TOPICS IN HETEROCYCLIC CHEMISTRY

02

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Heterocyclic Antitumor Antibiotics

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The series *Topics in Heterocyclic Chemistry* presents critical reviews on "Heterocyclic Compounds" within topic-related volumes dealing with all aspects such as synthesis, reaction mechanisms, structure complexity, properties, reactivity, stability, fundamental and theoretical studies, biology, biomedical studies, pharmacological aspects, applications in material sciences etc. Metabolism will be also included which will provide information useful in designing pharmacologically active agents. Pathways involving destruction of heterocyclic rings will also be dealt with so that synthesis of specifically functionalized non-heterocyclic molecules can be designed.
The overall scope is to cover topics dealing with most of the areas of current trends in heterocyclic chemistry which will suit to a larger heterocyclic community.
As a rule contributions are specially commissioned. The editors and publishers will, however, always be pleased to receive suggestions and supplementary information. Papers are accepted for *Topics in Heterocyclic Chemistry* in English.
In references *Topics in Heterocyclic Chemistry* is abbreviated *Top Heterocycl Chem* and is cited as a journal.

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Preface to the Series

Topics in Heterocyclic Chemistry presents critical accounts of heterocyclic compounds (cyclic compounds containing at least one heteroatom other than carbon in the ring) ranging from three members to supramolecules. More than 50% of billions of compounds listed in *Chemical Abstracts* are heterocyclic compounds. The branch of chemistry dealing with these heterocyclic compounds is called heterocyclic chemistry, which is the largest branch of chemistry and as such the chemical literature appearing every year as research papers and review articles is vast and can not be covered in a single volume.

This series in heterocyclic chemistry is being introduced to collectively make available critically and comprehensively reviewed literature scattered in various journals as papers and review articles. All sorts of heterocyclic compounds originating from synthesis, natural products, marine products, insects, etc. will be covered. Several heterocyclic compounds play a significant role in maintaining life. Blood constituent hemoglobin and purines as well as pyrimidines, are constituents of nucleic acid (DNA and RNA) are also heterocyclic compounds. Several amino acids, carbohydrates, vitamins, alkaloids, antibiotics, etc. are also heterocyclic compounds that are essential for life. Heterocyclic compounds are widely used in clinical practice as drugs, but all applications of heterocyclic medicines can not be discussed in detail. In addition to such applications, heterocyclic compounds also find several applications in the plastics industry, in photography as sensitizers and developers, and in dye industry as dyes, etc.

Each volume will be thematic, dealing with a specific and related subject that will cover fundamental, basic aspects including synthesis, isolation, purification, physical and chemical properties, stability and reactivity, reactions involving mechanisms, intra- and intermolecular transformations, intra- and intermolecular rearrangements, applications as medicinal agents, biological and biomedical studies, pharmacological aspects, applications in material science, and industrial and structural applications.

The synthesis of heterocyclic compounds using transition metals and using heterocyclic compounds as intermediates in the synthesis of other organic compounds will be an additional feature of each volume. Pathways involving the destruction of heterocyclic rings will also be dealt with so that the synthesis of specifically functionalized non-heterocyclic molecules can be designed. Each

volume in this series will provide an overall picture of heterocyclic compounds critically and comprehensively evaluated based on five to ten years of literature. Graduates, research students and scientists in the fields of chemistry, pharmaceutical chemistry, medicinal chemistry, dyestuff chemistry, agrochemistry, etc. in universities, industry, and research organizations will find this series useful.

I express my sincere thanks to the Springer staff, especially to Dr. Marion Hertel, executive editor, chemistry, and Birgit Kollmar-Thoni, desk editor, chemistry, for their excellent collaboration during the establishment of this series and preparation of the volumes. I also thank my colleague Dr. Mahendra Kumar for providing valuable suggestions. I am also thankful to my wife Mrs. Vimla Gupta for her multifaceted cooperation.

Jaipur, 31 January 2006 R.R. Gupta

Preface

The field of heterocyclic chemistry has grown significantly in the past decade, especially in areas involving the development of new synthetic methodologies and the discovery of medicinally active compounds. Much of the progress has come from discoveries made in academic and industrial laboratories.

This monograph contains six chapters, each providing an authoritative review of the past five to ten years research on a specific class of compounds and derivatives thereof. In each of the chapters, detailed synthetic methodologies are presented, along with highlights of the anticancer and antibiotic activities of selected compounds. The anticancer section comprises the first four chapters, which cover heterocyclic derivatives of stilbene and chalcone derivatives, followed by pyrrole-containing compounds related to the natural products lukianol A and B, derivatives of α-, β-, γ- and δ-carbolines, as well as diazo and diazonium DNA cleavage agents. The remaining two chapters describe recent developments in the field of novel synthetic antibacterial agents and β-lactam-based inhibitors of β-lactamases.

The editor gratefully acknowledges Dr. Toni Brown and Dr. Hilary Mackay for their assistance in assembling this monograph.

Holland, MJ, March 2006 Moses Lee

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Microwave-Assisted Synthesis of Heterocycles

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Synthesis of Biologically Active Heterocyclic Stilbene and Chalcone Analogs of Combretastatin

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Abstract Combretastatin A-4 (CA-4, **7**) has had a major impact in the field of medicinal chemistry as a potent bioactive molecule that binds to the cholchicine site of tubulin. However, its poor water solubility spurred a wealth of research into analogs to overcome these pharmakokinetic deficiencies. The focus of this chapter is the recent synthesis of novel and interesting biologically active heterocyclic analogs of CA-4, **7** that possess the stilbene and chalcone core. This review will also discuss alternative methods of synthesizing potentially biologically active derivatives of CA-4, **7**, reported in the last 5 years.

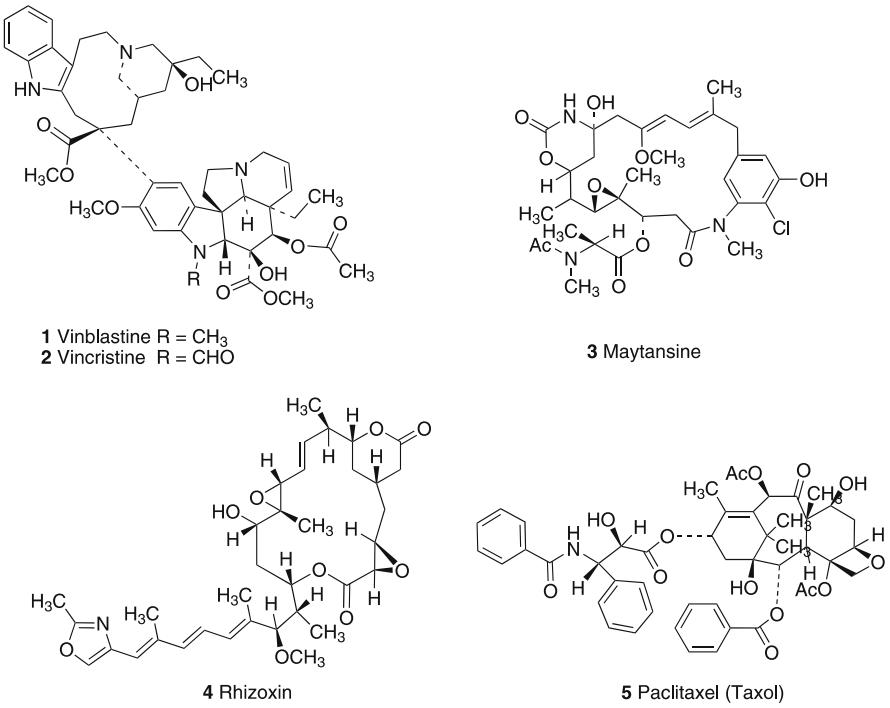
Keywords Chalcone · Colchicine · Combretastatin · Stilbene · Tubulin

1 Introduction

1.1 Tubulin

Tubulin, a globular protein of molecular weight 100 000, forms heterodimers in the presence of guanosine triphosphate. Microtubules are arrangements of the α,β-dimers into polymeric tubes and are hollow cylinders (outer and inner diameters, 24 nm and 15 nm, respectively). Microtubules are polar structures and are long protein fibers that exist in dynamic equilibrium with the tubulin dimer. Microtubules are vital components of the cell and are responsible for several important functions including intracellular transport, formation of the mitotic apparatus, mechanically stabilizing cellular processes, and formation of the mitotic spindle during cell division [1, 2]. A crystal structure of tubulin has been recently disclosed [3].

Antimitotic agents are tubulin binders that work by microtubule depolymerization or destabilization. There are currently five compounds in the standard agents database that are classified as tubulin binders: vinblastine (**1**), vincristine (**2**), maytansine (**3**), rhizoxin (**4**), and paclitaxel (Taxol, **5**). Taxol (**5**) is the only compound from this class that promotes the assembly of microtubules, resulting in highly stable, nonfunctional polymers and is used in the treatment of ovarian and mammalian cancer [4–6]; the others inhibit tubulin polymerization by binding to the same site of tubulin [7, 8]. Vinblastine (**1**) and vincristine (**2**) belong to a large class of compounds known as the vinca alkaloids, which were isolated from the Madagascar periwinkle



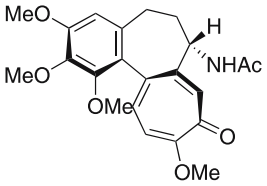
Structure **1** vinblastine, **2** vincristine, **3** maytansine, **4** rhizoxin and **5** paclitaxel (Taxol)

(*Catharanthus roseus*) [9]. Maytansine (**3**) is an ansa macrolyde isolated from *Maytenus ovatus* [10], and rhizoxin (**4**) is an antitumor macrolide isolated from the fungus *Rhizopus chinensis* [11]. Another very important tubulin interactive anti-cancer agent is colchicine (**6**), and this compound binds to a different binding site of tubulin but is also used in anti-cancer therapy.

1.2 Colchicine

Colchicine (**6**) was isolated by Pelletier and Caventou in 1820 and is the main alkaloid of the poisonous meadow saffron plant (*Colchicum autumnale L.*) [12–16]. Following some considerable debate over colchicine's structure [17–20] and its successful synthesis [21–26], colchicine was found to bind to tubulin at what is referred to as the colchicine binding site [1, 27].

Colchicine (**6**) is used in the treatment of a broad range of diseases including acute gout and Mediterranean fever [28] and induces depolymerization of tubulin. This compound (**6**) distorts the tubulin/microtubule equilibrium by binding to the tubulin dimer and halting mitosis in the metaphase. The reason this approach is such a successful target in cancer therapy is that



6 (-)-(a*R*,7*S*)-colchicine

Structure **6** colchicine

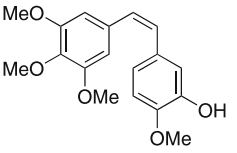
spindle poisons exert their influence when mitosis is in the metaphase—hence the large amount of research being performed in this area. However, the utility of colchicine (**6**) as an anti-cancer agent is seriously hampered by its toxicity [29]. Thus, research has focused on the discovery of molecules that are as effective as tubulin binders, but are less toxic than colchicine.

1.3 Combretastatins

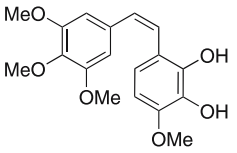
Combretastatins are a class of compounds originally derived from the African Willow tree (*Combretum caffrum*) and are powerful reversible inhibitors of tubulin polymerization. This class of molecules has been shown to bind to the colchicine binding site of tubulin, by the same mode of action as mentioned above (Sect. 1.2). Combretastatins consist of a *cis*-stilbene core structure. To date, there have been several compounds that have shown promise as potential anticancer drugs. However, development of these compounds as anticancer agents is limited by issues of chemical stability, bioavailibilty, toxicity, and solubility.

The most famous of these compounds is combretastatin A-4 (CA-4, **7**), isolated by Pettit et al. in 1989 [30]. Pettit's research led to the isolation and structural determination of a series of phenanthrenes, dihydrophenanthrene, stilbene, and bibenzyl compounds [31]. CA-4 (**7**), alongside CA-1 (**8**), was found to be an extremely active inhibitor of tubulin polymerization [30, 32]. The major problems associated with these compounds were poor bioavailability and low aqueous solubility [33, 34], and hence, research in the field was turned to designing better alternatives with the hope of eradicating the negative properties of these potent compounds.

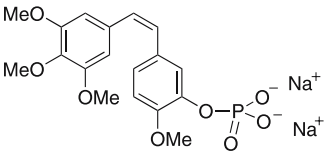
Following the synthesis of the sodium, potassium, and succinic acid esters of CA-4, which were not soluble in water [35], CA-4P (**9**), the disodium phosphate pro-drug was developed and is currently in phase II of clinical trials [36]. CA-4P is a promising candidate for combination anti-cancer therapy because it is inactive as a phosphate but is rapidly hydrolyzed in vivo to the active CA-4, **7** compound [31, 37].



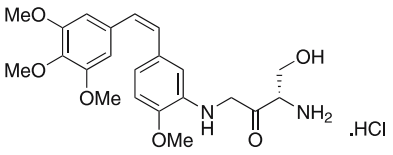
7 Combretastatin A-4 (CA-4)



8 Combretastatin A-1 (CA-1)



9 Combretastatin A-4P (CA-4P)



10 AVE8062 (AC-7700)

Structure **7** CA4, **8** CA-1, **9** CA-4P, **10** AVE8062 (AC-7700)

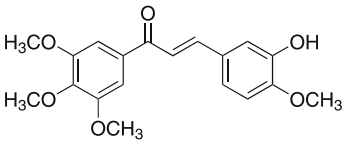
Other analogs of CA-4, **7** have been developed and are also in clinical trials. These include AVE8062 (formerly known as AC-7700, **10**), a water soluble analog [33].

1.4 Chalcones

Chalcones (including **11**) contain a 1,3-diaryl-α,β-unsaturated ketone moiety and have anti-cancer properties [38]. As analogs of CA-4, **7**, the mode of cytotoxic action of chalcones has been shown to be similar to the combretastatins. They bind to the colchicine site of tubulin and inhibit tubulin polymerization [39].

1.5 Focus of This Review

There are a number of reviews published in the field of tubulin binders as anti-cancer agents, but these mostly focus on the cytotoxicity of combretastatins and chalcones [1, 40, 41]. There has also been much published on



11

Structure

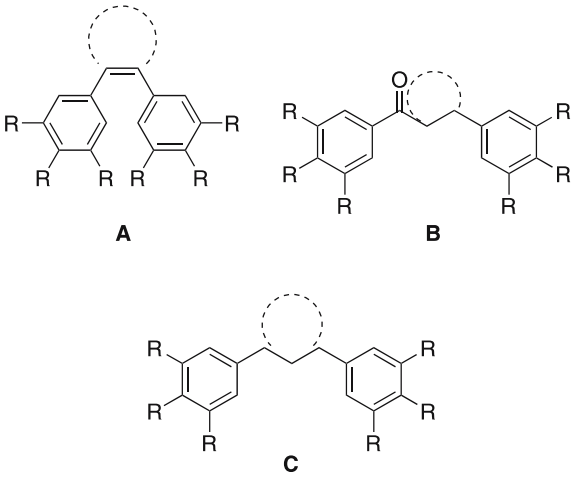


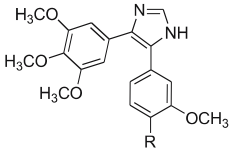
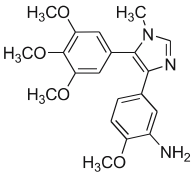
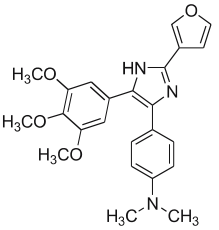
Fig. 1 General structure of a stilbene heterocyclic derivative (**A**), and two chalcone heterocyclic derivatives (**B** and **C**). (*Dashed circles* represent the location of the herocyclic ring)

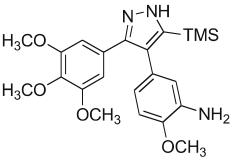
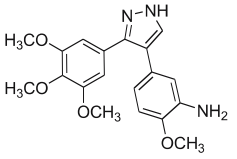
different analogs of these compounds that make variations to the phenyl rings either-side of the enone or stilbene core (e.g., benzophenone derivatives [42–44], and Lavendustin A derivatives [45]). As a result, this chapter will discuss heterocyclic analogs of these two classes of compounds with the main focus relating to the synthesis of biologically active heterocyclic analogs of the combretastatins and chalcones. Figure 1 shows the generic structures of the types of compounds to be included.

2 Stilbene Heterocyclic Derivatives

A number of biologically active stilbene compounds have been reported that contain different heterocyclic rings derived from the stilbene core of the molecule (Fig. 1A); these can be divided into two categories: aromatic and non-aromatic. Within each section a further division can be made: five- and six-membered rings. The five-membered aromatic rings include imidazole, pyrazole, triazole, furazan, oxazole, and thiazole. Non-aromatic rings include dihydrooxazole, furanone, dihydrofuran, oxazolone, and dihydrothiophene. A number of fused ring systems exist. These usually consist of a five-membered heterocycle fused to a phenyl ring, e.g., benzothiophene, benzofuran, and benzindole. In the aromatic six-ring category, biological activity was observed for pyrazine- and pyridine-containing molecules. No six-membered non-aromatic heterocyles with biological activity were found in the search.

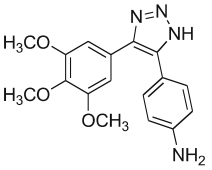
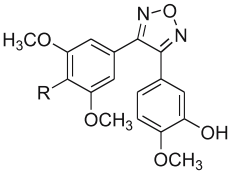
Table 1 Biologically Active Stilbene Derivatives

5-Membered aromatic rings		HCT-15 IC_{50} nM	NCI-H460 IC_{50} nM	Anti-tubulin IC_{50} μM
CA4 7	[34, 40]	1.7	3.0	1.2
	[34, 40]			
	17 R = NH_2	8.1	8.5	0.68
	R = OH	10	11	0.73
	[34, 40]			
	19	79	34	
	[34, 40]			
	23	67	190	

		Colon 26 IC_{50} nM	Anti-tubulin IC_{50} μM
	[50]	> 3000	> 10
	[50]		
	40	8.4	3

HCT-15 (human colon adenocarcinoma, MDR positive), NCI-H460 (human lung large cell carcinoma, MDR negative), Colon 26 (murine colon), B16 (murine melanoma), SH-SYSY (human neuroblastoma), HL-60 (human leukemia), A549 (human lung cancer), MCF7 (human breast cancer), SK-MEL-2 (human melanoma), HCT-116 (human colon carcinoma), A431 (human epidermal carcinoma), PC-3 (human prostate tumor)

Table 1 (continued)

		B16 IC_{50} nM
	[52] **43**	 56
		SH-SY5Y IC_{50} nm
CA4 7 	[54] [54] R = OCH_3 **53b** R = OH	5.8 1.4 17.5

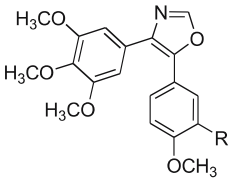
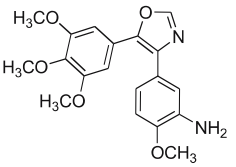
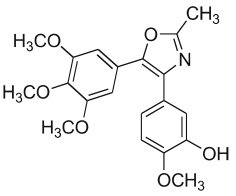
		HCT-15 IC_{50} nM	NCI-H460 IC_{50} nM	Anti-tubulin IC_{50} μM
	[34, 40] R = NH_2 **54** R = OH	 11 2.2	 9.2 2.3	 0.92 0.98
	[34, 40] **55**	 7.2	 11	
	[34, 40] **57**	 15	 35	

Table 1 (continued)

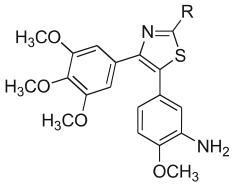
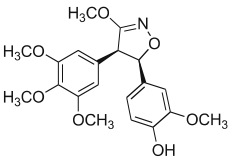
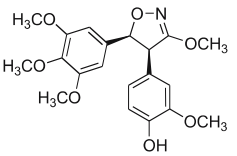
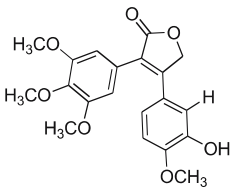
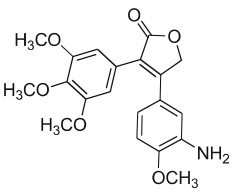
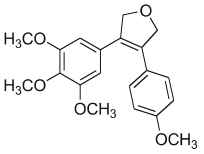
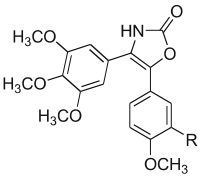
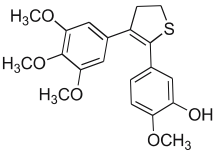
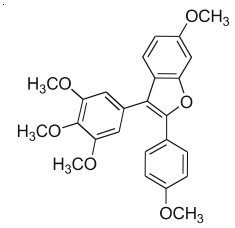
		Colon 26 IC_{50} nM	Anti-tubulin IC_{50} mM
	[50]		
	67b R = NH_2	57.5	1
	69 R = H	14.5	3
5-Membered non-aromatic rings		HL60 IC_{50} μM	
	[60]		
	70	0.1	
	[60]		
	71	0.25	
		A549 IC_{50} nM	MCF-7 IC_{50} nM	SK-MEL-2 IC_{50} nM
	[61]			
	78	16.3	11.4	10.2
	[61]			
	77b	5.3	4.7	3.3

Table 1 (continued)

		B16 IC_{50} ng mL^{-1}	HCT-116 IC_{50} ng mL^{-1}
	[68] **86**	> 1000	> 1000

		PC-3 IC_{50} nM	A549 IC_{50} nM	MCF-7 IC_{50} nM	B16 IC_{50} nM	HCT-16 IC_{50} nM
CA-4 7	[69]	2.7	2.1	2.7	1.0	0.9
	[69] R = OH	6.4	7.9	5.7	5.4	6.1
	91 R = NH_2	2.1	3.8	4.9	2.4	3.7

		MCF-7 IC_{50} nM	Anti-tubulin IC_{50} μM
	[70] **98**	390	3.6

5-Membered fused aromatic rings		MCF-7 IC_{50} nM	Anti-tubulin IC_{50} mM
CA-4 7	[73]	11	2.1
	[73] **108**	> 1000	> 40

There are a number of other compounds that make modifications to the A- and B-ring of the combretastatin derivatives; however, these molecules are outside the scope of this review.

Table 1 contains representative examples of compounds with biological activity in a variety of cell lines. The synthesis of the most potent compounds in each section will be discussed, followed by alternative methods of producing these important compounds.

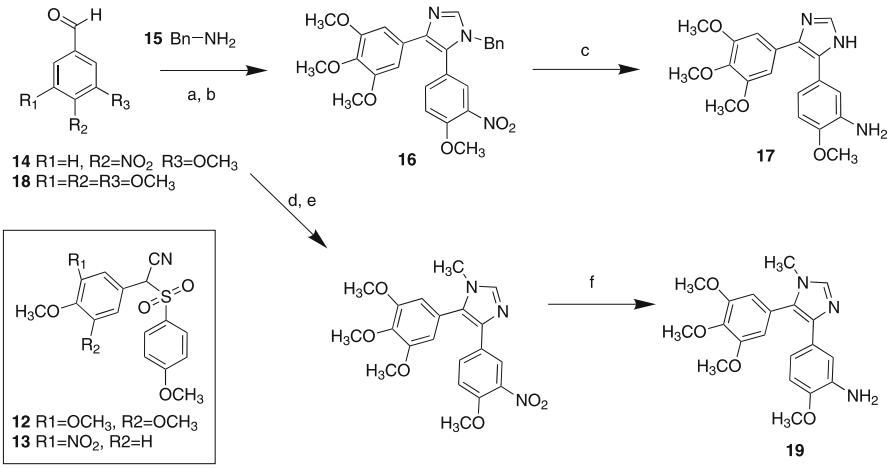
2.1 5-Membered Aromatic Rings

2.1.1 Imidazole Compounds

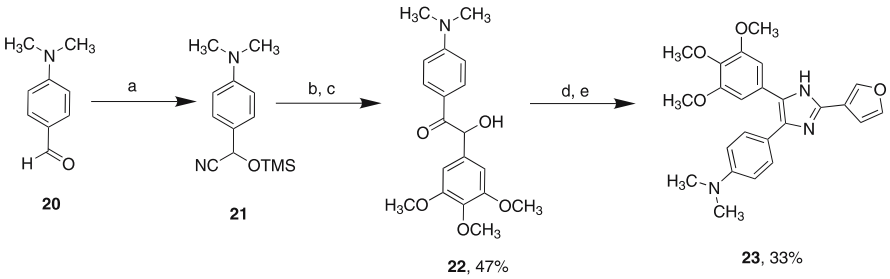
Synthesis of two different 4,5-disubstituted imidazole compounds were described by Wang et al. [34] and required the formation of tosmic reagents, **12** and **13** (Scheme 1). This reagent was formed via the reaction of substituted benzaldehydes with *p*-toluene sulfinic acid, formamide, and catalytic 10-camphorsulfonic acid to produce a methoxybenzenesulfonyl-formamido-intermediate. Further reaction with $POCl_3$ yielded the tosmic reagents **12** and **13**. Reaction of 3-nitro-4-methoxy-1-benzaldehyde (**14**) with benzylamine (**15**) produced an imine that was reacted with the aforementioned tosmic reagent **12**, to form the benzyl-protected imidazole **16**. Transfer hydrogenation with ammonium formate and palladium on carbon produced the imidazole-amino-stilbene analog **17**. Wang et al. [34] also described the synthesis of a second methylimidazole derivative **18**. A similar synthetic approach was followed except the tosmic reagent **13** was used, and a different imine intermediate was employed. The final amino-compound **19** was obtained by reduction using palladium on activated carbon catalytic hydrogenation (Scheme 1).

