion on the blood–brain barrier, any intracellular target requires passage across a cell membrane and similar constraints apply to that discussed above for the blood–brain barrier. Depending on the cell system, efflux and influx transporters may or may not play a significant role.

In attempting to define SAR more directly, many programmes looking at intracellular targets may use a direct enzyme assay and a cell-based assay. For instance to target HCV protease, drugs have to cross the liver cell membrane. Cheng *et al.*[40] used a protease assay to generate intrinsic Ki values and a replicon assay using a Hu-7 cell line to generate cell-based IC_{90} values. These assays often showed a lack of correlation across compounds due to the variability in permeability encountered. In an examination of permeability variability, a series of tetrapeptide/pentapeptide HCV NS3 protease inhibitors were evaluated. The structures included carbamates, esters and acids. The compounds ranged in molecular weight from 564 to 872 Da. The ratios of Ki : IC_{90} ranged from 0.5 to 0.0003. The lowest permeability compounds with the biggest difference between Ki and IC_{90} were acids with negative log $D_{7.4}$ values. Molecular weight showed no correlation with permeability, while there was a low correlation with log $D_{7.4}$ and the Ki : IC_{90} ratio. However, a parallel artificial membrane permeability assay (PAMPA) showed a reasonable correlation. PAMPA is a dodecane-impregnated membrane system, dodecane being a solvent that unlike octanol does not support hydrogen bonding. Although all

compounds had a high PSA ($\geq 163\,Å^2$), compounds with the cell-based IC_{90} closest to the Ki tended to have the lowest PSA and *vice versa*.

11.10 Permeability and Large Molecules

TC-1 is a BACE inhibitor derivative of the series outlined above that achieves good membrane permeability (Figure 11.13). Although it has a PSA of $136\,Å^2$, it achieves good membrane permeability as indicated by its A to B and B to A CaCo-2 membrane flux and its entry into the CNS. The compound has been used for proof of concept studies for BACE inhibition and Aβ lowering in rodents and rhesus monkey.[41] The permeability may be related to the fact that its PSA is comprised of substantially fewer hydrogen bond donors than other protease inhibitors as shown in Table 11.5.

The drug is now cleared almost exclusively by metabolism as described above, particularly by CYP3A enzymes. The rate of metabolism is very high, something that plagues many of the larger molecules, probably because of the flexibility of the active sites of CYP450s and the multiple sites of metabolism that a large molecule can present. Common features of the drug-metabolising enzymes include:[42]

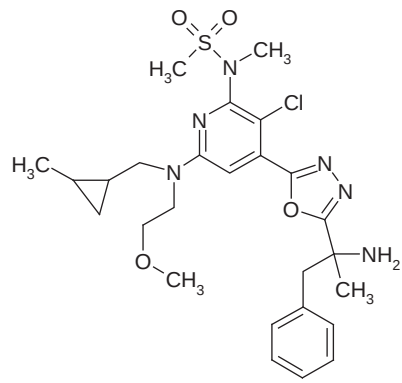


Figure 11.13 TC-1—a BACE inhibitor with good permeability properties. Although PSA remains high ($136\,Å^2$), it is possible that the difference between hydrogen bond donors and acceptors accounts for its permeability characteristics.

Table 11.5 Comparison of number of H-bond donors or acceptors for selected protease inhibitors.

	Number of HBAs	*No. of HBDs*	*PSA*	*MW*
Indinavir	9	4	118	614
Saquinavir	11	6	167	671
TC-1	8	2	136	563

- large substrate-binding cavities;
- binding of more than one substrate/effector;
- binding of substrates in alternative orientations and locations;
- rotation of a substrate at the active site;
- substantial substrate-induced conformational changes.

Studies of human CYP3A4 in complex with two well-characterized drugs, ketoconazole and erythromycin,[43] have shown the protein undergoes dramatic conformational changes upon ligand binding, with an increase in the active site volume by $>80\%$. Two distinct open conformations of CYP3A4 can be observed, which ketoconazole and erythromycin induce upon binding. Moreover, two molecules of ketoconazole can be observed in the CYP3A4 active site and, of particular importance to large molecules, multiple binding modes for erythromycin.