The synthesis of the furan-imidazole derivatives, shown in Scheme 2, were also described by Wang et al. [34]. Reaction of 4-(dimethylamino)benzaldehyde (**20**) with trimethylsilylcyanide (TMS)-CN in the presence of ZnI_2 produced the TMS cyanohydrin **21**. Compound **21** was treated with LDA followed by the addition of 3,4,5-trimethoxybenzaldehyde to give the benzoin intermediate **22**. Oxidation with $CuSO_4$ in aqueous pyridine, followed by reaction with 3-furaldehyde in acetic acid, produced the substituted imidazole **23**.

The synthesis of methylimidazole-thiophene compounds was reported by Santos et al. [46] and has been included for completeness, although no biological activity has been reported for these heterocycles. The formation of these imidizole-thiophenes (**24a–d**), occurs via the condensation of 2-formylthiophene (**25**) with benzil derivatives (**26a–d**) in the presence of ammonium acetate to yield the imidazole-thiophene compounds (**27a–d**). These compounds can then be *N*-methylated by treatment with iodomethane in



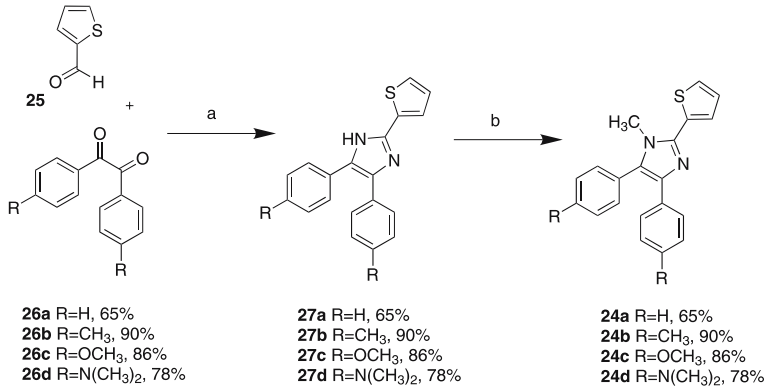
Scheme 1 **a** EtOH, catalytic AcOH, reflux; **b** EtOH/DME (6 : 4), K_2CO_3, **12**; **c** 5% Pd/C, $HCOONH_4$, MeOH, reflux; **d** MeOH, CH_3NH_2, reflux, catalytic AcOH; **e** K_2CO_3, EtOH/DME (6 : 4), **13**; **f** 5% Pd/C, H_2, EtOAc [34]



Scheme 2 **a** TMSCN, ZnI_2; **b** LDA, 3,4,5-trimethoxybenzaldehyde, THF, – 78 °C; **c** aq. HCl; **d** pyridine, $CuSO_4.5H_2O$, reflux; **e** 3-furancarboxyaldehyde, NH_4OAc, reflux [34]

the presence of potassium carbonate to produce compounds **24a–d** in yields ranging from 65 to 90%.

Santos et al. [46] also describe the synthesis of multiple analogs of these compounds (e.g. compounds **28a–31d**). In addition, a number of interesting derivatives of these compounds were synthesized using similar conditions employing microwave technology by Usyatinsky and Khemelnitsky [47]. After only 1.5 minutes in a domestic microwave oven, a wide variety of these central bi-aryl compounds obtained. Solid-phase synthesis employing a similar reaction as described in Scheme 3 was reported by Sarshar et al. [48]. The most interesting compound produced in this manner was compound **33**. These compounds, produced using solid-phase synthesis, were designed as modulators of P-glycoprotein-mediated multidrug resistance in CEM/VLB 1000 cells. They were found to be at least an order of magnitude more po-

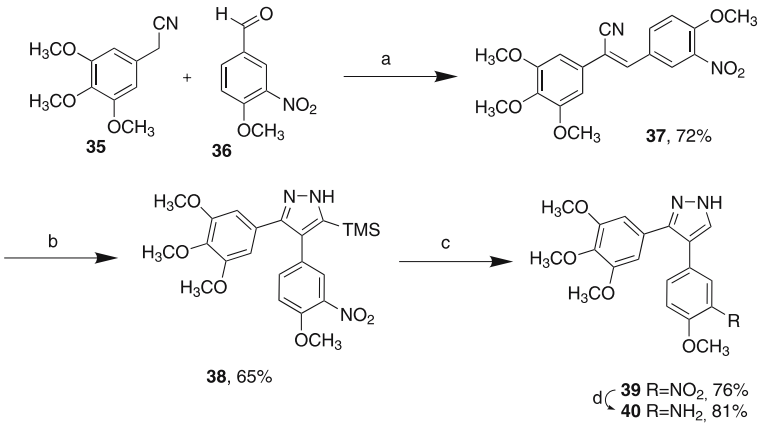


Scheme 3 **a** NH_4OAc, HOAc, 120 °C; **b** MeI, K_2CO_3, 55 °C [46]

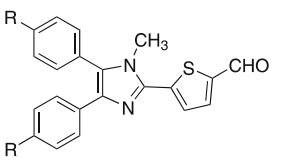
tent than a known drug, Verapamil, against a variety of resistant cell lines (compound **33**, ED_{50} = 0.08 μM, CEM/VLB 1000). Compound **33** completely resensitizes two cell lines (MCF7/ADR and MES-SA/DX5) in the presence of Taxol (**5**) [48]. Kozaki et al. have synthesized a series of dimer-type compounds (e.g., **34**) using chemistry similar to that described in Scheme 3 [49].

2.1.2 Pyrazole Compounds

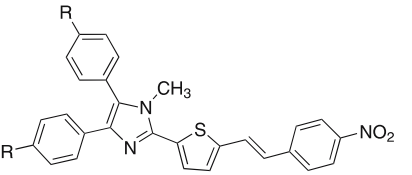
Synthesis of a pyrazole derivative with an amino group in position 3 in the B-ring was described by Ohsumi et al. [50]. Phenylacetronitrile (**35**)



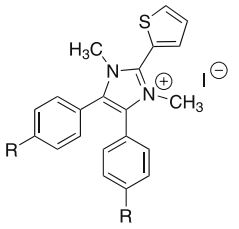
Scheme 4 **a** 1 M aq. NaOH, trimethyloctylammonium chloride, CH_2Cl_2, rt; **b** Lithium trimethylsilyl diazomethane, THF, – 78 °C; **c** 10% aq. KOH, EtOH, reflux; **d** Zn, AcOH, rt [50]



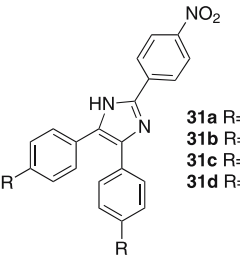
28a R=H, 75%
28b R=CH_3, 65%
28c R=OCH_3, 67%
28d R=$N(CH_3)_2$, 43%



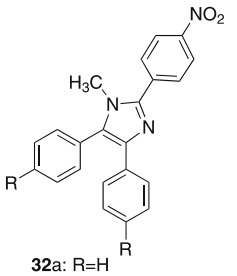
29a R=H, 68%
29b R=CH_3, 40%
29c R=OCH_3, 75%
29d R=$N(CH_3)_2$, 53%



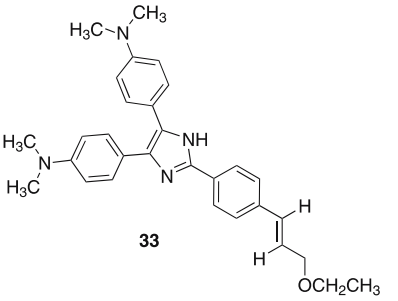
30 R=OCH_3



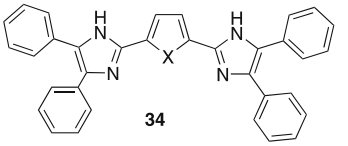
31a R=H
31b R=CH_3
31c R=OCH_3
31d R=$N(CH_3)_2$



32a: R=H
32b: R=CH_3
32c: R=OCH_3
32d: R=$N(CH_3)_2$



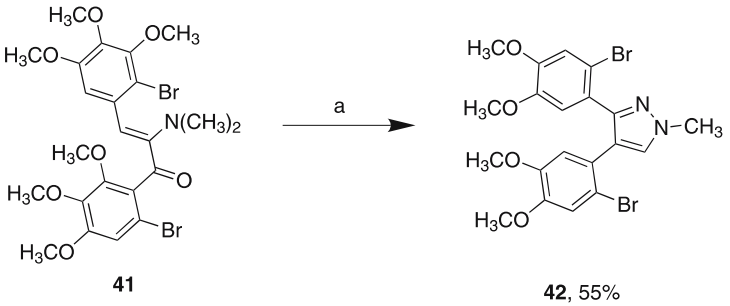
33



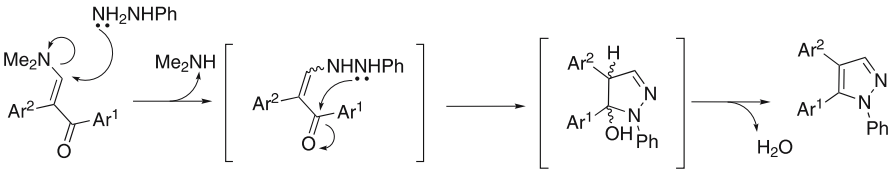
34

Structure

and nitrobenzaldehyde (**36**) were condensed in aqueous NaOH to give the *Z*-acrylonitrile intermediate (**37**) that was treated with lithium trimethylsilyl diazomethane to give the TMS-protected nitro-pyrazole derivative (**38**) in good yields. The TMS group was removed by aqueous 10% KOH to produce the nitro-compound (**39**) and the amino group was formed by re-



Scheme 5 **a** CH_3NHNH_2 [51]



Scheme 6 Mechanism of pyrazole synthesis [51]

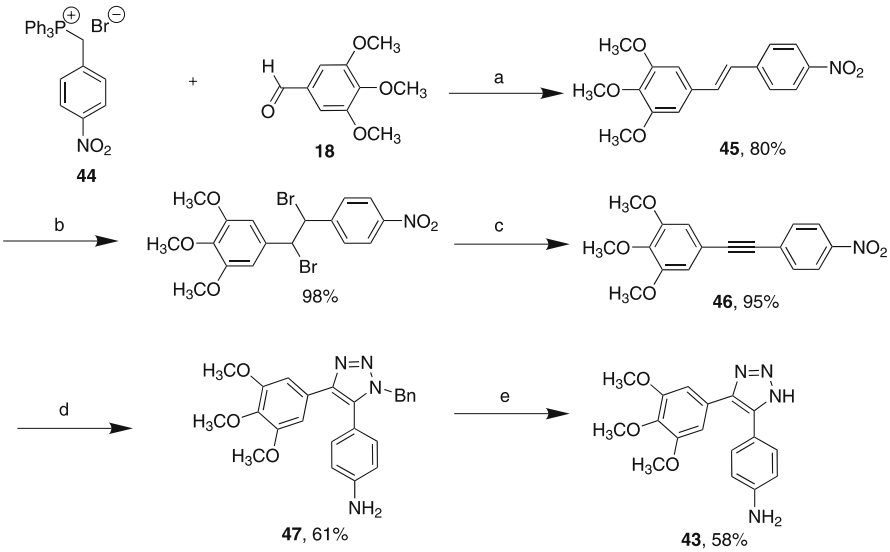
duction with Zn/AcOH to give the corresponding aniline derivative (**40**), Scheme 4.

A second method of producing pyrazole-containing compounds was described by Olivera et al. [51] and involves the use of an enaminoketone (**41**) (Scheme 5). The initial amine-exchange/heterocyclization produced pyrazole tautomers, so another method was attempted with NH_2NHMe. However, two isomers were produced with the methyl group on either nitrogen. Compound **42** was formed in 55% yield.

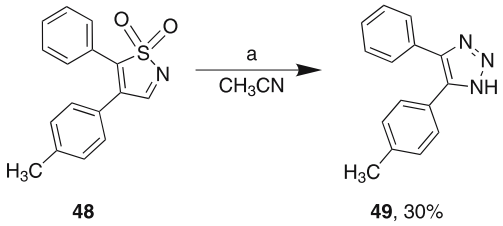
The authors also described a typical butyllithium organometallation reaction to produce the various compounds of interest. A possible mechanism for the reaction of enaminoketones with NH_2NHPh is shown in Scheme 6 [51].

2.1.3 Triazole Compounds

Synthesis of the amino-triazole derivative (**43**) was performed in the authors' laboratory by Pati et al. [52] (Scheme 7). Substituted benzyl bromide was reacted with triphenylphosphine to produce the phosphonium bromide starting material, **44**. The Wittig reagent, obtained by treatment with sodium hydride, was reacted with 3,4,5-trimethoxybenzaldehyde **18** to generate the nitro-stilbene **45** in good yields. The alkyne **46** was obtained by bromination of the stilbene, followed by didehydrobromination. Compound **46** was then reacted under thermal conditions with benzyl azides



Scheme 7 **a** DMF, rt, 16 h; **b** Br_2, CH_2Cl_2, 16 h; **c** KOtBu, *t*-BuOH, 50 °C, 5 h; **d** BnN_3, toluene, reflux, 18 h; **e** H_2, 10% Pd/C, THF, rt, atm. pressure, ~ 18 h [52]



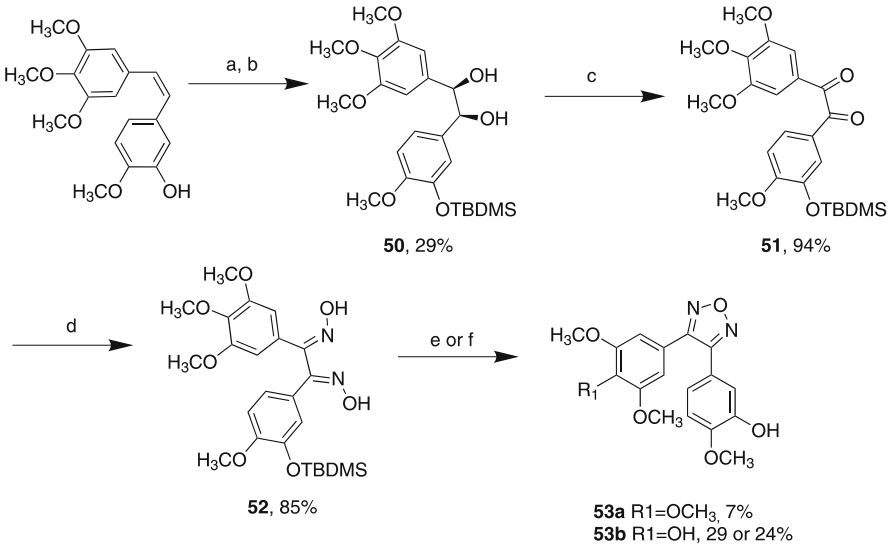
Scheme 8 **a** NaN_3, CH_3CN [53]

to form the benzyl protected-1,2,3-triazole **47** in modest yield. Removal of the protecting group by catalytic hydrogenation yielded the triazole amino-compound **43**. Although the reported cytotoxic activity of these compounds is modest, these compounds are interesting from the perspective of solubility and warrant further investigation in the realm of medicinal chemistry.

Synthesis of another triazole derivative was described by Clerica et al. [53]. This synthetic strategy involved reaction of an isothiazole derivative (e.g., compound **48**) with an equimolecular quantity of NaN_3 in a variety of solvents, e.g., different alcohols, THF, etc. Acetonitrile was used to produce compound **49** in a 30% yield, Scheme 8.

2.1.4
Furazan Compounds

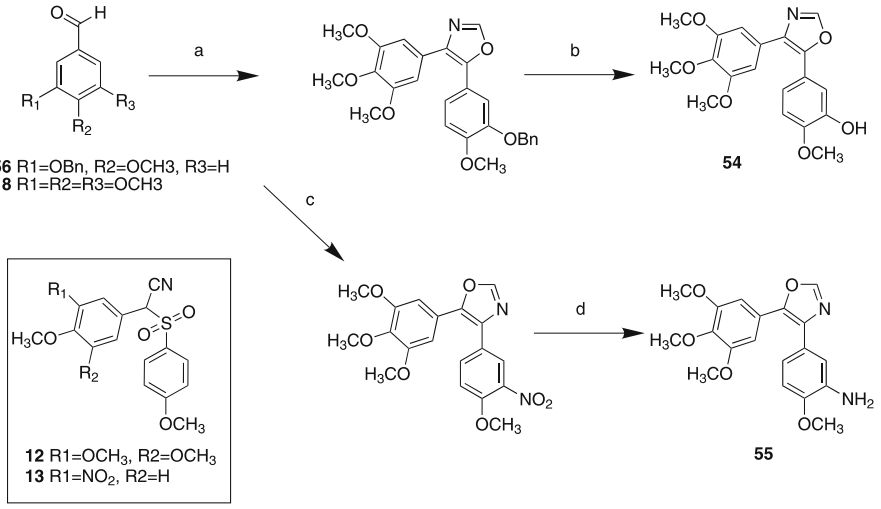
Another interesting class of five-membered aromatic heterocycles has recently been published by Tron et al. [54]. These compounds have biological activity in the nM range. An example of the formation of these furazan (1,2,5-oxadiazole) derivatives is shown in Scheme 9. The diol **50** was oxidized to the diketone **51** using TEMPO and sodium hypochlorite. Transformation to the bisoxime **52** was performed in an excess of hydroxylamine hydrochloride and pyridine at high temperature for several days. Basic dehydration of **52** formed two products (**53a** and **b**). A Mitsunobu reaction was then employed using toluene as solvent to form compound **53b** in 24% yield.



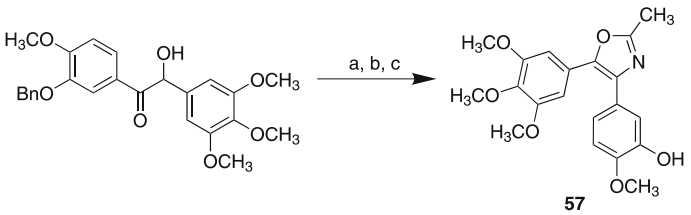
Scheme 9 **a** $TBDMSiCl_2$, imidazole, CH_2Cl_2; AD mix α (Sharpless asymmetric dihydroxylation reagent), methanesulfonamide, H_2O/*t*-butyl alcohol; **b** NaOCl, KBr, TEMPO in CH_2Cl_2/H_2O; **c** $NH_2OH \cdot HCl$, pyridine/EtOH, 90 °C; **d** NaOH, 1,2-propanediol, 160 °C; **e** PPh_3, DIAD, toluene, 0 °C, reflux [54]

2.1.5
Oxazole Compounds

The biologically active oxazole compounds were synthesized by Wang et al. [34], and two types of isomers were described: those with N1 pointing towards the A-ring (e.g., **54**) and those with N1 positioned closest to the B-ring (e.g., **55**), Scheme 10. Tosmic reagents **12** and **13** were used for this synthesis as described in Sect. 2.1.1, Scheme 1. The chemistry described



Scheme 10 **a** EtOH/DME (6 : 4), K_2CO_3, **12**; **b** 5% Pd/C, $HCOONH_4$, MeOH, reflux; **c** EtOH/DME (6 : 4), K_2CO_3, **13**; **d** 5% Pd/C, $HCOONH_4$, MeOH, reflux [34]



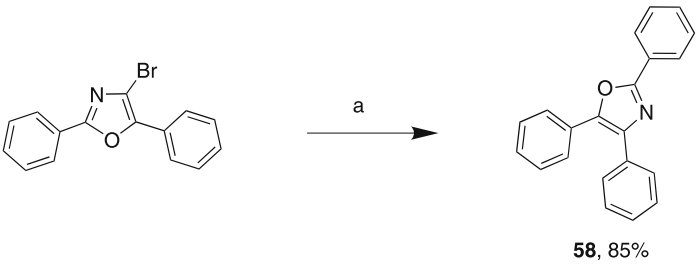
Scheme 11 **a** Ac_2O, DMAP, CH_2Cl_2; **b** HOAc, NH_4OAc, reflux, 6 h; **c** 5% Pd/C, 3 : 1 EtOH/EtOAc, H_2 [34]

in Scheme 1 was also used to form the final oxazole compounds **54** and **55**, except the starting benzaldehydes (**56** and **18**, respectively) were different.

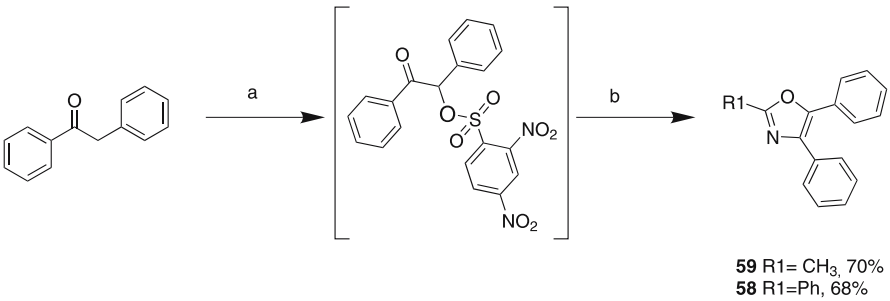
The formation of methyl-oxazole compounds was also described by Wang et al. [34] utilizing an analog of the keto-enol intermediate (**22**) described in Sect. 2.1.1, Scheme 2. Scheme 11 shows the synthesis of compound **57** which exhibits anti-tubulin activity of 7.7 μM [34]. In addition, a range of oxazole COX-2 inhibitors has been reported by Hashimoto et al. [55] employing similar chemistry.

Oxazole compounds can also be produced by use of the Stille reaction. Clapham and Sutherland describe the use of tri-2-furylphosphine/$Pd_2(dba)_3$-catalyzed Stille coupling reactions (Scheme 12) to produce a range of oxazole-containing derivatives, including **58**, with an 85% yield [56].

A highly efficient and interesting method of oxazole production was described by Lee et al. [57]. Scheme 13 describes the synthesis of compounds **59** and **58** using solvent-free microwave irradiation in yields of 70% and 68%, respectively.



Scheme 12 **a** phenyltributyltin, 90 °C, 8 h [56]



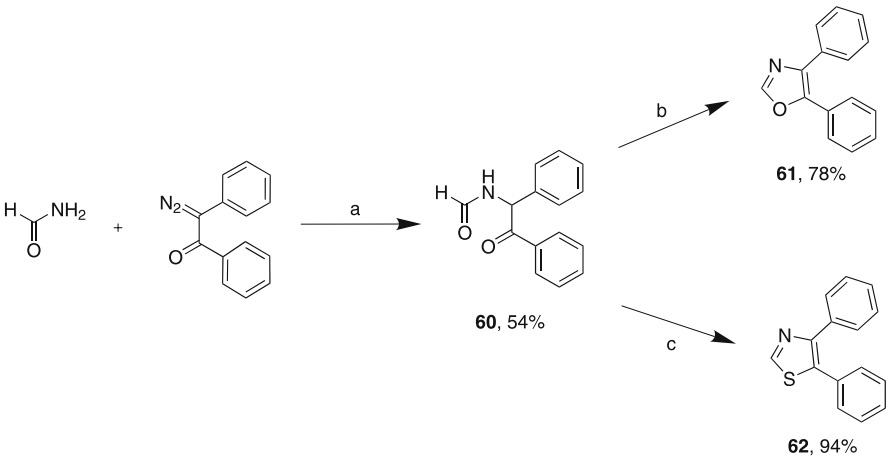
Scheme 13 **a** HDNIB, MWI, 20–40 s; benzamide or acetamide, MWI, 1–2 min [57]

2.1.6 Thiazole Compounds

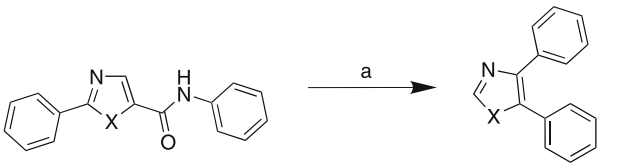
Davies et al. describe the preparation of both oxazole- and thiazole-containing derivatives of combretastatin. By formation of the ketoamide intermediate **60**, in a 54% yield (Scheme 14), both classes of compounds may be obtained by altering the last step of the reaction [58]. To produce the oxazole **61** a cyclo-dehydration reaction was performed using triphenylphosphine-iodine-triethylamine, and the thiazole compound **62** was formed by thionation using Lawesson's reagent, with an excellent yield (94%).

Scheme 15 shows the synthesis of an oxazole **63a** and thiazole **63b** derivative, accomplished by Yokooji et al. [59]. They employed arylation using tertiary phosphines and bromobenzene with Cs_2CO_3 in xylene to form these compounds.

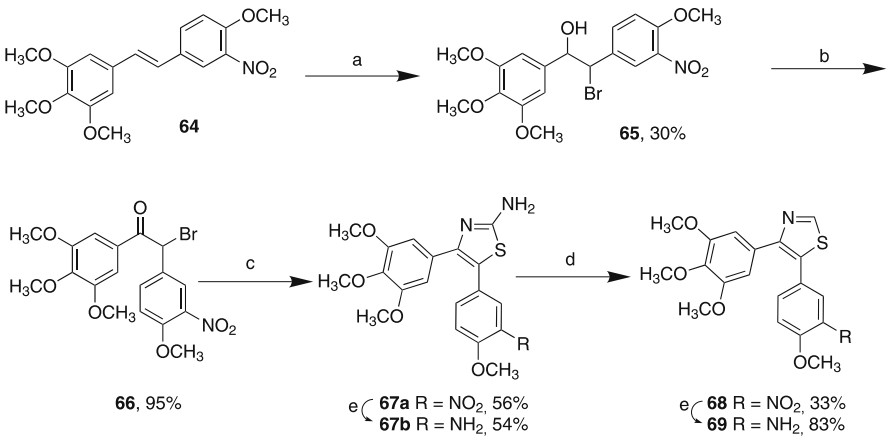
The synthesis of other biologically active thiazoles was described by Ohsumi et al. [50] and is shown in Scheme 16. Condensation of phosphonium bromide and 4-methoxy-3-nitrobenzaldehyde gave a 1:1 mixture of (*Z*)- and (*E*)-stilbenes. (*E*)-stilbene **64** was purified by crystallization and then converted to bromohydrin **65** by NBS-H_2O. Oxidation of the bromohydrin by DMSO-TFAA gave the bromoketone intermediate **66**, which was condensed with thiocarbamoyl compounds in the presence of Na_2CO_3 in DMF to give the corresponding 2-substituted thiazole derivatives (**67a** and **b**). Compound **67a**



Scheme 14 **a** cat. $Rh_2(OAc)_4$, 1,2-dichloroethane, reflux; **b** Ph_3P, I_2, Et_3N; **c** Lawesson's reagent [58]



Scheme 15 **a** $Pd(OAc)_2$, P(biphenyl-2-yl)(*t*-Bu)$_2$, Cs_2CO_3/*o*-xylene, reflux, 48 h [59]



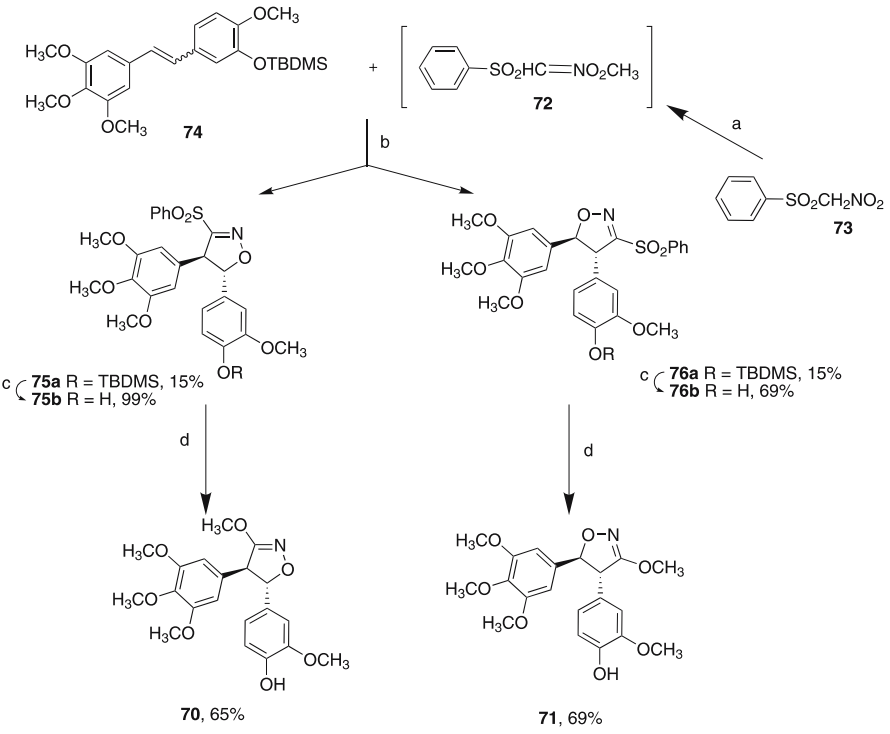
Scheme 16 **a** NBS, DMSO-H_2O, rt; **b** DMSO, TFAA, CH_2Cl_2, – 78 °C; **c** thiourea, Na_2CO_3, DMF, rt; **d** (i) $NaNO_2$, H_2SO_4, AcOH, 5 °C (ii) H_3PO_2, rt; **e** Zn, AcOH, rt [50]

was converted to a diazonium salt then reduced by H_3PO_2 to give the nitrothiazole compound (**68a**); the aniline compound **69** was formed by reduction of the nitro group using Zn/AcOH.

2.2
5-Membered Non-Aromatic Rings

2.2.1
Dihydroisoxazole Compounds

The synthesis of two different derivatives of methoxy-dihydroisoxazole compounds with biological activity have been described by Simoni et al. [60]. The first derivative has the iminium and the methoxy group nearest the A-ring **70** and the other has the iminium and methoxy closest to the B-ring **71**. Methyl nitronic ester **72** was prepared by treating the corresponding nitro compound **73** with ethereal diazomethane. The nitronic ester **72** was reacted with both the *cis*- and *trans*-TBDMS-protected (TBDMS: t-butyldimethylsilyl) combretastatin derivatives (**74**) in the presence of *p*-toluene sulfonic acid in refluxing CH_2Cl_2

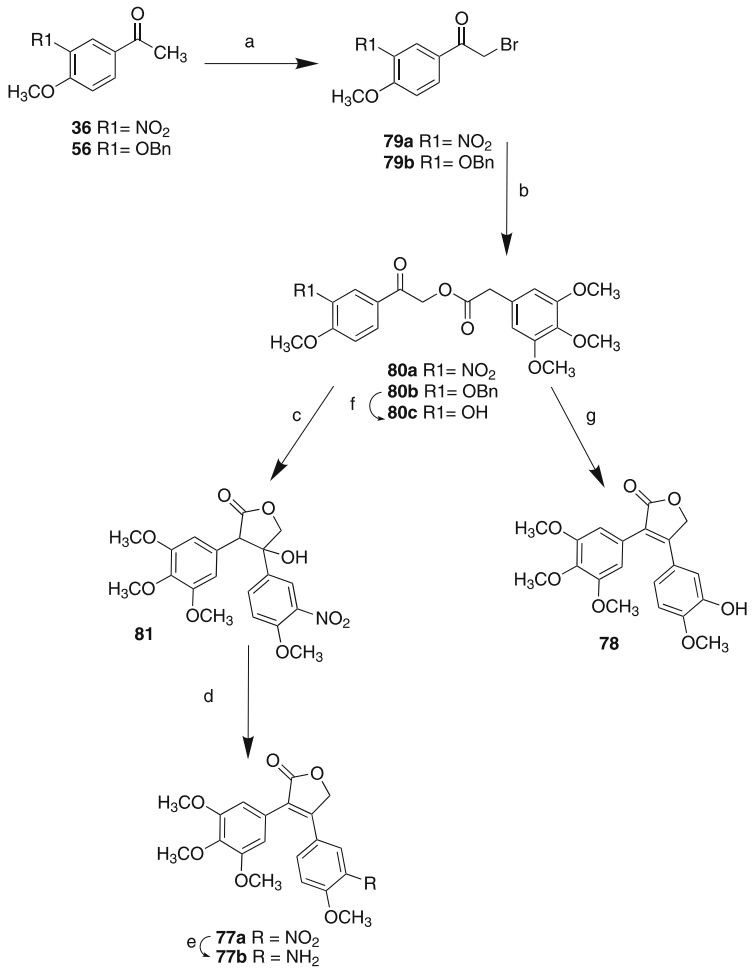


Scheme 17 **a** Diazomethane, Et_2O; **b** CH_2Cl_2, *p*-Ts-OH, reflux; **c** TBAF, CH_2Cl_2; **d** Na, CH_3OH or MeLi, THF [60]

to produce the isooxazolines **75a** and **76a**. Following treatment with sodium methoxide, these compounds were converted to the 3-alkoxyisoxazolines **70** and **71** in good yields (Scheme 17).

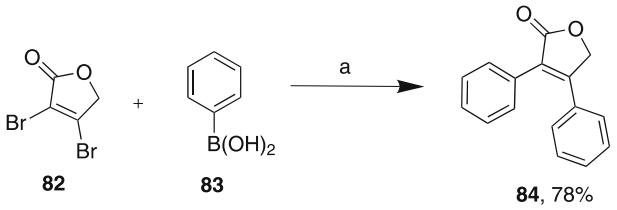
2.2.2 *H*-Furan-2-one Compounds

Kim et al. describe the synthesis of biologically active furanone compounds (Table 1) involving the formation of an amino and a hydroxyl compound

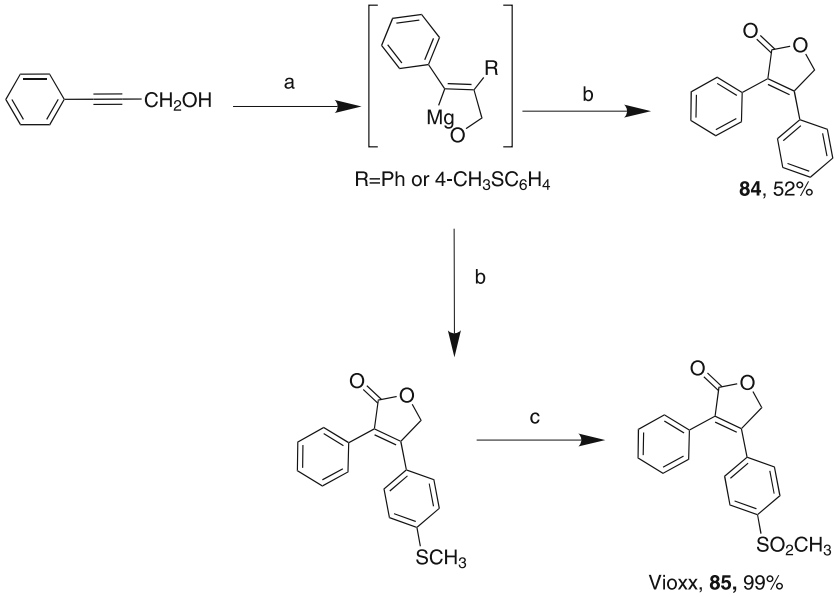


Scheme 18 **a** Br_2, AcOH, HCl, rt; **b** 3,4,5-trimethoxyphenylacetic acid, NEt_3, CH_3CN, rt; **c** DBU, CH_3CN, 0 °C; **d** *p*-TsOH, benzene, reflux; **e** Zn, AcOH, rt; **f** 10% Pd/C, THF, rt; **g** TEA, *p*-TsOH, 4 Å molecular sieve, CH_3CN, reflux [61]

(**77b** and **78**, respectively, Scheme 18) [61]. In both cases the formed α-bromo acetophenone, **79a** or **79b,** was reacted with 3,4,5-trimethoxyphenylacetic acid in the presence of triethylamine to give the required phenacylacetates **80a** and **80b**. The hydroxylphenylacylacetate **80c** was obtained by debenzylation using catalytic hydrogenation with 10% Pd/C, followed by an Aldol-type condensation and subsequent dehydration with triethylamine and *p*-toluenesulfonic acid to produce the hydroxyl compound **78**. Formation of the amino-compound proceeded via an Aldol-type cyclization mediated by DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) to give the 4-hydroxy-4-(4-methoxy-3-nitrophenyl)-3-(3,4,5-trimethoxyphenyl)dihydrofuran-2-one **81**. This was dehydrated with *p*-toluene sulfonic acid in refluxing benzene to give the nitro compound, converted to the amino-compound **77b** by reduction using Zn/AcOH. A number of compounds have been produced using a similar method with varying R-groups on the aromatic rings [62–64].



Scheme 19 **a** CSF, $PdCl_2(PPh_3)_2$, toluene, H_2O, $BnEt_3NCl$, 3 h [65]



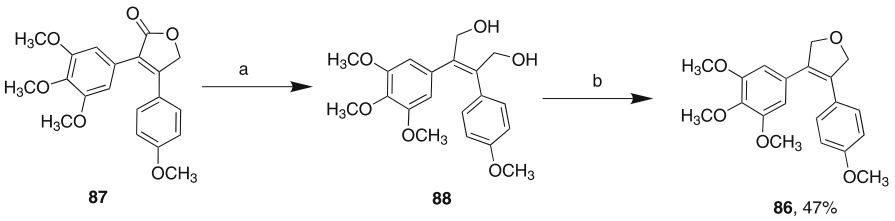
Scheme 20 **a** 3.2 equiv PhMgCl or 4-$MeSC_6H_4MgCl$, C_6H_{12}, 80 °C, 19 h; **b** CO_2; **c** *m*-CPBA, 0–21 °C [67]

Zhang et al. reported the use of densely functionalized molecules through Suzuki cross-coupling reactions [65]. This synthesis involves the reductive amination of mucaholic acids to form the unexpected lactone (e.g., **82**). Compound **82** can then be reacted with phenylboronic acid (**83**) to form the 2,3-diaryl-α,β-unsaturated-γ-lactone **84** as outlined in Scheme 19 in a 78% yield. A similar procedure is outlined in the work of Bellina et al. [66].

Another route to the synthesis of the furanone-containing compounds (e.g., **84**, Scheme 18) is via magnesium-mediated carbometallation of propargyl alcohols, as described by Forgione et al. [67]. Scheme 20 demonstrates this procedure as a feasible means of producing the Merck anti-inflammatory drug Vioxx, **85**.

2.2.3 Dihydrofuan Compounds

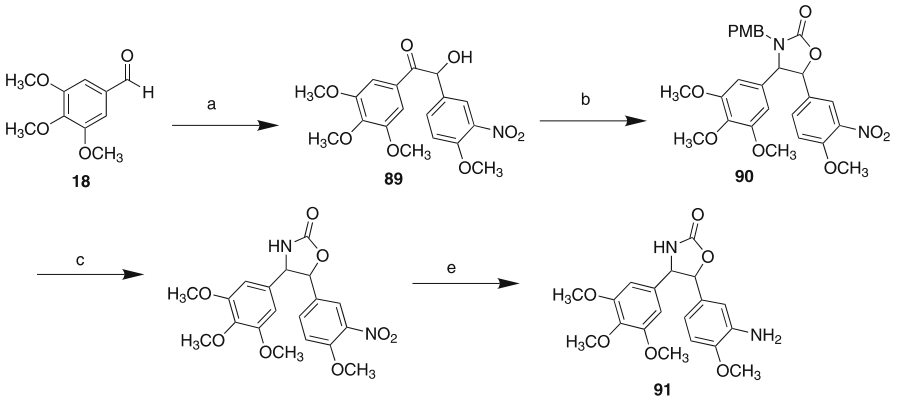
Scheme 21 shows the synthesis of a dihydrofuran derivative **86**. Synthesis of this compound was described by Nam et al. [68] utilizing a furanone compound **87** synthesized by Kim et al. [61] via a similar synthetic approach as described in Scheme 17. The lactone was reduced using lithium aluminum hydride to give the diol **88** and intramolecular etherification using the Mitsunobu reaction afforded the dihydrofuran **86** in moderate yield (47%).



Scheme 21 **a** $LiAlH_4$, Et_2O, 3 h, rt; **b** PPh_3, DEAD, THF [68]

2.2.4 3-*H*-oxazol-2-one Compounds

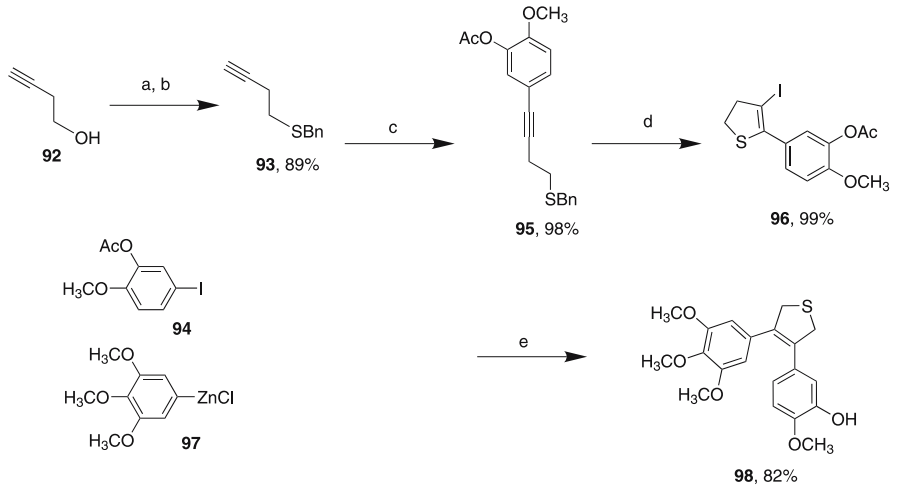
The synthesis of 3-*H*-oxazol-2-ones was described by Nam et al. [69]. The substituted benzoin **89** was formed from the coupling of 3,4,5-trimethoxybenzaldehyde **18** with 3-nitro-4-methoxybenzaldehyde, Scheme 22. Reaction with PMB-isocyanate and subsequent cyclization gave the protected oxazolone derivative **90**. The PMB group was removed by reflux in TFA and reduction of the nitro-group was performed using Zn to give the combretoxazolone-aniline **91**.



Scheme 22 **a** (i) TMS-CN, ZnI_2, THF; (ii) LiHMDS, Ar_2CHO, THF, – 78 °C; **b** (i) PMB-NCO, toluene, 80 °C, 3 h; (ii) AcOH, reflux, 8 h; **c** TFA, reflux, 3 h; **d** Zn, CH_3COOH [69]

2.2.5 Dihydrothiophene Compounds

Synthesis of the dihydrothiophene derivatives was described by Flynn et al. [70] (depicted in Scheme 23) and involved the conversion of 3-butynol **92** to benzyl 3-butynal sulfide **93**. Sonogashira coupling of the sulfide **93** with acetic acid 5-iodo-2-methoxyphenyl ester **94**, produced the intermediate **95**. Treatment of compound **95** with iodine resulted in a rapid and



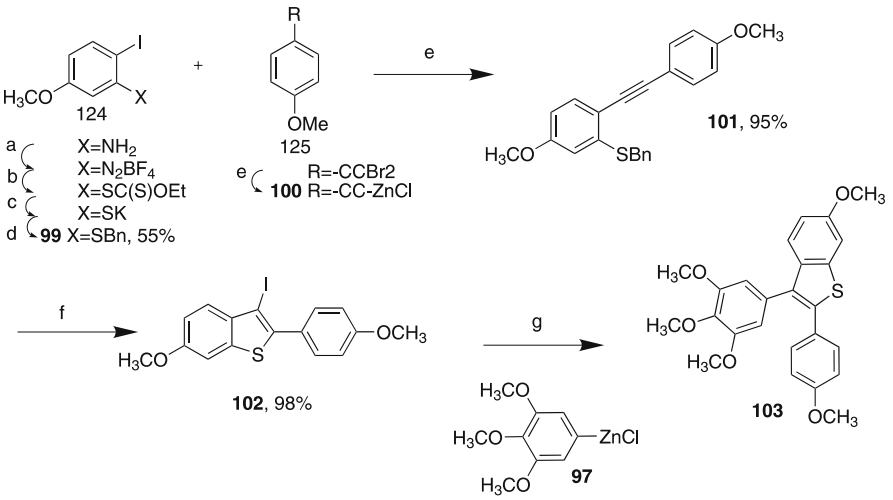
Scheme 23 **a** KOH, TosCl, CH_2Cl_2; **b** NaH, BnSH, THF, 18 °C; **c** **94**, $Pd(PPh_3)_2Cl_2$, 2.0 mol %, CuI 4.0 mol %, DMF/Et_3N 3 : 1, 18 °C; **d** I_2, CH_2Cl_2; **e** **97**, (from 3,4,5-trimethoxyiodobenzene, 2 equiv *t*-BuLi, 1 equiv $ZnCl_2$), $Pd(PPh_3)_2Cl_2$ 5.0 mol %, THF, 18 °C, 4 h followed by MeOH, K_2CO_3 [70]

efficient 5-*endo-dig*-iodocyclization to produce the acetic acid 5-(3-iodo-4,5-dihydrothiophen-2-yl)-2-methoxyphenyl ester **96**. Cross-coupling of the obtained vinyliodide (**96**) with arylzinc (**97**) via in situ hydrolysis of the acetate group produced the thiophene compound **98**.

2.3 Fused Non-Aromatic 5-Membered Compounds

2.3.1 Methoxybenzothiophene Compounds

Methoxybenzothiophene compounds contain a thiophene moiety with a benzene ring fused at the 2,3-position. These compounds do not possess biological activity but have been included for completeness. The synthesis of these compounds is described by Flynn et al. [71] and shown in Scheme 24. Sulfide **99** was prepared by a process involving a multi-step reaction consisting of diazotation, xanthate substitution, methanolysis, and benzylation with an overall 55% yield. Reagent **99** was then coupled to an ethynyl zinc species **100** (obtained directly from β,β-dibromostyrene by the addition of 2 equiv of *n*-BuLi and zinc chloride) giving the aryne intermediate **101**. Reaction with iodine produced the rapid 5-*endo-dig*-iodocyclization to give 3-iodobenzo[*b*]thiophene **102**. Negishi coupling of **102** with arylzinc **97** produced the target compound **103** with a good yield. Yue and Larock also report the synthesis of these compounds using similar chemistry [72].

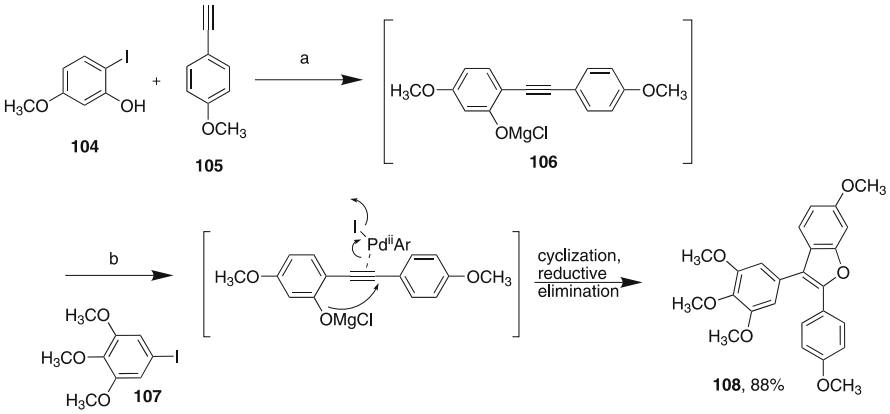


Scheme 24 **a** HBF_4, $NaNO_2$, H_2O; **b** KSC(C)OEt, DMF; **c** MeOH, KOH **d** aq. KOH, BnCl, *n*-Bu_4NHSO_4 cat., CH_2Cl_2; **e** 2 × *n*-BuLi, THF, then $ZnCl_2$, $Pd(PPh_3)_2Cl_2$ 2 mol %, **99**; **f** I_2, CH_2Cl_2; **g** **97** (from 3,4,5-trimethoxyiodobenzene, 2 × *t*-BuLi, THF and $ZnCl_2$), $Pd(PPh_3)_2Cl_2$ 2 mol % [71]

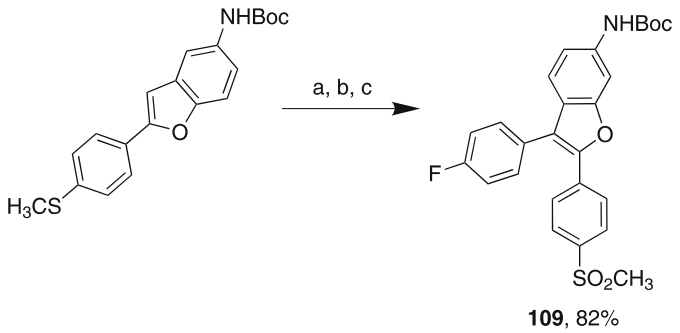
2.3.2
Methoxybenzofuran Compounds

These compounds contain a furan ring fused to a benzene moiety in the 2,3-position. This synthesis was also described by Flynn et al. [73] and is shown in Scheme 25 involved the coupling of 2-iodo-5-methoxyphenol **104**, 4-methoxyphenylethyne **105** to form the intermediate *o*-alkynylphenolate **106**. Aryl iodide **107** was added to the phenolate in DMSO with heat. Oxidative addition, palladium(II)-induced cyclization and reductive elimination resulted in the product **108** with an 88% yield.