The example of indinavir[41] shows the many orientations a large molecule can adopt in CYP3A4 and the number of vulnerable sites such a molecule can contain. Major CYP3A4 metabolites of indinavir include pyridine *N*-oxidation, indane hydroxylation, removal of the methylpyridine, and to a lesser extent para phenyl hydroxylation. These can be carried out as a single oxidation or multiple consecutive oxidations.

The flexibility of the proteins and their resultant promiscuity has meant that progress in understanding metabolism from knowledge of the protein structure has been hampered. The concept that X-ray crystallography of CYPs and their substrates would lead to better drug design has not been fulfilled. It is likely that, in future, medicinal chemists will follow the largely empirical process of eliminating vulnerable sites of metabolism in compounds wherever possible. We have described the need for better ways of fluorination to aid in this process. The use of fluorine (due to its size, electronic characteristics and possible hydrogen bonding role) is widespread; Figure 11.14 illustrates the use of fluorine to help stabilise a thrombin inhibitor[44] and a bradykinin B antagonist.[45]

11.11 Conclusion: Beyond PSA and ADME Space

In this chapter we have suggested that most of the future drug targets will come from known gene families. Some of these have binding sites that dictate the ligands having properties on the edge of those required for acceptable ADME properties. Moreover, some of the most attractive targets reside in locations requiring even greater constraints on physicochemical properties such as the CNS. One of the problems in the way most physicochemical properties are calculated is that the fragmental methods used do not allow, in most cases, for conformational differences and proximity effects. On closer examination of the drug atazanavir (Figure 11.4), it could be envisaged that this molecule has a conformation that allows for internalisation of some of its polar groups through intramolecular hydrogen bonding. The internal hydrogen bonding network could nullify hydrogen bonding (PSA) particularly when in an aprotic

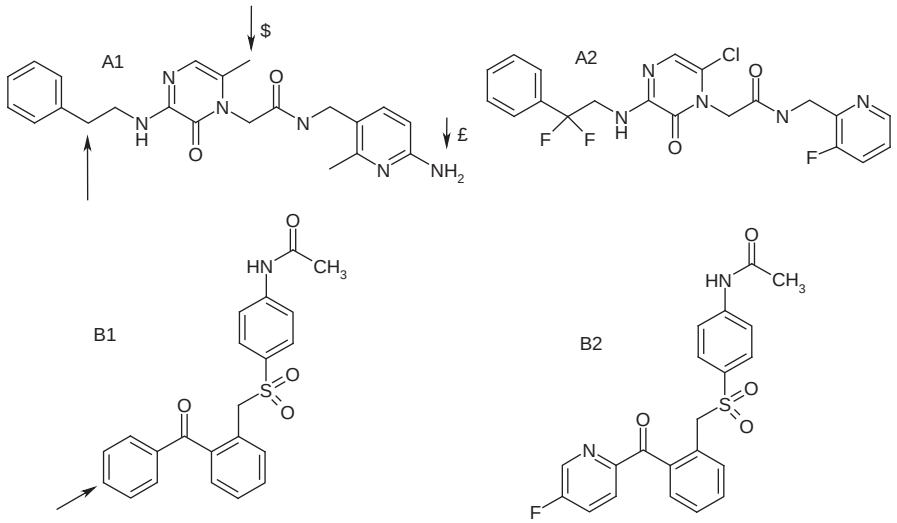


Figure 11.14 Stabilisation to oxidation using fluorine (and chlorine, $) and conjugation (removal of functionality, £) for A1 and A2, a thrombin inhibitor, and B1 and B2, a bradykinin receptor antagonist.

lipophilic environment such as a membrane interior. Such behaviour has been demonstrated for cyclosporin, a drug that significantly changes conformation moving from solvent into aprotic environments. At present there is a paucity of methods to predict such behaviour and yet, if available, compounds could be designed against targets requiring considerable hydrogen bonding interactions and yet were permeable to membranes.

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Subject Index