Formation of the fluorinated analog was described by Dai and Lai and involved a Suzuki coupling [74] to produce compound **109** shown in Scheme 26. This compound is of interest as a known COX-2 inhibitor.



Scheme 25 **a** MeMgCl 2 equiv, $Pd(PPh_3)_2Cl_2$ 3 mol %, THF, 65 °C, 1.5 h under $N_{2(g)}$; **b** cool to rt, add **107** and DMSO, then heat to 80 °C, 16–18 h [73]



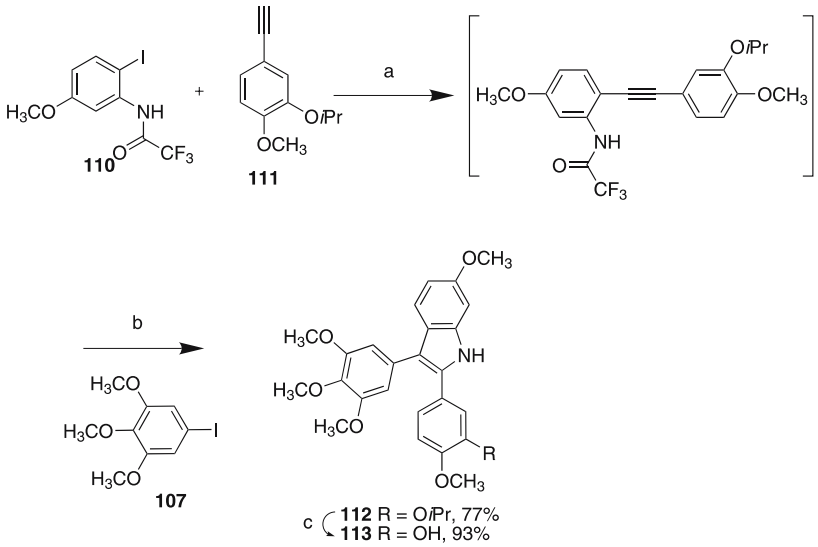
Scheme 26 **a** NBS (N-bromosuccinimide), THF-MeCN (1 : 2), – 20–0 °C; **b** $4\text{-}FC_6H_4B(OH)_2$, $Pd(PPh_3)_4$, CsF, $PhCH_3 - H_2O$ (4 : 1), reflux, 18 h; **c** Oxone, THF, rt, 3 h [74]

2.3.3
Methoxyindole Compounds

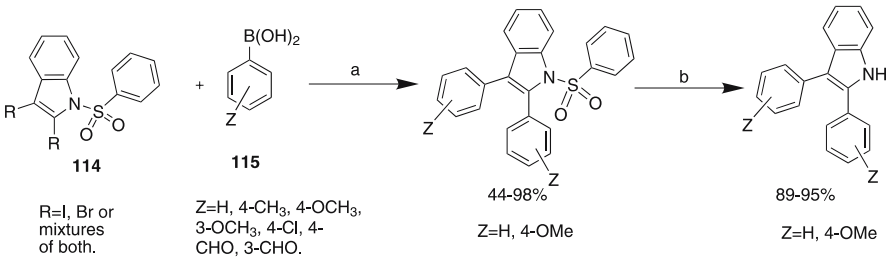
The indole compound was described by Flynn et al. [73] and is prepared in a similar manner as the thiophene **103** and furan **108**. One method involved a similar synthesis as described in Scheme 25, using the relevant starting material. However, an alternative synthesis involved a one-pot, room-temperature synthesis, Scheme 27. The *o*-iodotrifluoroacetanilide **110** was coupled to the alkyne **111** under Sonogashira conditions in MeCN. K_2CO_3 and the aryliodo compound **107** was added and the reaction stirred to produce the protected product **112** with a 77% yield. Deprotection to the corresponding phenol **113** was performed using $AlCl_3$.

An alternative method of producing indole-containing compounds involves a bis-Suzuki reaction of 2,3-dihaloindoles **114** with 2 equiv of boronic acids **115** with 10 mol % $Pd(OAc)_2$ [75]. The paper describes the difference in electronic effects of the boronic acids. Electron-rich boronic acids give better yields (85–95%) whilst the electron-deficient boronic acids give poorer yields (44–55%). Scheme 28 shows the general synthesis of these compounds.

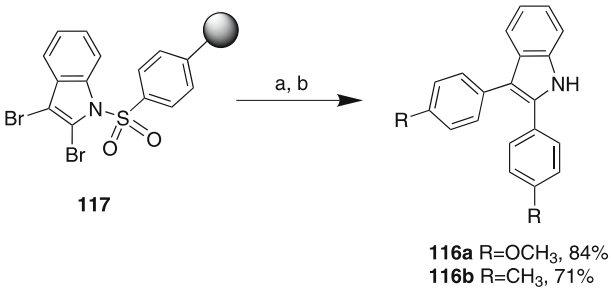
Another Suzuki coupling reaction was described by Zhang et al., to produce arylindoles **116a** and **b**, using solid-phase synthesis [76]. The synthesis was achieved by palladium-mediated coupling/intramolecular indole cyclization of resin-bound 2-trimethylsilylindole **117**, Scheme 29.



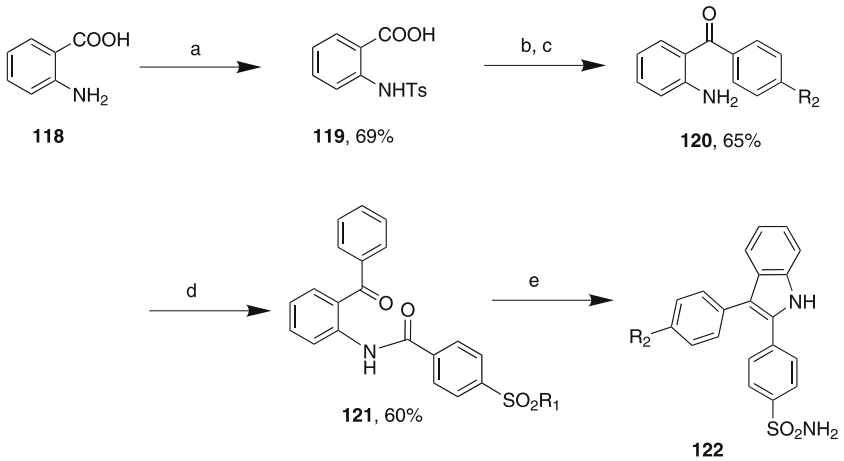
Scheme 27 **a** $Pd(PPh_3)_2Cl_2$ 3 mol %, Et_3N 2 equiv, CuI 6 mol %, CH_3CN, 18 °C, 1 h under $N_{2(g)}$; **b** K_2CO_3 5 equiv, **107**, 18 °C, 18 h, **c** $AlCl_3$ 4 equiv, CH_2Cl_2 [73]



Scheme 28 **a** $Pd(OAc)_2$, 10 mol %, P(*o*-tol)$_3$, K_2CO_3, aq. acetone or DMF; **b** Mg, MeOH, 89–95% [75]



Scheme 29 **a** $ArB(OH)_2$, [Pd]; **b** TBAF [76]



Scheme 30 **a** Na_2CO_3, TsCl, 60–85 °C; **b** (i) PCl_5, 50 °C, (ii) $AlCl_3$, C_6H_6, 80–90 °C; **c** H_2SO_4, 120 °C; **d** 4-$NH_2SO_2C_6H_4$ – COCl, THF, Et_3N, rt; **e** Zn, $TiCl_4$, THF, reflux [77]

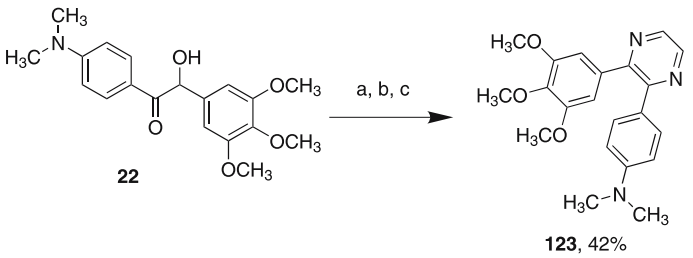
Another series of COX-2 inhibitors was described by Hu et al., and the key step in this reaction is the construction of the indole skeleton by the McMurry coupling reaction [77]. The chemistry described in this paper is shown

in a general manner in Scheme 30. *o*-Aminobenzoic acid **118** was subjected to Friedel-Crafts conditions, following tosyl protection **119**. Hydrolysis with concentrated H_2SO_4 gave the substituted 2-aminobenzophenone **120** that was acylated with 4-aminosulfonylbenzoyl chloride to give amide-linked intermediate **121**. The cyclization step was achieved by McMurry condensation to produce the indole compound **122**.

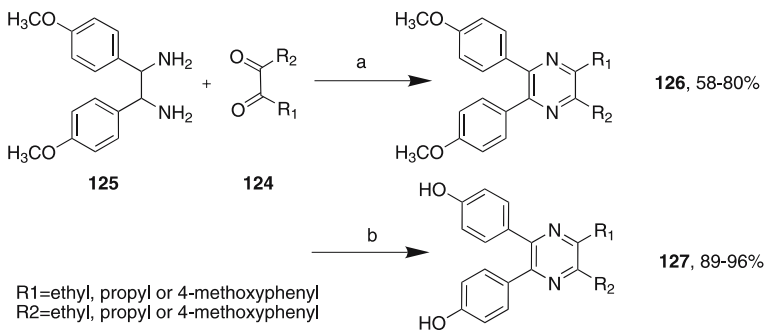
2.4 Aromatic 6-Membered Compounds

2.4.1 Pyrazine Compounds

The synthesis and biological testing of the pyrazine compound **123** was described by Wang et al. [34]. The same benzoin intermediate **22** was formed as described in Scheme 2. A three-step reaction was then performed to obtain the desired pyrazine **123**, shown in Scheme 31: (i) oxidation of $CuSO_4$ in aqueous pyridine, (ii) reaction with ethylenediamine in EtOH, and (iii) aromatization in the presence of elemental sulfur.



Scheme 31 **a** Pyridine, $CuSO_4 \cdot 5H_2O$, H_2O, reflux; **b** ethylenediamine, EtOH, reflux; **c** elemental sulfur, 140 °C, 30 min [34]



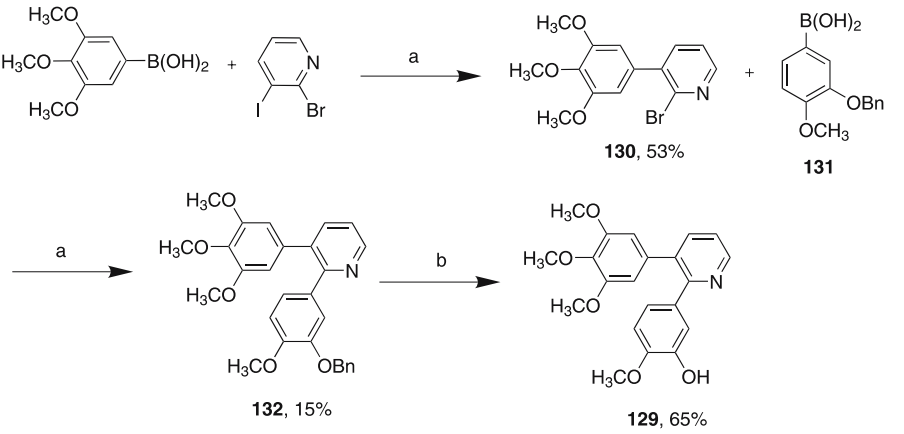
Scheme 32 **a** AcOH, O_2, reflux, 6 h; **b** $BF_3 \cdot SMe_2$ [78]

An alternative method of synthesizing the pyrazine compounds was described by Ghosh et al., and the synthesis is shown is Scheme 32 [78]. Reaction of a 1,2-dione (**124**) with a 1,2-diamine (**125**) to form an imine intermediate followed by spontaneous oxidation of the dihydropyrazine intermediate, formed the protected pyrazine compound **126.** The free phenol **127** was obtained by removal of the methyl-protecting groups with a boron trifluoride-dimethyl sulfide complex. Similar compounds were prepared via the same method by Simoni et al. [79].

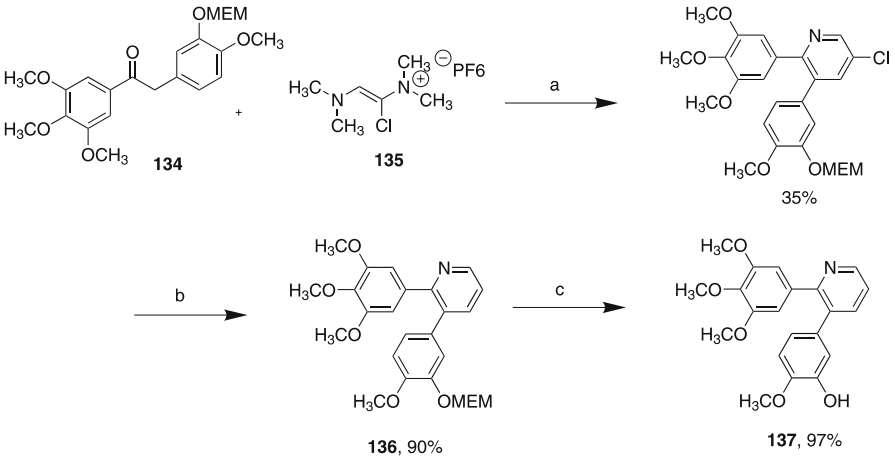
2.4.2 Pyridine Compounds

The synthesis and biological activity of these pyridine-containing compounds, in which the nitrogen points toward the B-ring, was described by Simoni et al. [60]. A double Suzuki cross-coupling strategy was employed as previously described by the same group [79], and the synthesis is shown in Scheme 33. The desired diphenyl compound **129** was obtained in good yields from a Suzuki coupling in toluene; tetrakis(triphenylphosphine)palladium(0) was employed as the catalyst for the reaction, and Na_2CO_3 provided the basic environment. The subsequent Suzuki cross-coupling between the bromophenyl derivative **130** and boronic acid (**131**) produced pyridine **132.** Hydrogenation in the presence of Pd/C produced the deprotected hydroxyl compound **129**.

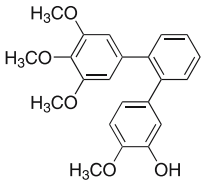
Synthesis of the pyridine derivative, in which the nitrogen is closest to the A-ring was also described by Simoni et al. [60], is shown in Scheme 34 and was more productive than the synthesis described in Scheme 33. The keto-compound **134** was reacted with the vinamidinium hexafluorophosphate salt (CDT-phosphate) **135**, *tert*-BuOK, ammonium acetate, and an equimolar amount of DABCO (1,4diazabicyclo[2.2.2]octane). Hydrogenation using 10%



Scheme 33 **a** $Pd(Ph_3P)_4$, aq. Na_2CO, toluene/EtOH; **b** H_2, Pd/C, EtOH [60]



Scheme 34 **a** *t*-ButOK, DABCO, NH_4OAc, THF, 6 h reflux; **b** TFA, CH_2Cl_2, 5 h rt.; **c** H_2, 10% $Pd/CaCO_3$, 1 M NaOH/EtOH 1 : 1, 18 h, rt [60]



Structure

palladium over $CaCO_3$ yielded the protected compound **136**. Treatment with TFA (trifluoroacetic acid) in CH_2Cl_2 removed the MEM-protecting group to yield the hydroxyl product **137** with a good yield.

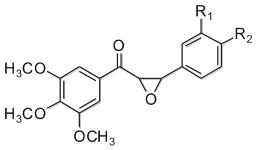
Synthesis of the phenyl derivative (**138**) was also described by Simoni et al. [60] and employs the same synthetic strategy as for the pyridine derivative (**137**) described in Scheme 34. No biological activity was reported for this compound, but it was included in order to provide a broader coverage of stilbene derivatives.

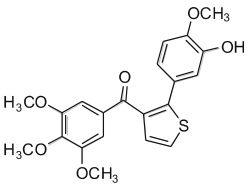
The aforementioned section described the synthesis of a wide range of biologically important heterocyclic derivatives of combretastatin. The next part of this chapter will focus on the synthesis of heterocyclic chalcone derivatives.

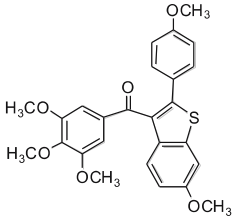
3
Heterocyclic Chalcone Derivatives

There are considerably fewer examples of heterocyclic chalcone analogs of combretastatin than in the heterocyclic stilbene derivative category. Of these,

Table 2 Biologically active chalcone derivatives

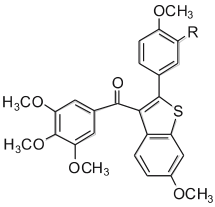
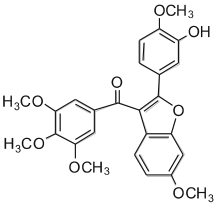
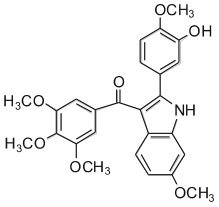
Alkene-functionalized 3-membered heterocycles		B16 IC_{50} μM	L1210 IC_{50} μM
	[80]		
	140a $R_1 = OCH_3$	25	5
	$R_2 = H$	29	3.9
	140b $R_1 = H$		
	$R_2 = NO_2$		

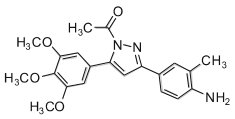
Alkene-functionalized 5-membered non-fused aromatic compounds		Anti-tubulin IC_{50} μM	Colchicine binding inhibition (%) 5 μM	MCF-7 IC_{50} nM
	[70]			
	142	1.0	67	300

		Colchicine Binding Inhibition (%) 5 μM	OVCAR-3 GI_{50} $\mu g\,mL^{-1}$	A498 GI_{50} $\mu g\,mL^{-1}$	NCI-H460 GI_{50} $\mu g\,mL^{-1}$
CA-4 7	[83]	100			
	[83]				
	145	23	0.19	0.46	0.13

HCT-15 (human colon adenocarcinoma, MDR positive), NCI-H460 (human lung large cell carcinoma, MDR negative), B16 (murine melanoma), MCF7 (human breast cancer), HL-60 (human leukemia), L1210 (murine leukemia), OVCAR-3 (human ovarian cancer), A498 (renal human cancer), hACAT-1 (acyl-CoA: cholesterol acyl transferase-1 from Hi5 cells), hACAT-2 (acyl-CoA: cholesterol acyl transferase-1 from Hi5 cells), CA46 (Burkitt lymphoma)

Table 2 (continued)

Alkene-functionalized 5-membered fused aromatic compounds	Anti-tubulin IC_{50} μM	Colchicine binding inhibition (%) 5 μM	CA46 IC_{50} nM	
	[71]			
	145 R = H	> 40		2000
	154 R = OH	3.5	6	500
CA-4, 7	[73]	2.1	91	11
	[73]			
	157	1.3	80	42
	[73]			
	159	1.6	54	45

Enone-functionalized 5-membered aromatic compounds		HCT-15 IC_{50} μM	NCI-460 IC_{50} μM	
	[85]			
	167	365	1000	

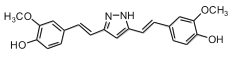
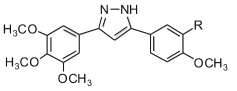
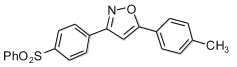
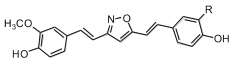
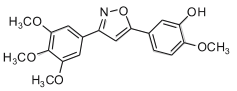
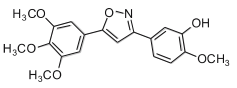
		Anti-oxidant activity IC_{50} μM	COX-1 inhibition (%) 100 μM	COX-2 inhibition (%) 100 μM	Anti-inflammatory inhibition 75 mg kg^{-1}
	[87]				
	170	9.70	87.0	61.0	68.8

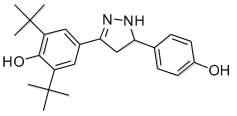
Table 2 (continued)

		B16 IC_{50} μM	L1210 IC_{50} μM
	[80]		
	180 R = NO_2	32	37
	178 R = NH_2	5	2.4

	Antimicrobial activity zone inhibition @ 25 μg mL^{-1} *Bacillus subtillus*
 [92]	
182	9

		Anti-oxidant activity IC_{50} μM	COX-1 inhibition (%) 100 μM	COX-2 inhibition (%) 100 μM	Anti-inflammatory inhibition (%) 75 mg kg^{-1}
	[87]				
	184a R = OCH_3	10.71	80.8	58.1	60.3
	184b R = H	18.96	47.4	35.0	

	HL60 IC_{50} μM	Apoptotic activity AC_{50} μM
 [60]		
189	3	4.5
 [60]		
190	3	4

Enone-functionalized 5-membered non-aromatic compounds	hACATI IC_{50} μM	hACAT2 IC_{50} μM
 [95]		
197	32.4	68.5

only a relatively small number have been assessed for biological activity. As a result the remaining part of this chapter will focus on those analogs that: a) possess biological activity, b) closely resemble CA-4, **7** structurally, c) have an interesting synthetic scheme, and/or d) have the potential to possess antitumor activity.

There are two distinct classes of compounds that fit the criteria mentioned above: alkene-functionalized chalcone derivatives (Fig. 1B) and enone-functionalized chalcone derivatives (Fig. 1C). Within each class, both aromatic and non-aromatic compounds exist. Those compounds functionalized at the alkene include: i) 3-membered heterocycles, e.g., epoxide and aziridine compounds, ii) 5-membered aromatic derivatives including fused and non-fused compounds, and iii) 6-membered aromatic pyrazine compounds. The enone-functionalized compounds include: i) 5-membered aromatics such as pyrazole and isoxazole compounds, ii) 5-membered non-aromatic compounds for example pyrazolines and isoxazolines, and iii) 6-membered non-aromatics where a discussion of heterocyclic and non-heterocyclic compounds will be given for completeness.

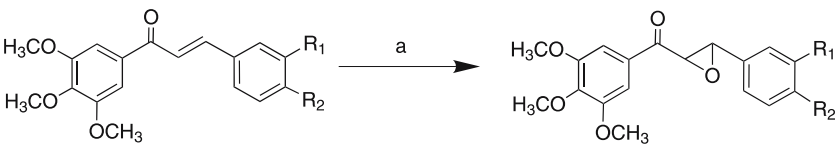
Table 2 shows a representative example of compounds that possess biological activity. The synthesis of the most active compound in each class will follow plus any alternative methods of producing them.

3.1 Alkene Functionalized Chalcone Derivatives

3.1.1 3-Membered Heterocycles

a) Epoxide Compounds

Synthesis of biologically active epoxides were reported by Le Blanc et al. and were synthesized initially as a route into the pyrazole compounds shown in Sect. 3.2.1a Scheme 48 [80]. Scheme 35 shows the synthesis of two of the most active epoxides **139a** and **139b**. They were formed from the reaction of chalcones (**140a** and **140b**, respectively) by reaction with H_2O_2 and K_2CO_3 in MeOH



140a R1=OCH_3, R2=H
140b R1=H, R2=OCH_3

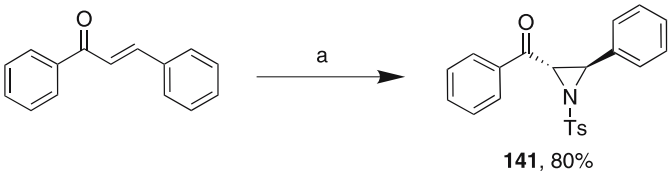
139a R1=OCH_3, R2=H, 89%
139b R1=H, R2=OCH_3, 78%

Scheme 35 **a** H_2O_2, K_2CO_3, CH_3OH, rt, 3 h [80]

at room temperature for 3 hours. These conditions improve on those stated by Bhat et al. [81]. They report a similar reaction except NaOH and EtOH were used. The reaction time was significantly longer at 15 versus 3 hours.

b) Aziridine Compounds

The aziridines are the nitrogen analogs of the epoxides and undergo similar electrophilic reactions. No biological data were obtained for these compounds nor were they used as precursors to any CA-4, **7**, analogs. They have been included since the synthesis is noteworthy, and they could be interesting intermediates. Xu et al. stereoselectively aziridinated chalcones using the nitrene precursor (PhINTS) and a copper catalyst to form compound **141** (Scheme 36) [82].



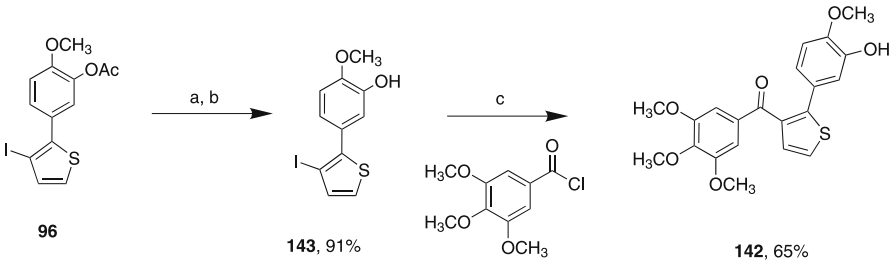
141, 80%

Scheme 36 **a** PhINTS, AnBOX, CuOTf, CH_2Cl_2, 5 h [82]

3.1.2 5-Membered Aromatic Rings

a) Non-Fused Thiophene Compounds

Flynn et al. described the synthesis of thiophene-containing analogs of CA-4, **7** [70]. The synthesis of compound **142** was performed using intermediate **96** (a description of the formation of this intermediate is given in Scheme 23). Aromatization of **96** with DDQ and acetate hydrolysis yielded the hydroxyl intermediate **143**. Dilithiation of **143** and reaction with 3,4,5-



96

143, 91%

144

142, 65%

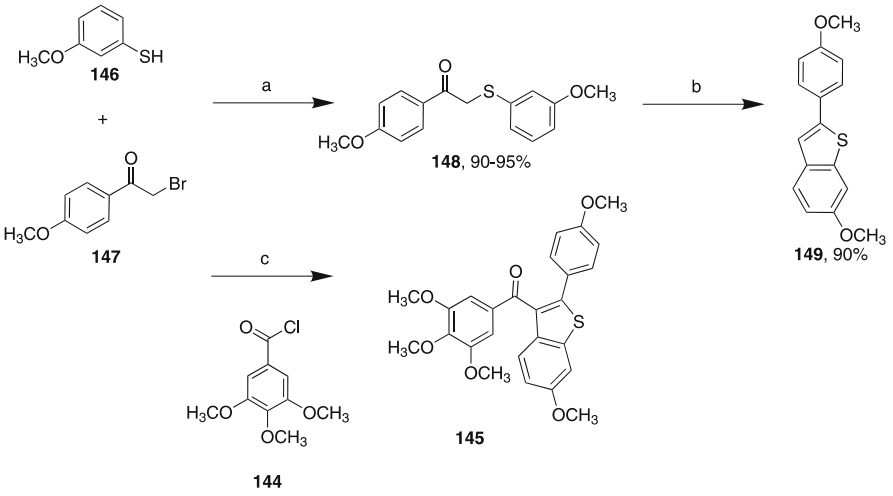
Scheme 37 **a** DDQ, CH_2Cl_2; **b** MeOH, K_2CO_3; **c** *t*-BuLi, – 78 °C, then **144** [70]

trimethoxybenzoyl chloride **144** produced the thiophene compound **142** in 65% yield (Scheme 37).

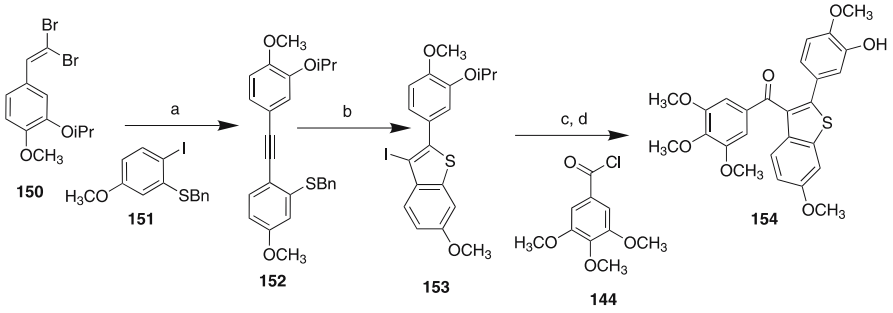
b) Fused Thiophene Compounds

Pinney et al. reported the synthesis of benzothiophene CA4 analogs and an example synthesis is given in Scheme 38 [83]. Benzothiophene (**145**) was produced by reacting aromatic thiol **146** with α-bromoacetophenone **147** to generate the sulfide **148**. Compound **148** was then cyclized to the benzothiophene **149** using polyphosphoric acid and heat. Formation of **145** was achieved by Friedel-Crafts aroylation of **149** with the methoxybenzoyl chloride **144.**

2,3-disubstituted benzo[*b*]thiophenes were similarly synthesized and were described by Flynn et al. [71]. Dibromostyrene **150** is coupled to iodoben-



Scheme 38 **a** NaOH, EtOH; **b** PPA, heat; **c** **144**, $AlCl_3$, CH_2Cl_2 [83]

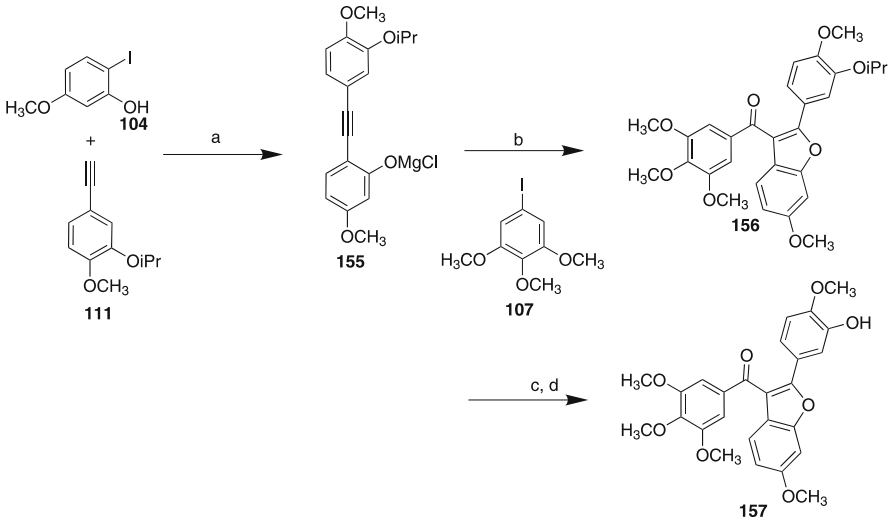


Scheme 39 **a** $2 \times t$-BuLi, THF, then $ZnCl_2$, $Pd(PPh_3)_2Cl_2$ 2 mol %, **151**; **b** I_2, CH_2Cl_2; **c** *t*-BuLi, THF, **144**; **d** $AlCl_3$ 3 equiv, CH_2Cl_2 [71]

zenesulfide **151**. The diaryl alkyne **152** then reacts with iodine to give the 5-*endo*-*dig*-iodocyclization to the 3-iodobenzo[*b*]thiophene **153**. The iodobenzo[*b*]thiophene is then coupled with the aryl acid chloride **144** to form the target compound **154** (Scheme 39).

c) Fused Benzofuran Compounds

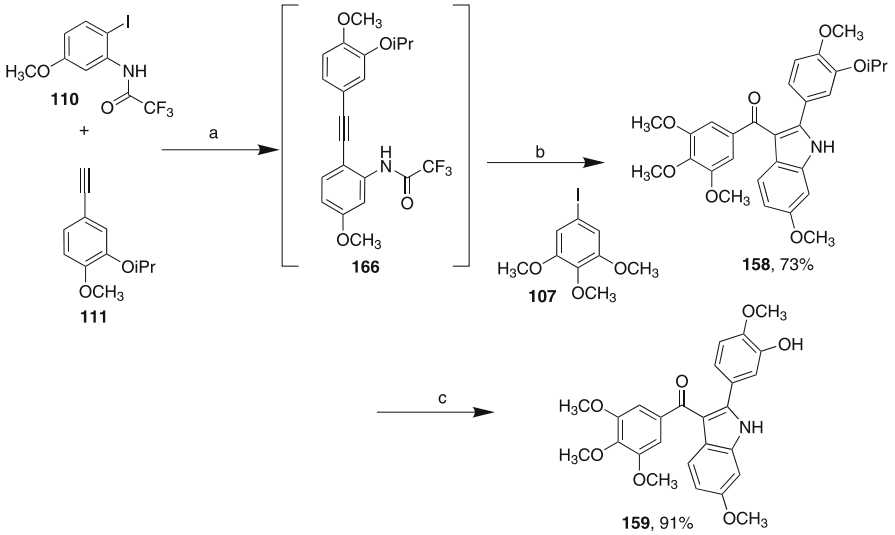
The synthetic approach to the benzo[*b*]furan is similar to that of the thiophenes described in Scheme 39. The synthetic approach was described by Flynn et al. [73], and an example synthesis is given in Scheme 40. The appropriate iodophenol **104** is coupled to the aryl alkyne **111**. The intermediate **155** is subsequently cyclized in the presence of an appropriately substituted aryl iodide, e.g., **107** under an atmosphere of carbon monoxide gas, to give the benzo[*b*]furan chalcone derivative **156**. Deprotection of the hydroxyl produces the target compound **157**.



Scheme 40 **a** CH_3MgCl 2 equiv, $Pd(PPh_3)_2Cl_2$ 3 mol %, THF, 65 °C, 1.5 h, $N_{2(g)}$; **b** cool to rt, **107**, DMSO, 80 °C, 16–18 h, $CO_{(g)}$; **c** $AlCl_3$, CH_2Cl_2 [73]

d) Fused Indole Compounds

Flynn et al., also described the synthesis of the fused indoles [73]. The *o*-iodotrifluoroacetanilide **110** was coupled to aryl alkyne **111** under Sonogashira conditions followed by subsequent reaction with aryl iodide, **107** with gaseous carbon dioxide produced the fused indole **158**. Lewis acid dealkylation with aluminum trichloride produced the deprotected alcohol **159**.



Scheme 41 **a** $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, CH_3CN, 18 °C, 1 h, $N_{2(g)}$; **b** **107**, K_2CO_3, 18 °C, 18 h, $CO_{(g)}$; **c** $AlCl_3$, CH_2Cl_2 [73]

3.1.3
6-Membered Aromatic Derivatives

a) Pyrazine Compounds

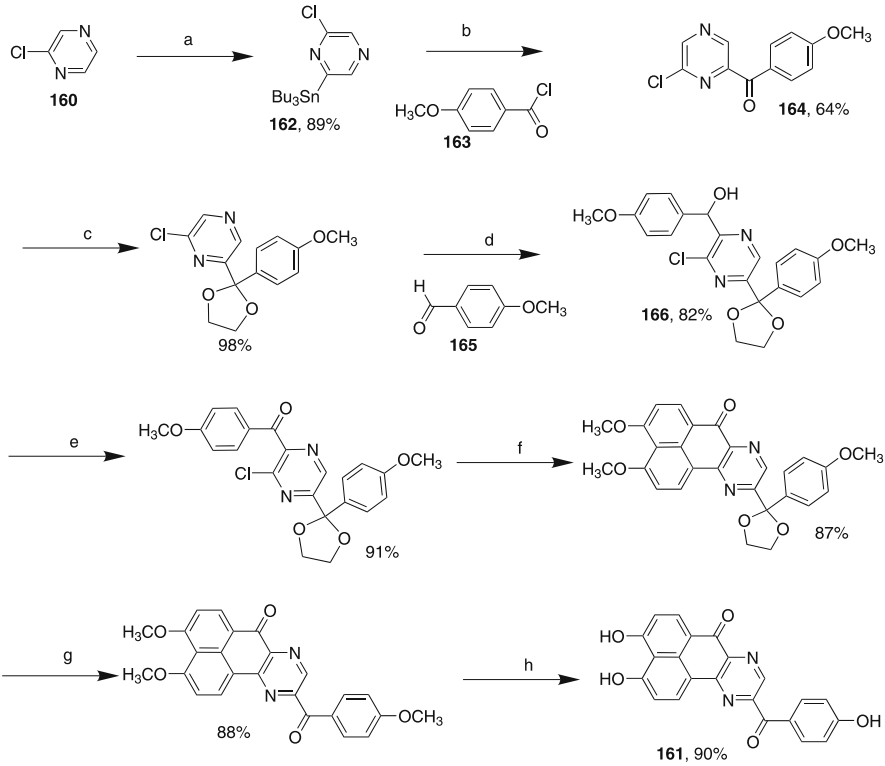
Buron et al., published the synthesis of botryllazine derivatives containing a pyrazine core [84]. Scheme 42 describes the synthesis of these compounds. Chloropyrazine **160** was employed as the starting material for the synthesis of the pyrazine chalcone analog **161**. 2-Chloro-3-tributylstannylpyrazine **162** was the key intermediate and was coupled with acid chloride **163** to produce the ketone **164**. Following protection and subsequent reaction with **165**, pyrazine **166** was generated. Oxidation, deprotection, and demetallation produced the pyrazine of interest **161**.

3.2
Enone Functionalized Chalcone Derivatives

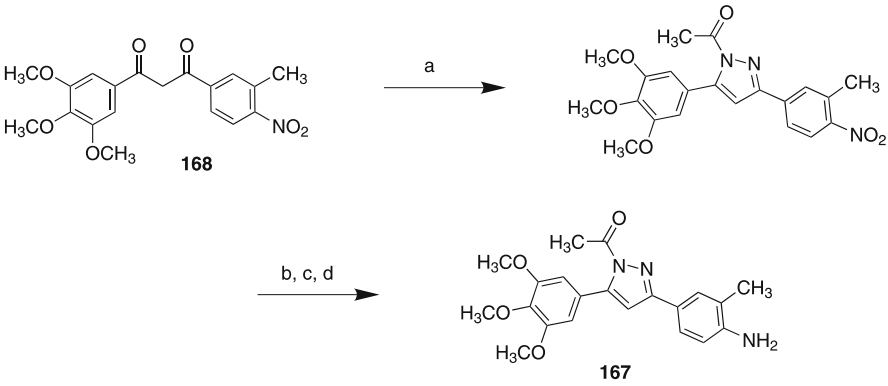
3.2.1
5-Membered Aromatic Rings

a) Pyrazole Compounds

Szczepaikiewicz et al. [85] reported the synthesis of pyrazoles (e.g., **167**) from the diketone **168** with hydrazine hydrate, shown in Scheme 43. Nigram et al. executed a similar synthetic sequence [86].



Scheme 42 **a** (i) LTMP, THF, – 100 to – 40 °C, 2.5 h, Bu_3SnCl (ii) H_2O, HCl, THF, –40 to 0 °C; **b** **163**, 10% $Pd(PPh_3)_4$, $PhCH_3$, 110 °C; **c** ethylene glycol, APTS; **d** LTMP, THF – 75 °C, **165**; **e** MnO_2, THF, rt; **f** *p*-$CH_3OC_6H_4B(OH)_2$, $Pd(PPh_3)_4$, EtOH, K_2CO_3, $PhCH_3$, 8 h; **g** 6 M aq. HCl, CH_3OH, 65 °C, 2 h; **h** pyridinium hydrochloride, 210 °C, 1.5 h [84]

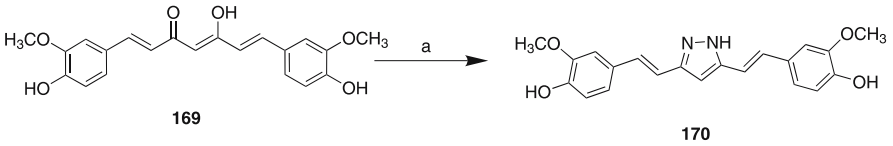


Scheme 43 **a** Hydrazine hydrate, CH_2Cl_2/EtOH; **b** Ac_2O; **c** H_2/Pd – C, EtOAc; **d** separate regioisomers by flash chromatography [85]

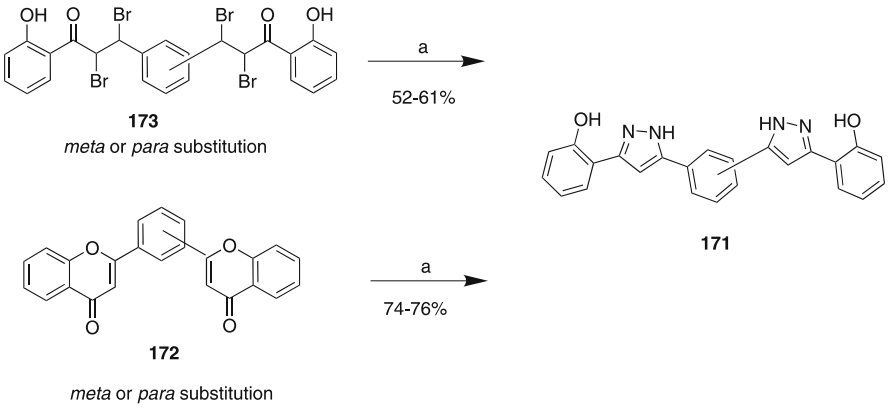
Similarly, Scheme 44 indicates that Selvan et al. utilized β-hydroxy enones (e.g., **169**) to synthesize pyrazoles (e.g., **170**) [87]. Although this example is a curcumin analog and not a chalcone derivative, it has been included as this class of compounds exhibited anti-oxidant and COX-1/COX-2 activity.

Scheme 45 shows a general reaction of the pyrazole chemistry reported by Pinto et al. [88]. This group generated bis-pyrazoles (e.g., **171**) from bis chromones (e.g., **172**) and dibrominated bis-chalcones (e.g., **173**) using similar reaction conditions as stated in Schemes 43 and 44.

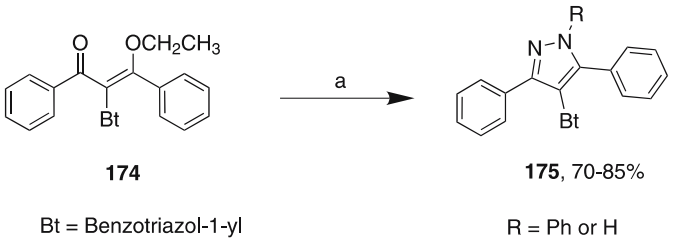
Fattah et al., utilized β-keto vinyl ethers (e.g., **174**) in the presence of hydrazine as precursors to pyrazoles (e.g., **175**) [89]. Scheme 46 gives a general illustration of this reaction.



Scheme 44 **a** Hydrazine hydrate, acetic acid [87]



Scheme 45 **a** Hydrazine hydrate, MeOH, reflux, 24 h [88]

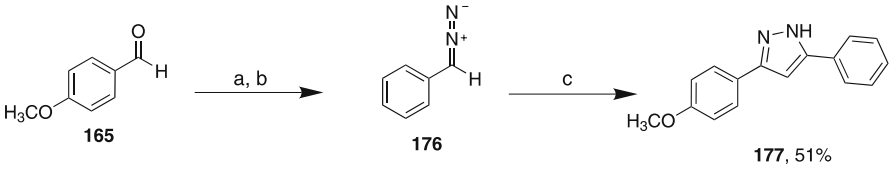


Scheme 46 **a** Hydrazine hydrate or phenyl hydrazine, EtOH, reflux [89]

A different method of generating pyrazoles was reported by Aggarwal et al. and is shown in Scheme 47 [90]. Reaction of diazo compound **176** (derived from benzaldehyde **165**) with an alkynylbenzene enabled cyclization to pyrazole **177**.

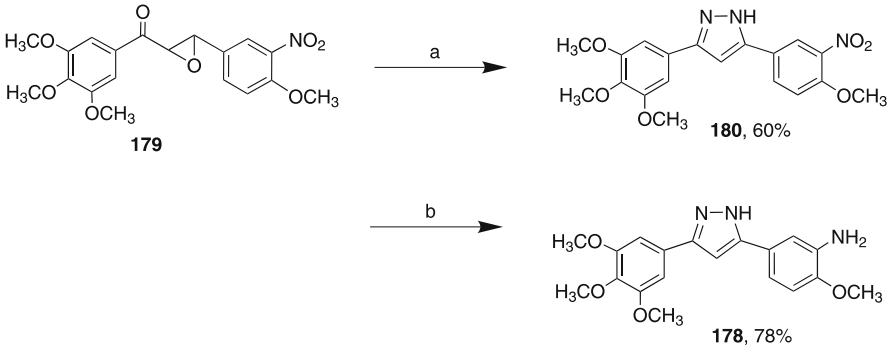
Pyrazoles were synthesized in the authors' laboratory by Le Blanc et al. from the epoxy-ketone as already stated in Sect. 3.1.1a, Scheme 35 [80]. The synthetic strategy employed by Le Blanc et al. [80] was based upon that the strategy published by Bhat et al. [81] who also described the synthesis of pyrazoles but did not report cytotoxic evaluation on the synthesized compounds. Scheme 48 shows the synthesis of the most active compound (**178**). Dissolution of the epoxide (**179**) with a xylenes followed by treatment with *p*-toluenesulfonic acid and hydrazine hydrate produced the pure nitro-pyrazole **180** in good yield (60%). Catalytic hydrogenation with palladium on activated carbon allowed the amino-pyrazole (**178**) to be obtained in a pure form. This synthesis allowed relatively large numbers of compounds to be produced as the crude product was sufficiently pure. Yield, reaction time, and purification compared to reported approaches were improved [50, 61, and 81]. Cytotoxicity of these pyrazole analogs was disappointing. The planarity of these compounds may account for this, as CA-4, **7** is a twisted molecule.

To try and address this issue of conformation, Forrest et al., in the authors' laboratory examined methyl- and phenyl-substituted analogs of the pyrazoles mentioned above, employing a similar synthesis [91]. These molecules are



165 **176** **177**, 51%

Scheme 47 **a** *p*-Toluenesulfonyl hydrazide, acetonitrile, rt, 3 h; **b** 5 M aq. NaOH; **c** phenylacetylene, 50 °C, 48 h [90]



179 **180**, 60% **178**, 78%

Scheme 48 **a** Hydrazine hydrate, *p*-TsOH, xylenes/CH_2Cl_2, reflux; **b** H_2, 5% Pd/C, THF, rt [80]

still under investigation as the cytotoxicity of these compounds was found to be comparable to the unsubstituted pyrazoles (Table 2).

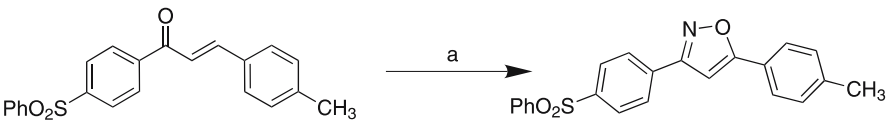
b) Isoxazole Compounds

Moustafa and Ahmed reported the synthesis of isoxazoles [92, 93]. These compounds are related to pyrazoles except that an oxygen replaces the amine nitrogen. Scheme 49 shows the synthesis of **182** from the corresponding chalcone **183** by reaction with hydroxylamine. Although these compounds were not tested for cytotoxicity as tubulin binders, they were found to possess antibacterial and anti-fungal activity.

The isoxazoles **184a** and **b** were synthesized by Selvam et al., and the synthesis is described in Scheme 50 [87]. As with Scheme 49, this group utilized hydroxyl amine reacted with the β-hydroxy enones (**169** and **185**) to form the isoxazoles (**184a** and **b**).

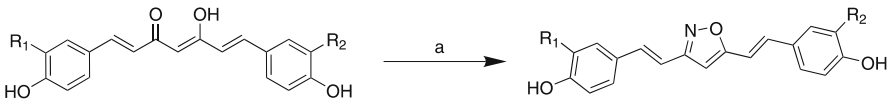
A more elaborate approach was taken by Kaffy et al. [94]. The goal of the research was a series of compounds with greater stability and a higher affinity for endothelial cells within tumor vessels than CA-4, **7**; however, the paper described a method that was purely synthetic. The synthetic strategy involved a 1,3-dipolar cycloaddition of a nitrile oxide **186** with a substituted aryl alkyne **187** to form the oxazole **188**.

Simoni et al., described the synthesis of isoxazole analogs of CA-4, **7** [60]. The synthetic approach was similar to that of Kaffy et al. Two isomers could be produced by following the synthetic route shown in Scheme 52, the nitrogen pointing to the A-ring (**194**) and the oxygen pointing to the A-ring (**195**). The same starting materials and the same reaction conditions were used for both compounds; the difference lay in which set of reaction conditions were applied to which starting material. To produce oxime **192**- and



183 **182**

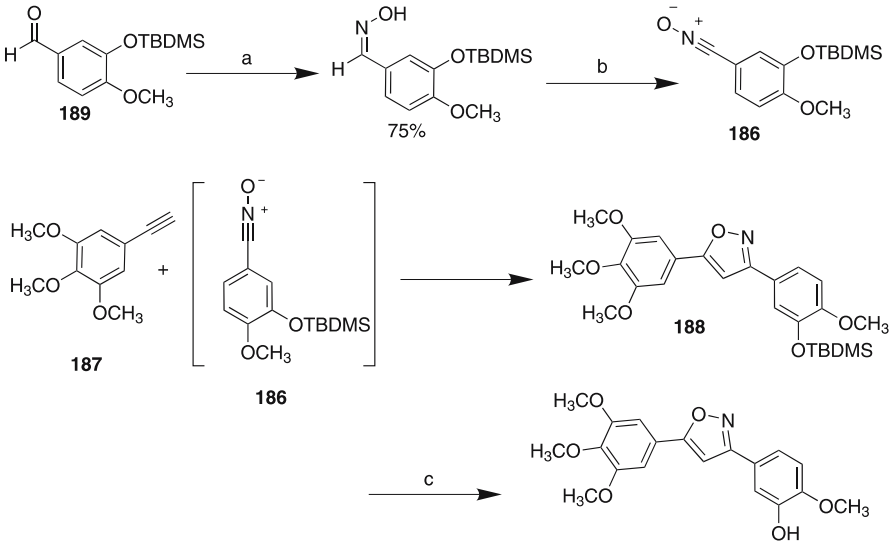
Scheme 49 **a** Hydroxylamine hydrochloride, sodium acetate/acetic acid, EtOH, reflux, 6 h [92, 93]



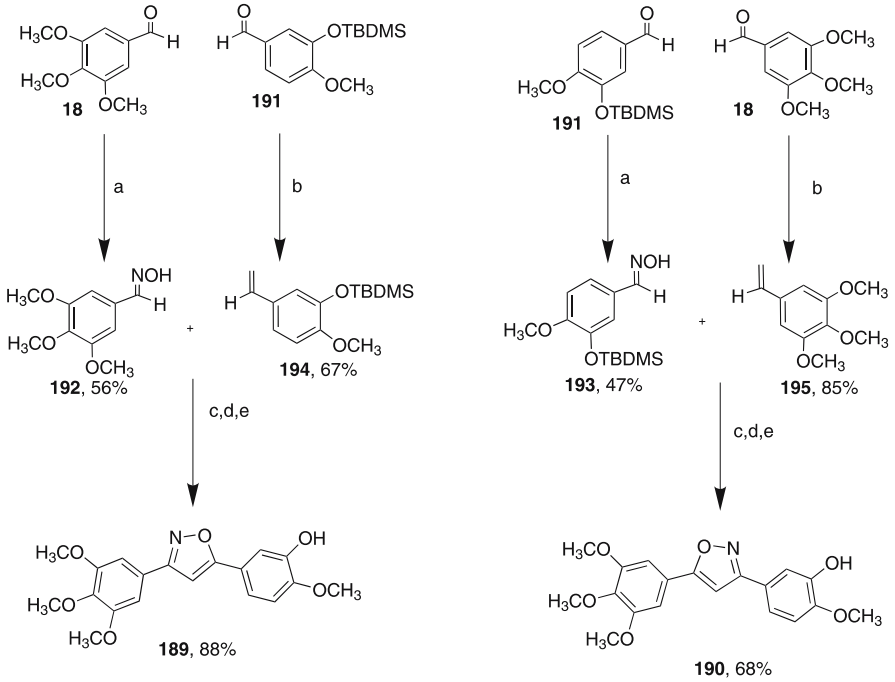
169 R1=R2=OCH_3
185 R1=OCH_3, R2=H

184a R1=R2=OCH_3
184b R1=OCH_3, R2=H

Scheme 50 **a** Hydroxylamine hydrochloride, acetic acid, 85 °C, 6 h [87]



Scheme 51 **a** Hydroxylamine hydrochloride, pyridine, EtOH, reflux, 1 h; **b** aq. NaOCl, Et_3N, CH_2Cl_2, 0 °C, rt, 24 h; **c** TBAF [94]



Scheme 52 **a** Hydroxylamine hydrochloride, sodium bicarbonate, CH_3OH/H_2O 3 : 1; **b** $CH_3PPh_3^+Br^-$, NaH, THF; **c** $CHCl_3$, pyridine, NCS, TEA; **d** TBAF, CH_2Cl_2; **e** MnO_2, benzene, reflux [60]

193-substituted aldehydes, **18** and **191**, respectively, were reacted with hydroxylamine in aqueous MeOH. The olefins **194** and **195** were prepared by a Wittig reaction of **191** and **18**, respectively, with methyltriphenylphosphonium bromide. The nitrile oxides of the oximes (**192** or **193**) following Torsell's procedure underwent a [3 + 2] regioselective cycloaddition with required olefin (**194** or **195**) to produce the isoxazoline intermediates. Oxidation was performed using MnO_2 in a benzene solution, and the corresponding isoxazoles (**189** or **190**) were obtained.

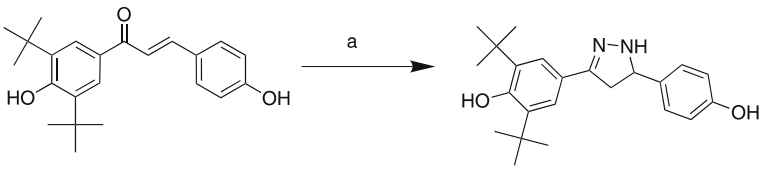
3.2.2 5-Membered Non-Aromatic Ring Compounds

a) Pyrazoline Compounds

Pyrazoline compounds are partially unsaturated pyrazoles. Jeong et al. [95, 96] and Moustafa and Ahmad [92] described the formation of these compounds from chalcones (e.g., **196**) using hydrazine hydrate to form the pyrazolines (e.g., **197**, Scheme 53 [95]). Chimenti et al. also described the synthesis of the pyrazolines from reaction of hydrazine with chalcones but included acetic acid in the reaction mixture [97].

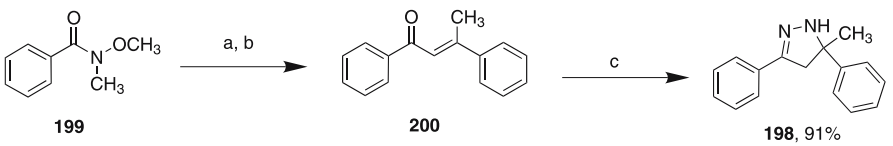
Scheme 54 shows the synthesis reported by Cox et al. of the pyrazoline compound **198** [98]. The Weinreb amide (e.g., **199**) was reacted with a terminal alkyne followed by a reaction of the resulting alkyl ketone (**200**) with an aryl cuprate to produce the pyrazoline **198**. Cox et al. employed the use of microwave technology in this reaction. Kidwai and Misra also employed microwave technology to produce pyrazoline compounds [99].

Although none of the pyrazolines have been tested as tubulin binders Jeong et al. reported the activity of pyrazolines for their lipid peroxidation inhibitory



196 **197**, 96%

Scheme 53 Hydrazine hydrate, EtOH, rt-reflux [95, 96]



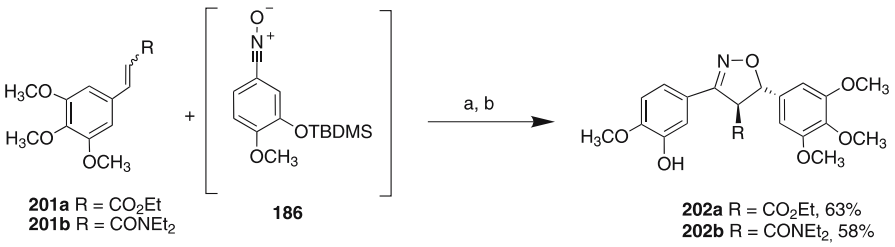
199 **200** **198**, 91%

Scheme 54 **a** *n*-BuLi, $HCCCH_3$, THF, – 78 °C to rt, 3 h; **b** Ar′Li, CuBr-DMS, THF, – 78 °C, 3 h; **c** hydrazine hydrate, EtOH, microwave, 150 °C, 30 min [98]

properties against hACAT-1 and hACAT-2 [95, 96]. Chimenti et al., examined the pyrazolines against anti-bacterial strains [97] and β-alkyl pyrazolines synthesized by Cox et al., were reported to exhibit inhibitory properties against mitotic kinesins that are essential to formation of the mitotic spindle [98]. Breslin et al., synthesized a host of pyrazolines that were found to be active in inhibiting mitotic kinesins with IC_{50} values of less than 50 μM [100]. These compounds typically contained a difluoro-A-ring, with a variety of substituents on the B-ring. This observation led to an examination of the bioactive properties of pyrazoline analogs of CA4, **7** by the authors. Early studies carried out by Dickson et al. showed that these pyrazolines have improved IC_{50} values when compared to pyrazole derivatives and CA-4, **7** [101].

b) Isoxazoline Compounds

Isoxazolines are partially unsaturated isoxazoles. In most cases these compounds are precursors to the isoxazoles, and as a result, the synthesis can also be found in Sect. 3.2.1b. Kaffy et al., used a 1,3-dipolar cycloaddition of a nitrile oxide (**186**) with the respective styrene (**201a** or **b**) to generate isoxazolines (**202a** or **b**, respectively). Depending on the substitution of the vinyl portion of the styrene molecule, either 3- or 4-substituted isoxazolines could be formed (Scheme 55) [94]. Simoni et al. employed similar chemistry to produce isoxazolines [60]. Kidwai and Misra emplyed microwave technology to treat chalcones with hydroxylamine and basic alumina [99]. The isoxazoles synthesized by Simoni et al. possess anti-proliferative and apoptotic activity in the micromolar range [60].

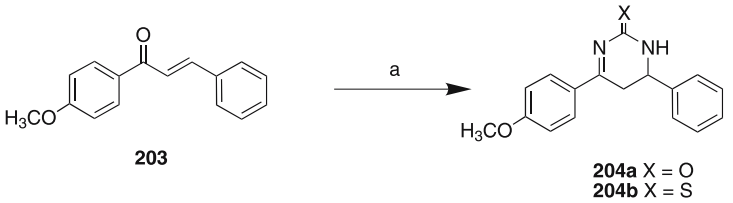


Scheme 55 **a** Et_3N, aq. $NaOCl/CH_2Cl_2$, 0 °C, rt, 24 h; **b** TBAF [94]

3.2.3
6-Membered Non-Aromatic Ring Compounds

a) Dihydropyrimidine Thione Compounds

Heterocyclic rings can be produced from the reaction of a chalcone **203** under basic conditions with urea or thiourea, generating the corresponding diaryl guanidinium structure **204a** or **204b** as displayed in Scheme 56 by Kidwai and



203

204a X = O
204b X = S

Scheme 56 **a** (i) Urea, HCl, EtOH, reflux, 8 h or thiourea, NaOH, EtOH, reflux, 5 h; (ii) urea or thiourea, EtOH, neutral alumina, microwave [99]

Misra [99]. Scheme 56 indicates another route to the same compounds also reported by Kidwai and Misra but employing microwave technology [99].

This section has illustrated a number of chalcone-derived analogs of combretastatins. For the most part there have been limited biological studies with these compounds; however, the synthesis was included for completeness and to indicate that they warrant further investigation.

4 Conclusions

The synthesis of biologically important heterocyclic stilbene and chalcone derivatives of combretastatins has been discussed. Combretastatins have been shown to be inhibitors of tubulin polymerization. In many cases the compounds described in this chapter were included because of an interesting synthesis or structure, although limited biological data were found. It is the author's opinion that a great number of the compounds contained within this review are worthy of further investigation as potential tubulin binders.

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Pyrrole Natural Products with Antitumor Properties

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Abstract This review examines the antitumor activity and synthetic strategies for pyrrole-containing natural products possessing the oxygenated 3,4-diaryl or 3,4-diheteroaryl substitution pattern during the period of 1995–2005. The specific pyrrole-containing natural products are discussed in an order according to their characteristic structural features, which include the dihydroisoquinoline and δ-lactone framework in some cases. The presence or absence of methoxy or hydroxy groups at appropriate positions on aromatic rings or heteroaromatic rings is discussed and such factors have been correlated to antitumor activity and multidrug resistance reversal activity for various structural classes.

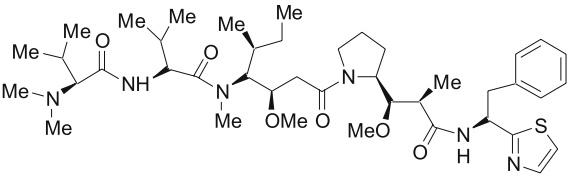
Keywords Pyrrole · Natural products · Antitumor agents · Multi-drug resistance

Abbreviations

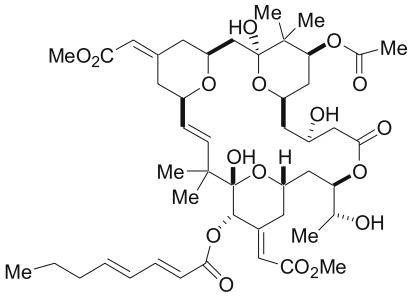
DMFA	*N*,*N*-dimethylformamide dimethylacetal
DPPA	Diphenylphosphorylazide
ED_{50}	Effective dose for 50% inhibition of cell culture growth
MDR	Multi drug resistance
MIC	Minimum inhibitory concentration
PTSA	*p*-Toluenesulfonic acid

1 Introduction

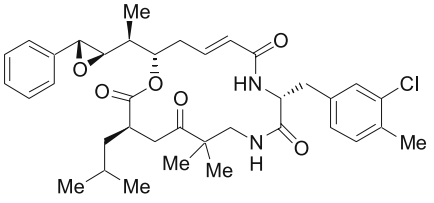
The development of new chemotherapeutic agents for a particular disease state continues to depend to a significant degree on the discovery of a "lead compound", which can be further developed into a viable drug candidate. A very important aspect of this process is to find novel substances that will provide a new mode of action for the treatment of a specific disease. From a historical standpoint, the isolation of novel substances from natural sources has often provided this lead compound. The number of new drugs and their origin for the period 1981–2002 has recently been summarized [1], and, of 79



Dolastatin-10

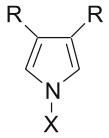


Bryostatin-1



Cryptophycin-52

Fig. 1 Marine Natural Products in Clinical Trials



R = Aryl or Heteroaryl groups
X = H or alkyl/aryl grouping

Fig. 2 General Structural Features of Pyrrole Containing Marine Natural Products

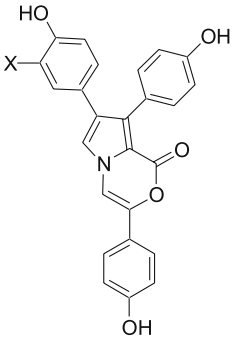
new pharmaceuticals listed under the category of antitumor agents, 40 have their origin in the area of natural products chemistry. One area of natural products, which is attracting significantly more attention, involves substances derived from marine organisms [2]. At least three substances [Dolastatin-10, Bryostatin-1 and Cryptophycin-52 (see for example Fig. 1)] of marine natural product origin are currently in Phase II clinical trials for the treatment of various forms of cancer.

It is interesting to note that during the period from 1995 to 2005 a significant number of marine natural products possessing a pyrrole ring system have been isolated, characterized, and bioassayed. Many of these pyrrole-derived natural products contain an oxygenated 3,4-diaryl substitution pattern (Fig. 2), which appears to be directly related to a variety of interesting biological properties.

Once such natural product derived drug templates are found through appropriate screening techniques, the need to find efficient and versatile syntheses of the natural products and their analogs from readily available starting materials is crucial to the development of a new drug. Major or minor alterations in the natural product structure may be required to maximize desirable pharmacokinetic and pharmacodynamic properties of the lead compound and to minimize the undesirable properties of the substance. The focus of this review will be to examine the antitumor activity and synthetic strategies for pyrrole-containing natural products possessing the oxygenated 3,4-diaryl substitution pattern during the period of 1995–2005. The specific pyrrole-containing natural products will be taken up according to their characteristic structural features. It should be noted that there are numerous pyrrole natural products, which are also of significant biological interest but do not conform to the oxygenated 3,4-diaryl scaffold and these substances have in some cases been reviewed elsewhere [3–5].

2
Lukianols

Lukianol A (1) and B (2) (Fig. 3) were discovered in 1992 by Scheuer and coworkers [6] during the isolation of compounds from the extracts of a Pacific



1 Lukianol A X = H

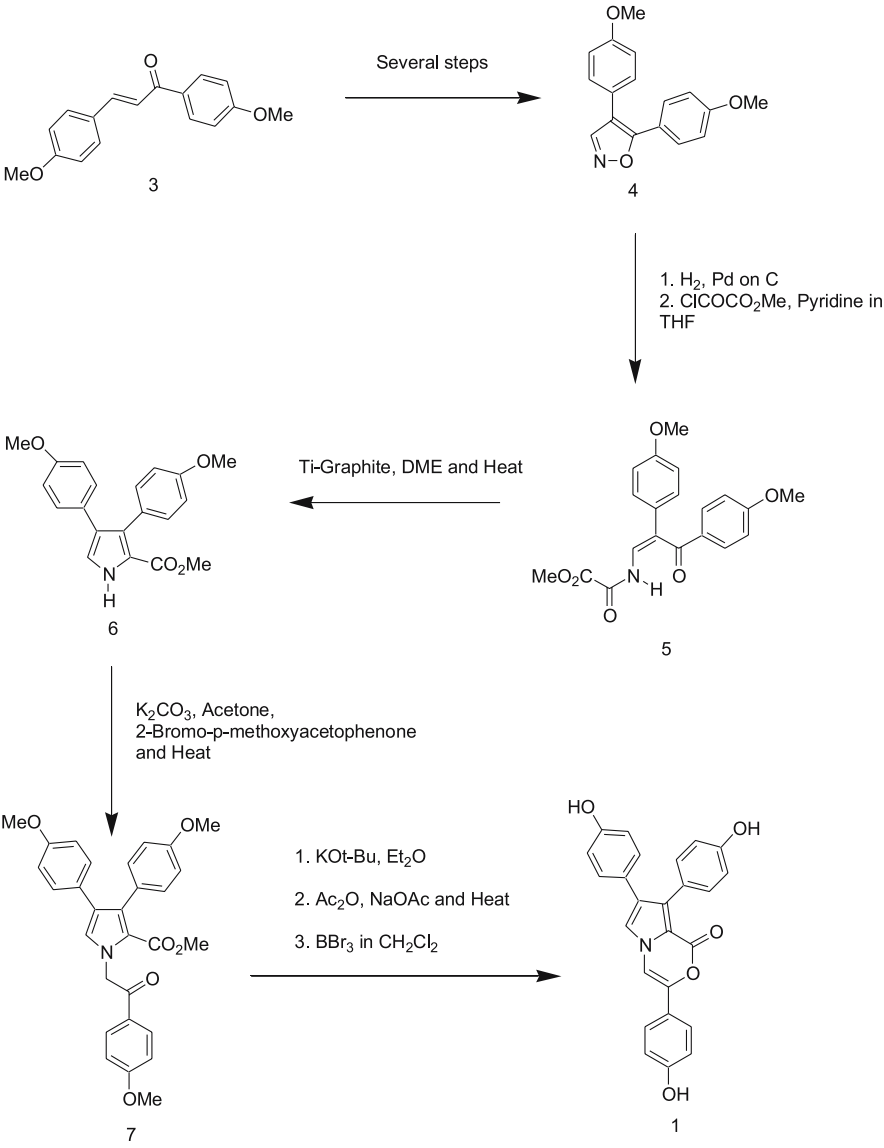
2 Lukianol B X = I

Fig. 3 General Structure of Lukianol Natural Products

tunicate. A subcategory of tunicates are referred to as ascidians [7], which are vertebrate animals and are among the more highly evolved marine organisms routinely examined for natural product purposes. The term tunicate is derived from the fact that their body resembles a tunic. The two lukianols were examined for activity against a human epidermatoid carcinoma cell line in which case lukianol A exhibited a minimum inhibitory concentration (MIC) of 1 μg/mL while lukianol B exhibited an MIC value of 100 μg/mL.

Lukianol A was first synthesized (Scheme 1) by Furstner and coworkers [8] in 1995 in which case a disubstituted α,β-unsaturated ketone (3) was used as the starting material. This material was epoxidized, rearranged, trapped as an oxazole, subsequently cleaved via hydrogenolysis, and acylated to give a tricarbonyl compound (5). A key step involving a titanium mediated McMurry-type ring closure of 5 produced 2-carbomethoxy-3,4-diarylpyrrole (6) in good yield. It should be noted that this last reaction creates the oxygenated 3,4-diarylpyrrole scaffold, which is presumed to be critical for biological activity. The transformation of this pyrrole (6) to lukianol A (1) is completed by alkylation at nitrogen, ring closure and demethylation with boron tribromide.

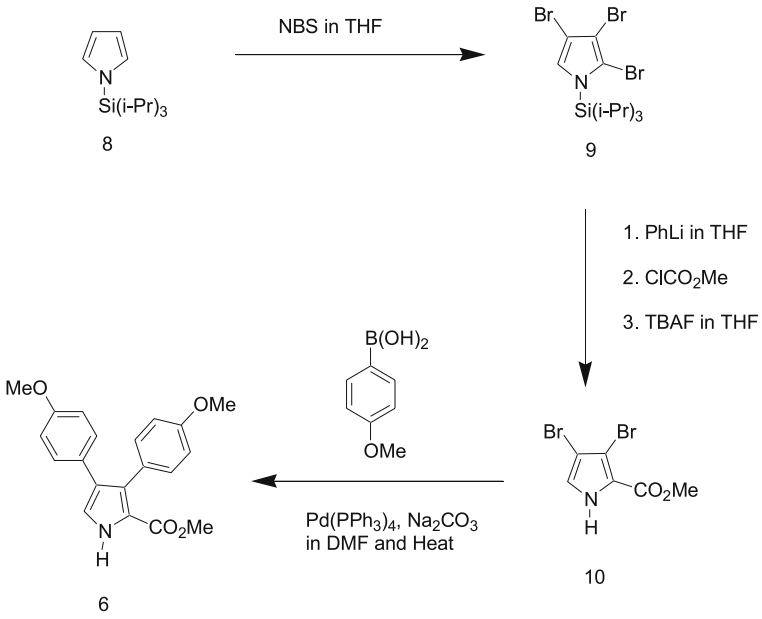
Banwell and coworkers [9] reported their synthesis of lukianol A in 1997 and suggested therein that lukianol A and related compounds might be functioning as antimitotic agents by virtue of their close structural relationship to combretastatin A-4, which is known to bind to the colchicine site of tubulin. The Banwell synthesis is depicted in Scheme 2. The synthesis commences with the tribromination of an N-triisopropylsilyl protected pyrrole (8) followed by selective metalation of the 2 position, carbomethoxylation and subsequent N-deprotection. The resulting 2,3,4-trisubstituted pyrrole (10) is subjected to Suzuki cross-coupling conditions with the appropriate aryl



Scheme 1 Furstner Group Synthesis of Lukianol

boronic acid to give the key lukianol synthon (6) prepared by Furstner and thereby constituting a relay synthesis of the natural product.

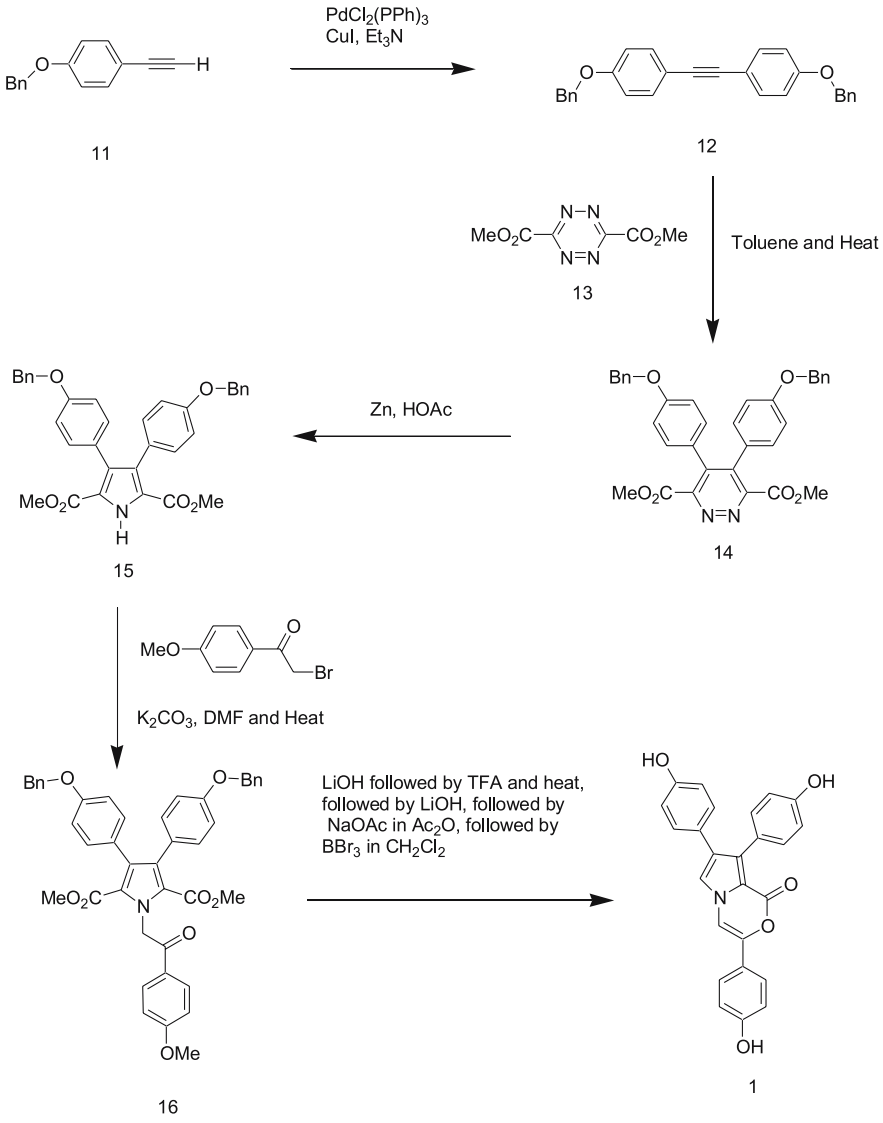
Boger and his research group [10] have also developed a very efficient and flexible synthesis of lukianol A as depicted in Scheme 3. The key transformation for the formation of the tetrasubstituted pyrrole precursor (15) involves formation of a symmetrically substituted diazine (14) by a Diels–



Scheme 2 Banwell Group Synthesis of Lukianol

Alder/retrograde Diels–Alder reaction sequence of a diaryl alkyne with a 3,6-dicarbomethoxy tetrazine. The resulting diazine (14) is then reduced, cleaved and cyclized with Zn/acetic acid to the 2,3,4,5-tetrasubstituted pyrrole (15), which is then N-alkylated with α-bromo-4-methoxyacetophenone to give a pentasubstituted pyrrole (16). The synthesis of lukianol A is completed by ester hydrolysis, decarboxylation, ring closure and deprotection.

A clear advantage of this methodology is the ability to generate analogs quickly and easily thereby making it useful for the preparation of related natural products, which will be evident later in this review. In this paper, Boger also reported the activity of lukianol A and several synthetic precursors against a select group of cancer cell lines whereby IC_{50} values in the range of 1–20 μM were observed. Perhaps more importantly, Boger also examined the multidrug resistance (MDR) reversal activity of lukianol, related analogs and synthetic precursors in comparison to verapamil, which is a well-known MDR reversal agent. Since multidrug resistance cancer cell lines are a challenging problem in chemotherapy, the ability to inhibit cellular P–glycoprotein efflux pumps with suitably functionalized small molecules becomes quite significant. Although lukianol A did not demonstrate MDR reversal activity, a precursor (15) did exhibit some reasonable activity in this regard. It is also of interest to note that the MDR reversal activity of these pyrrole-containing compounds occurs at non-cytotoxic concentrations. Another general trend observed by Boger and others is that phenolic hydroxyl

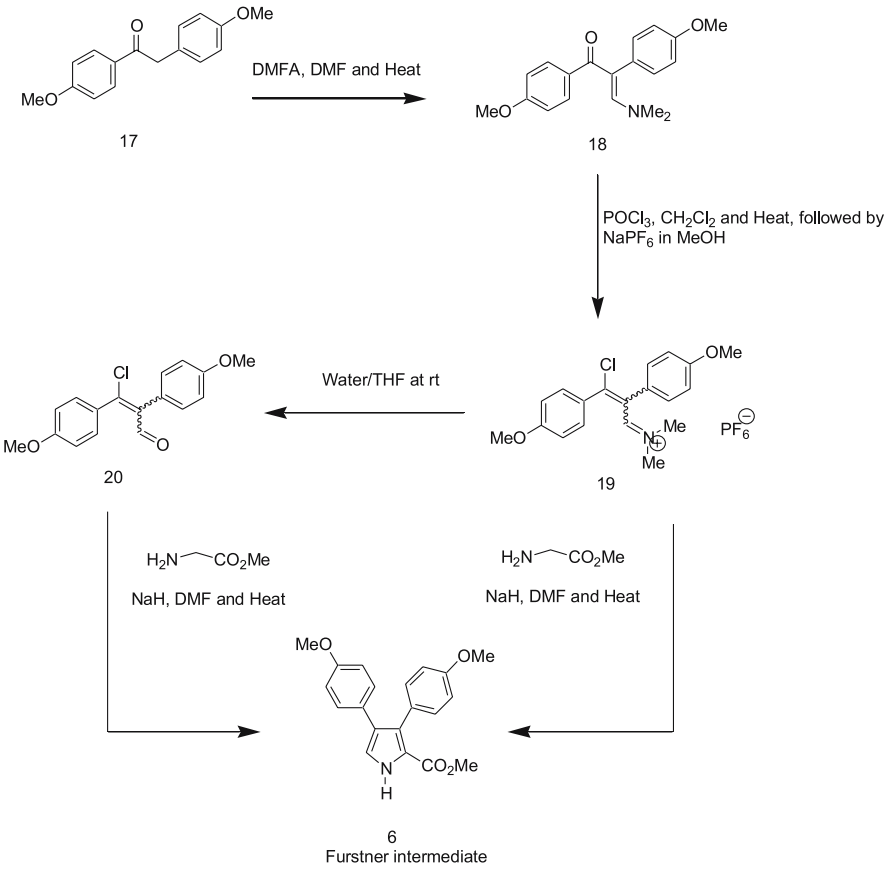


Scheme 3 Boger Group Synthesis of Lukianol

groups in such pyrroles are normally associated with cytotoxic behavior while the O-protected derivatives are associated with MDR reversal behavior. The observance of this kind of activity is a continuing theme for many of the oxygenated 3,4-diarylated pyrrole-containing natural products.

Our own research group [11] has been interested in using vinylogous iminium salt derivatives for the preparation of highly functionalized pyrroles, thereby providing another alternative to the synthesis of lukianol A.

Our synthesis (Scheme 4) begins with the treatment of desoxyanisoin (17) with DMF acetal to give a vinylogous amide (18), which is then converted to a diarylsubstituted vinylogous iminium salt (19) as an E/Z mixture of isomers in a 60/40 ratio, respectively. This salt is crystallized cleanly with sodium hexafluorophosphate in methanol. This compound (19) is condensed with glycine methyl ester hydrochloride under basic conditions to give the Furstner intermediate (6) which was previously converted to lukianol A. It is postulated that that the iminium salt (19) forms a Schiff's base with glycine and this is followed by an anionic ring closure with elimination of chloride and dimethyl amine to give the desired 2,3,4-trisubstituted pyrrole (6). Alternatively, the iminium salt can be hydrolyzed to the corresponding β-chloroenal (20), which also reacts readily with glycine methyl ester hydrochloride under basic conditions to produce the Furstner intermediate (6). Kim and coworkers [12] have reported a modification of our synthetic strat-

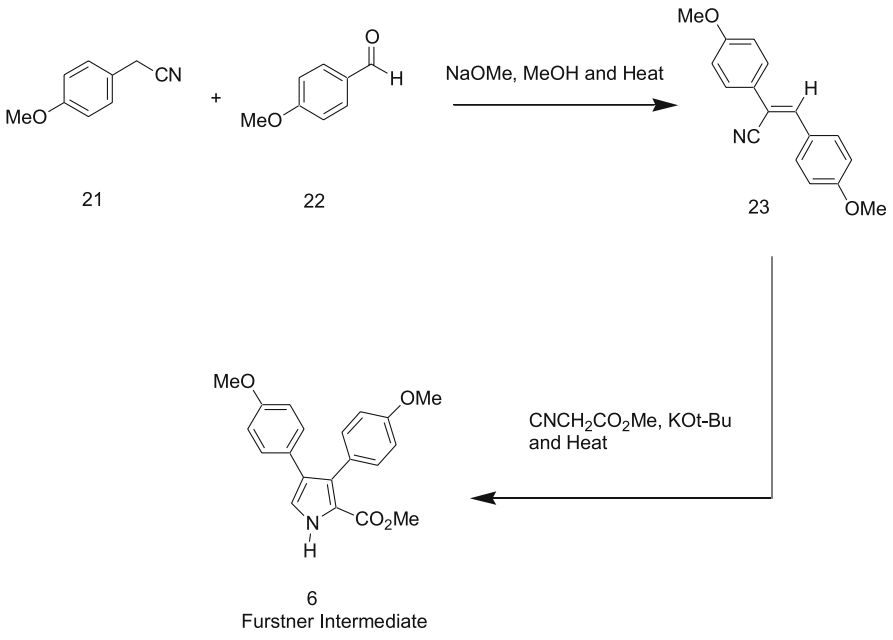


Scheme 4 Gupton Group Synthesis of Lukianol

egy by condensing the dimethyl ester of aminomalonate with our vinylogous amide (18) in an acidic two-step process which involves heating in acetic acid in the first step, followed by heating in acetic acid/water. In collaboration with our group and the Hall and Burnham groups [13–16], our key pyrrole intermediate (6) was evaluated against a number of cancer cell lines in which case ED_{50} values in the range of 3–20 μM were observed. It was also determined that when L1210 lymphocytic leukemia cell metabolism was examined over a 60-min period upon treatment with this pyrrole (6), DNA and protein synthesis were inhibited. Inhibition of the enzymes DNA polymerase α, certain RNA polymerases, ribonucleoside reductase, dihydrofolate reductase, PRPP amido-transferase, IMP dehydrogenase as well as purine de novo synthesis was also noted during this study. Additional synthetic analogs, which contained only one aryl group and in some cases no methoxy or no hydroxyl groups, continued to exhibit cancer line ED_{50} values in the range of 3–20 μM.

The Bullington group [17] at Johnson and Johnson Pharmaceutical have also developed a very efficient and concise synthesis (Scheme 5) of the Furstner intermediate (6) to lukianol A. The synthesis relies on the condensation of benzyl nitriles with aromatic aldehydes under basic conditions to give the corresponding electron deficient alkenes (23).

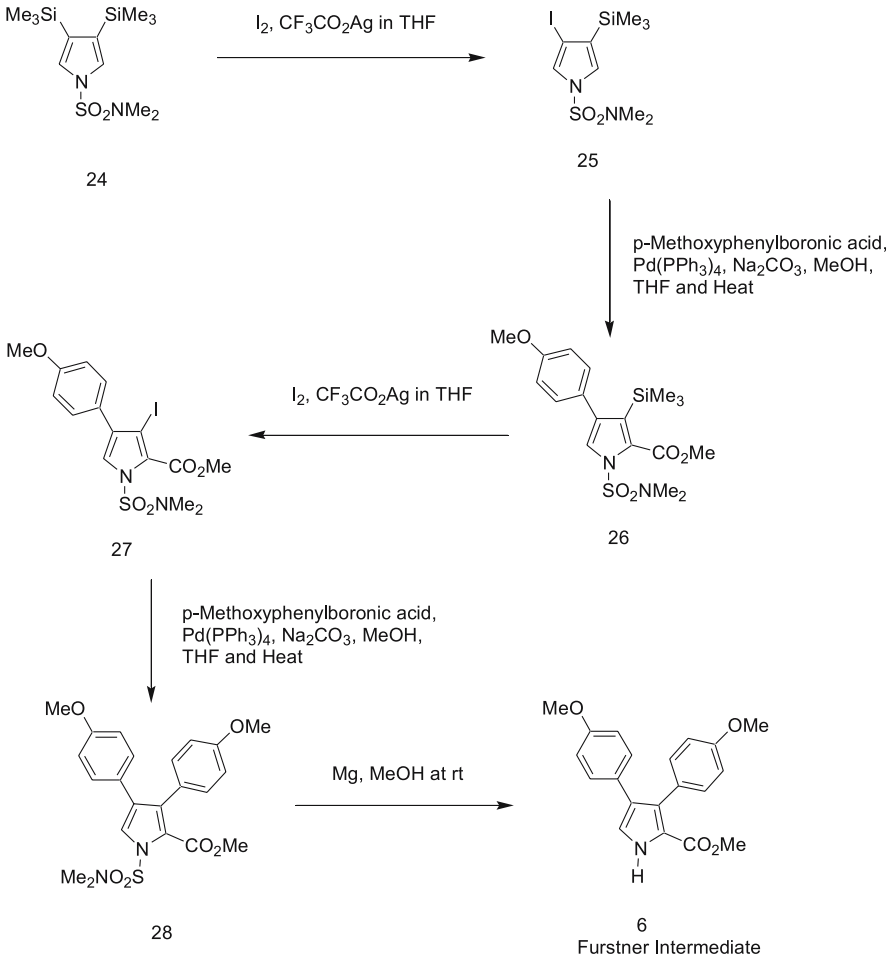
Methyl isocyanoacetate is known to react with such electron deficient alkenes under basic conditions to give 2-carboalkoxy pyrroles (6) via



Scheme 5 Bullington Group Synthesis of Lukianol

a Michael-type addition followed by ring closure and elimination. One of the strengths of this approach is the ease of incorporation of various aromatic groups into the pyrrole ring with control of regiochemistry.

The final method (Scheme 6) available for the preparation of the Furstner intermediate (6) for conversion to lukianol A was developed by Wong and his group [18]. It is somewhat reminiscent in a general sense to the cross-coupling strategy used by Banwell. A 3,4-bis(trimethylsilylated)pyrrole (24) protected as an N-sulfonamide is monoiodinated to differentiate the reactivity of the 3,4-positions. This iodopyrrole (25) undergoes Suzuki cross-coupling with 4-methoxyphenylboronic acid at the iodo-bearing carbon. This monoarylated pyrrole (26) then undergoes replacement of the remaining

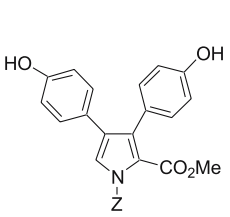


Scheme 6 Wong Group Synthesis of Lukianol

TMS group and the Suzuki cross-coupling reaction is again repeated. This sequential process allows for the introduction of different aromatic groups at positions 3 and 4 of the pyrrole system. The synthesis of the Furstner intermediate (6) is completed by removal of the sulfonamide group.

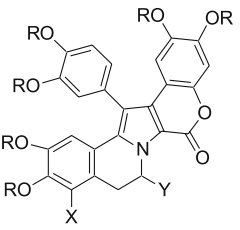
3 Lamellarins

The lamellarins (Fig. 4) are another group of the 3,4-diarylpyrrole-containing natural products of marine origin. The first isolation and structure determination of these substances was reported [19] by Faulkner and Clardy in 1985. As a consequence of their significant biological activity, this class of compounds has attracted great attention. Bailly has recently provided an excellent review [20–24] detailing the origin, structure, and relevant biological information for the lamellarins. There are at present over 30 compounds that are considered to be lamallarin-type alkaloids having been isolated from prosobranch mollusks and ascidians. From a structural standpoint, the lamellarins fall into one of three categories (Fig. 4). Lamellarins O, P, Q, and R have a motif (29) closely related to the lukianols. At least 16 of the remaining lamellarins can be characterized by a tetrahydroisoquinoline structure (30) and another eight examples exhibit the dihydroisoquinoline framework (31). Bailly, Steglich and others have suggested that the biogenesis of these and related 3,4-diarylpyrroles derives from 3,4-dihydroxyphenylalanine (DOPA) secondary metabolites and that substances with a dihydroisoquinoline structural type (31) have a clear specificity for acting on DNA manipulating enzymes.



29

Z = oxygenated phenyl group connected by a one or two carbon tether to nitrogen; a carbonyl group may be present in the tether.

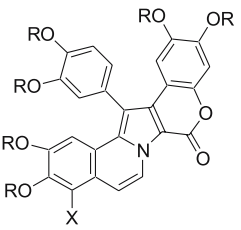


30

X = MeO or HO or H

Y = HO or H

R = Me or H



31

X = MeO or HO or H

R = Me or H or SO_3Na

Fig. 4 General Structure of Lamellarin Natural Products

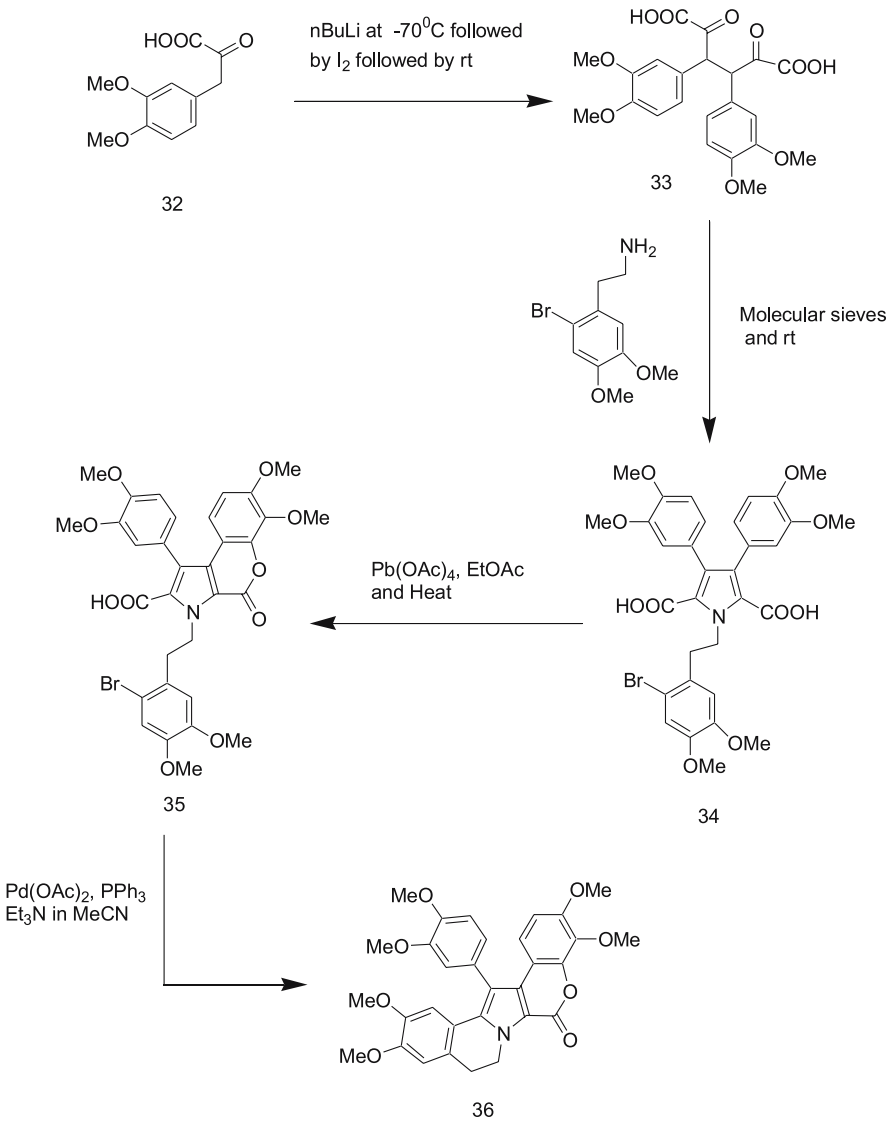
Bailly's review [20–24] covers in great detail the origins and nature of lamellarin biological activity and a brief treatment of this topic will be mentioned here. Compounds exhibiting the dihydroisoquinoline structure (31), which bear a sulfate group, show impressive activity as inhibitors of HIV-1 integrase [25] whereas little cytotoxicity for cancer cell lines is observed. Other compounds of this structural type, such as lamellarin D, are known to be inhibitors of mammalian topoisomerase I, which is the site of action for the important anticancer agent camptothecin. Additional lamellarins of this class (31) are also known to bind to DNA, and in the presence of certain metals, will produce DNA cleavage. Ishibashi [26] and Quesada [27] have reported that lamellarins of type 30 and 31 exhibit IC_{50} values in the range of 10 ηM for a variety of cancer cell lines and they have correlated this activity to the presence or absence of certain methoxy and hydroxy group substitution. More recently, Bailly and coworkers [28] have pointed out that lamellarin D can act as a potent apoptotic agent against P388 leukemia cells. As was the case for the lukianols, the lamellarins as reported by Quesada [27], exhibit very promising activity as MDR reversal agents at non-cytotoxic concentrations for cancer lines resistant to normal chemotherapy. Consequently, lamellarins with a structural scaffold containing the tetrahydroisoquinoline (30) or dihydroisoquinoline (31) framework have been an important target for many synthetic chemists and these syntheses will be presented in some detail.

In 1997, Steglich reported (Scheme 7) the synthesis [29] of lamellarin G trimethyl ether (36) based on the biosynthetic proposal that such compounds arise from 3,4-dihydroxyphenylalanine (DOPA) secondary metabolites.

The coupling reaction of appropriately substituted arylpyruvic acids with themselves in the presence of base and iodine at low temperature produced the desired symmetrical 1,4-diketone (33) in good yield. Reaction of this diketone with a highly functionalized arylethylamine in the presence of molecular sieves generates the pentasubstituted pyrrole (34). Subsequent treatment with lead tetraacetate produces a pyrrololactone (35) through oxidative lactonization. The last step involving the formation of the isoquinoline framework is quite unique and involves an intramolecular Heck-type reaction with loss of a carboxylic acid group to produce lamellarin G trimethyl ether (36). Steglich has also used a similar strategy [30] for the preparation of lamellarin L whereby two different arylpyruvic acids were coupled together in the first step of the synthesis.

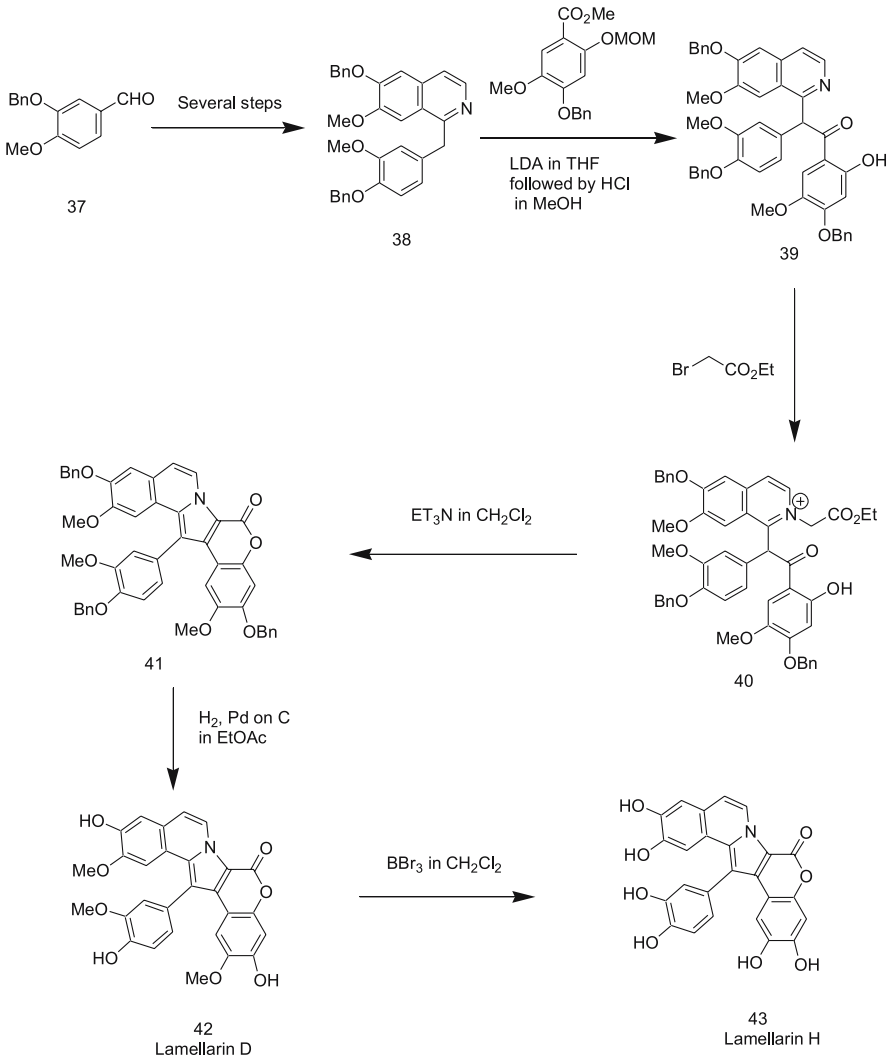
Ishibashi and coworkers [26] have reported a very efficient method for synthesizing a variety of lamellarin alkaloids and the preparation of lamellarin D and H are presented in Scheme 8.

An appropriately functionalized isoquinoline (38) bearing a benzyl group is prepared from an aromatic aldehyde (37). The benzyl position is metalated with LDA and the resulting carbanion is reacted with a highly substituted methyl benzoate to produce a ketone (39). The isoquinoline nitrogen is alkylated with ethyl α-bromoacetate and the resulting quaternary salt is cyclized



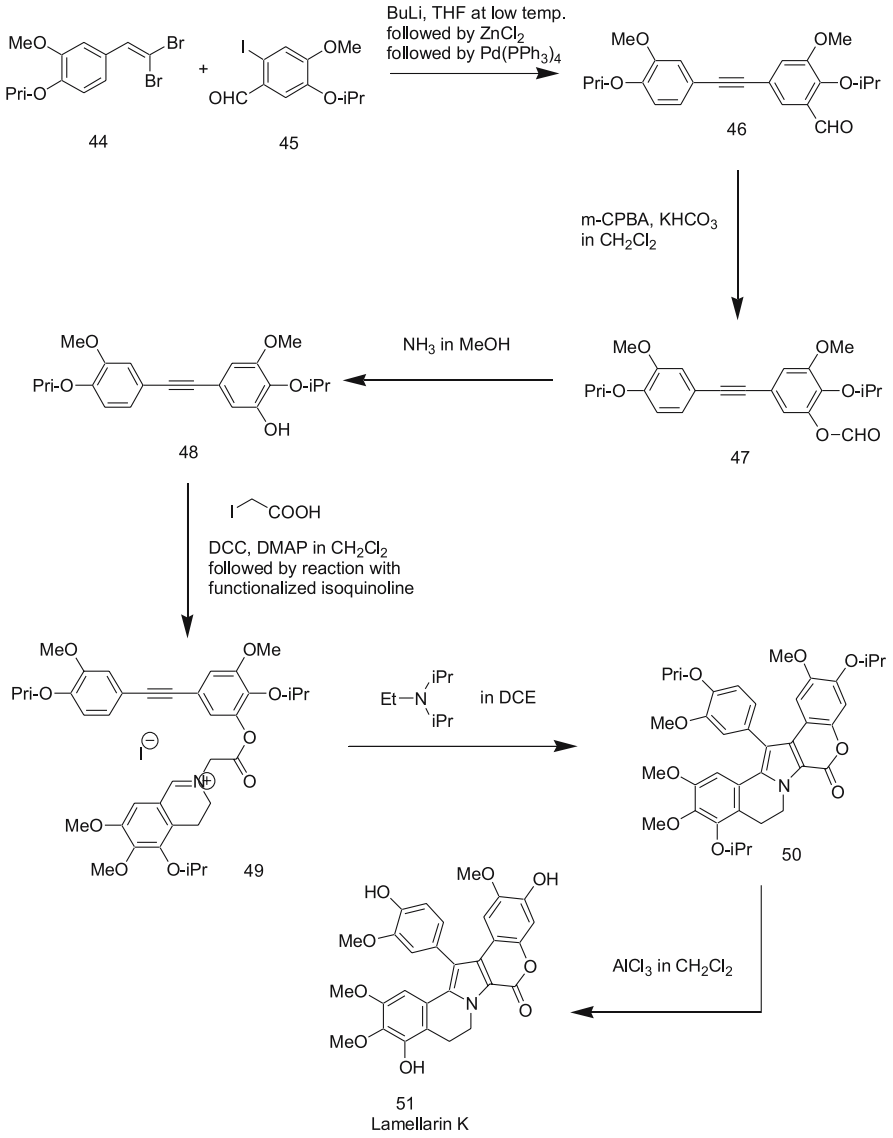
Scheme 7 Steglich Group Synthesis of Lamellarin Natural Products

with triethyl-amine to the lamallarin scaffold (41). The O-benzyl protecting groups are removed from the pyrroloisoquinoline (41) via hydrogenolysis to give lamellarin D (42), which could be transformed to lamellarin H (43) by treatment with boron tribromide. Assuming the benzylated isoquinolines (38) can be easily prepared with some degree of structural diversity, this synthetic strategy should provide a wide range of lamellarin analogs in a very efficient manner.



Scheme 8 First Ishibashi Group Synthesis of Lamellarin Natural Products

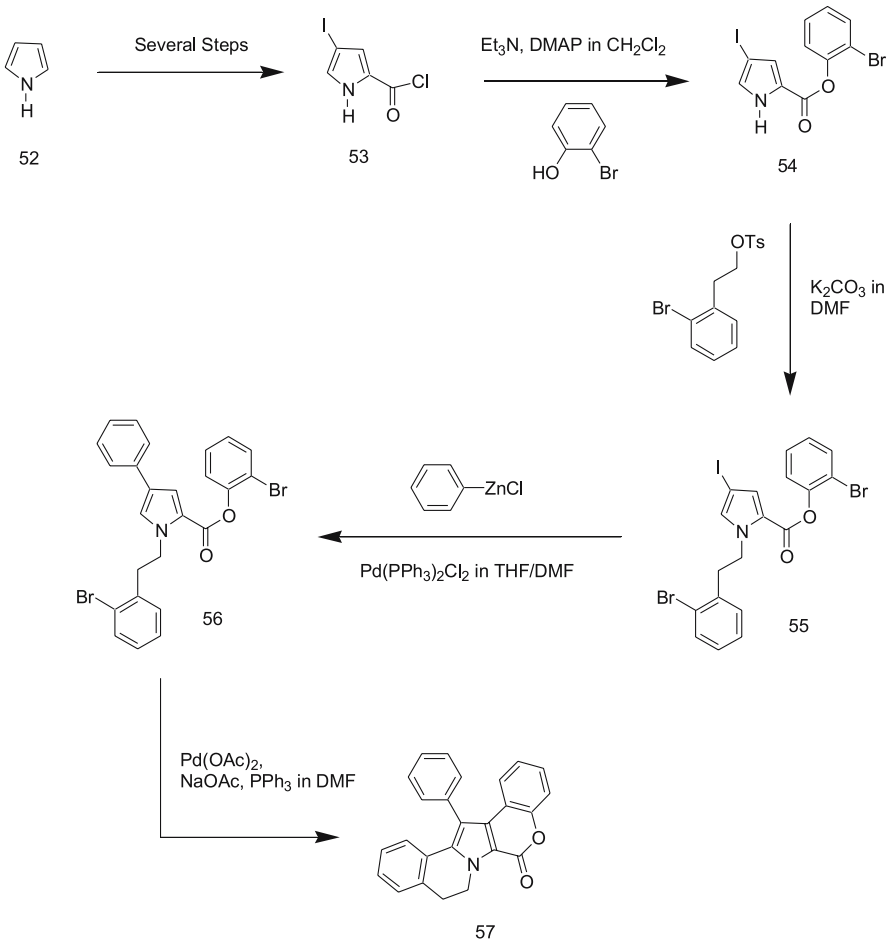
Banwell's group [31] has also made significant contributions to the synthesis of lamellarin-type alkaloids and their preparation of lamellarin K is described in Scheme 9. The preparation of unsymmetrical alkynes (46) bearing oxygenated phenyl groups is accomplished by cross-coupling chemistry. A phenolic group (48) is then unmasked via a Bayer–Villager oxidation and subsequent ammonolysis. The phenolic group is esterified with α-iodoacetic acid and the resulting α-iodoacetic acid ester is reacted with an appropriately substituted dihydroisoquinoline to give a quaternary salt (49). Upon treatment of this salt (49) with base an azomethine ylide forms and undergoes



Scheme 9 First Banwell Group Synthesis of Lamellarin Natural Products

intramolecular cycloaddition to the alkyne to form the lamellarin scaffold (50). Selective deprotection of the i-propoxy groups by aluminum trichloride in methylene chloride produces lamellarin K (51).

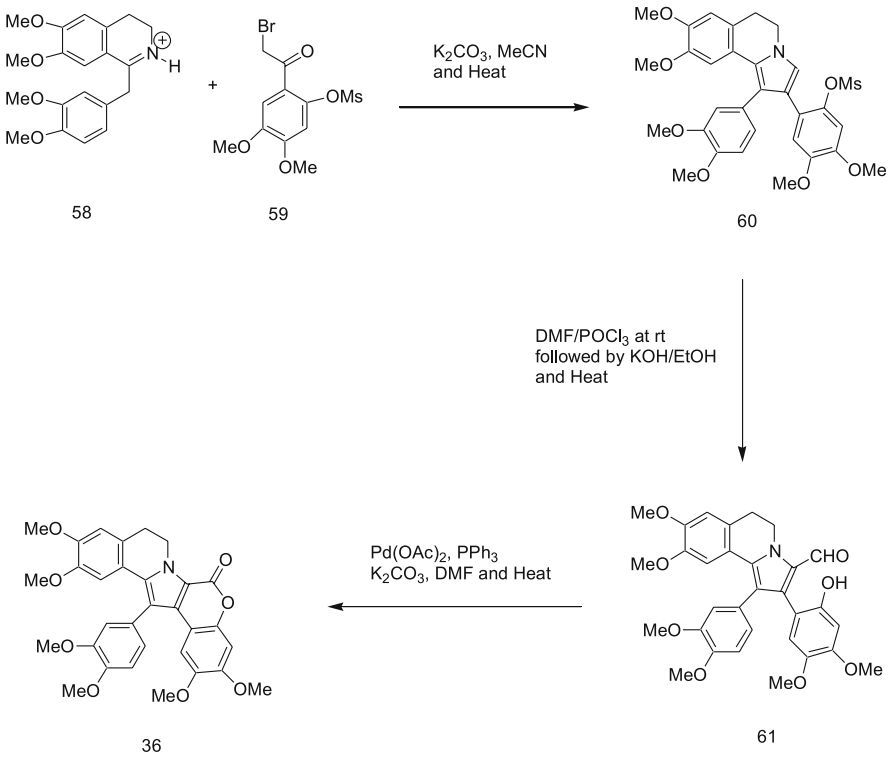
Banwell's group has provided an alternative strategy [32] for obtaining the lamellarin framework and it is presented in Scheme 10. The methodology involves the formation of a 2,4-disubstituted pyrrole (53) containing an acid



Scheme 10 Second Banwell Group Synthesis of Lamellarin Natural Products

chloride at carbon 2 and an iodo group at carbon 4. The acid chloride is esterified with 2-bromophenol and this product (54) is then alkylated on nitrogen with the tosylate ester of o-bromophenethyl alcohol to give a trisubstituted pyrrole (55). This pyrrole is subjected to a Negishi cross-coupling reaction to yield a key pyrrole synthon (56), which is then subjected to an intramolecular Heck-type coupling reaction producing the lamellarin scaffold (57). Banwell has referred to this process as a "double-barrelled approach" since two Heck reactions are accomplished in a one-pot process. Although the yield is rather low for the last step, the rapid assembly of the lamellarin skeleton is quite impressive.

Ruchirawat has also been active in the construction of the lamellarins and his route [33] to lamellarin G trimethyl ether (36) is depicted in Scheme 11.



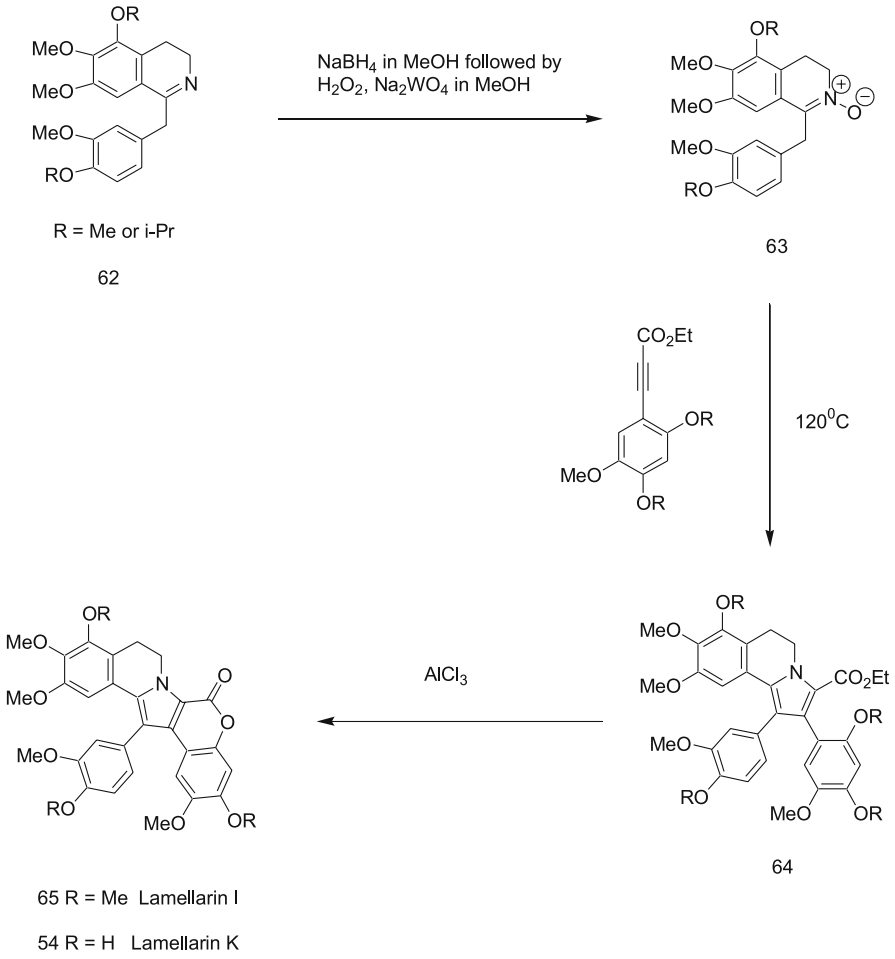
Scheme 11 Ruchirawat Group Synthesis of Lamellarin Natural Products

In this route a dihydroisoquinoline (58) is N alkylated with a highly functionalized α-bromoacetophenone (59) to give a quaternary salt (60), which is treated with base and cyclizes to a pyrroloisoquinoline (60). The pyrrole nucleus is then formylated under Vilsmeier–Haack conditions at position 5 and a proximate mesylated phenolic group is deprotected with base to yield a pentasubstituted pyrrole (61). Subsequent oxidative cyclization of this formylpyrrole produces the δ-lactone portion of lamellarin G trimethyl ether (36). This sequence allows for rapid and efficient analog synthesis as well as the synthesis of the natural product.

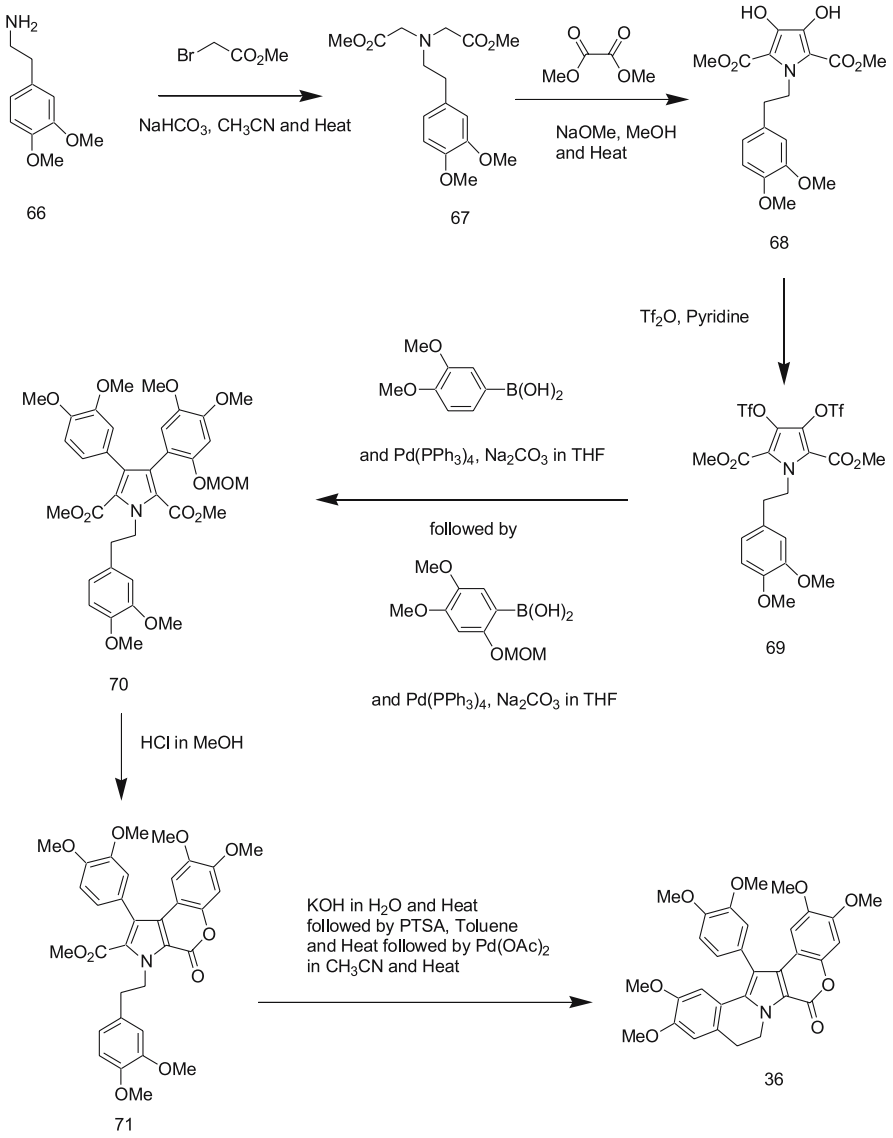
Ruchirawat has also developed a second generation and third generation variant of Scheme 11. The second generation strategy [34] utilizes the basic structure of compound 60 whereby a bromine is present at the 5-position of the pyrrole and the nearby mesylate group is replaced with a carbethoxy substituent allowing for selective metalation and ring closure to lamellarin G trimethyl ether (36). The third-generation strategy [35] employs an ethoxycarbonyl-β-nitrostyrene for construction of the desired pentacyclic precursors (61).

Guitian and coworkers [36] have also employed dihydroisoquinoline starting materials (62) for their approach (Scheme 12) to the lamellarins. The dihydroisoquinoline nitrogen is oxidized and the N-oxide (63) undergoes a 1,3-dipolar cycloaddition with an alkynyl ester to generate the desired lamellarin precursor (64) after rearrangement of the initial adduct. Deprotection of an isopropyl protected phenol and subsequent lactonization with aluminum trichloride yields either lamellarin I (65) or K (54) depending on the nature of the phenolic protecting groups that are employed.

Ishibashi and Iwao have reported [37] a novel route (Scheme 13) to lamellarin G trimethyl ether that utilizes a much different strategy than the one outlined in Scheme 8. The synthesis begins with the dialkylation of a phenethyl amine with ethyl α-bromoacetate to yield an aminodiester (67).



Scheme 12 Guitian Group Synthesis of Lamellarin Natural Products

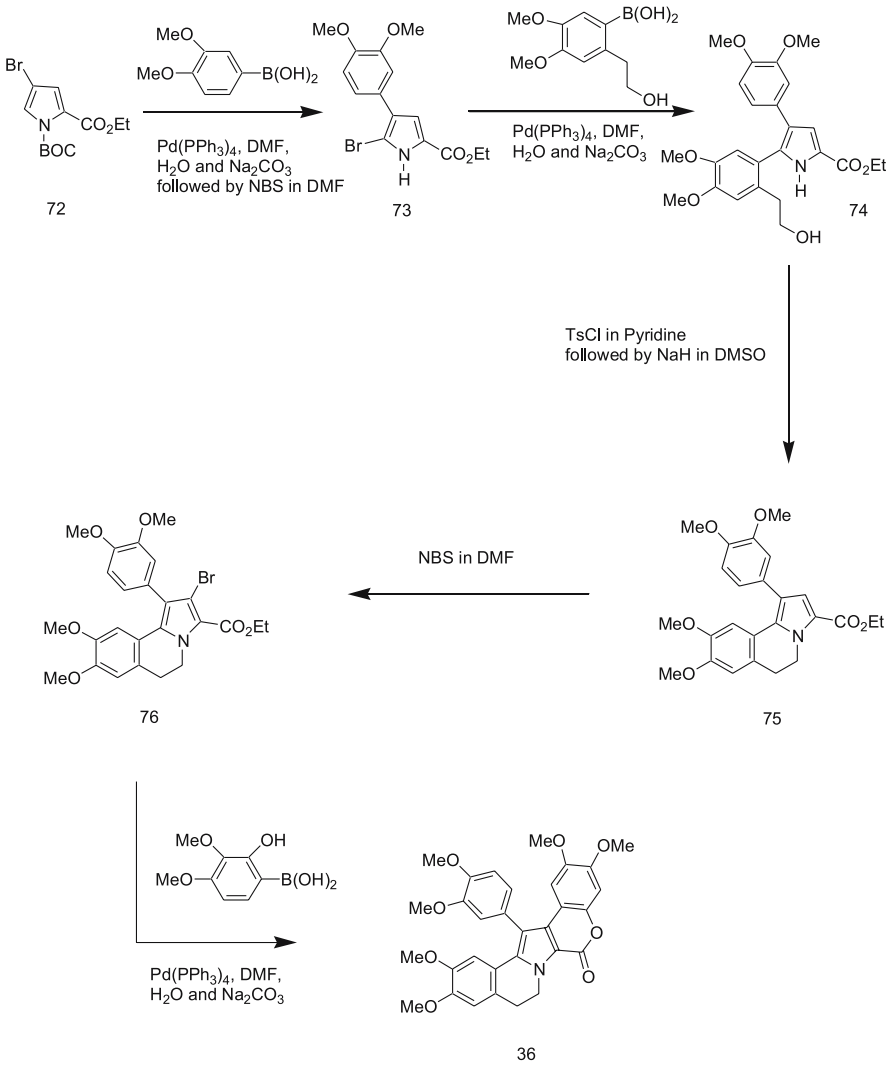


Scheme 13 Second Ishibashi Group Synthesis of Lamellarin Natural Products

This aminodiester (67) is condensed under basic conditions with dimethyl oxalate to give a pentasubstituted system (68) bearing hydroxyl groups at positions 3 and 4 of the pyrrole. The hydroxyl groups are triflated and sequentially cross-coupled with highly oxygenated boronic acids to yield a pentasubstituted pyrrole (70). This pyrrole (70) is hydrolyzed, cyclized and decarboxylated to give an intermediate, which is subjected to Heck cross-

coupling conditions. This allows for the formation of the dihydroisoquinoline portion of the molecule and completes the transformation to lamellarin G trimethyl ether (36).

Handy and coworkers [38] have also offered a very useful approach (Scheme 14) to lamellarin G trimethyl ether (36) and they have referred to this strategy as being "modular in nature". The route starts with an N-protected 4-bromo-2-carboethoxypyrrole (72) and involves introduction of three different aryl groups via three sequential, regiospecific halogenations, which are



Scheme 14 Handy Group Synthesis of Lamellarin Natural Products

followed by three Suzuki cross-coupling reactions. This procedure allows for great diversity for the introduction of alternative aromatic groups. In addition, Handy and Zhang [39] have provided a recent and excellent review of the lamellarin alkaloids.

Alvarez and coworkers [40] have used a somewhat similar approach to the method of Handy with the exception that the dihydroisoquinoline framework is constructed first and bromines are then incorporated at positions 3 and 4 of the pyrrole followed by Suzuki cross-coupling reactions to introduce the highly oxygenated aryl groups. Due to the great interest in making analogs of the lamellarin family of alkaloids for drug-development studies, Alvarez and coworkers [41, 42] and Ruchirawat and coworkers [43] have successfully applied polymer-supported methods to the strategies previously described in this section. Clearly, the lamellarin scaffold is a privileged structure for antitumor activity as well as other disease targets.

4 Ningalins

The isolation and characterization of ningalins A–D (Fig. 5) from a dark purple *Didemnum* ascidian were first reported by Fenical [44] and Kang in 1997. Structurally, the ningalins somewhat resemble the lamellarins in that ningalins A and B possess either one or two δ-lactone rings and in their report Fenical and Kang suggested that ningalin A may well be a biogenic precursor to many of the lamellarins. Ningalins C and D are very unique in that they contain the 2,5-pyrroledione core and are closely related to purpurononе [45] and the polycitrins. Purpurononе only slightly differs in structure from Ningalin D in that the N substitution corresponds to a 4-hydroxyphenethyl group. These 2,5-pyrroledione natural products are quite interesting biologically in that they inhibit ATP-citrate lyase [45], which is a significant target for hypercholesterolemia therapy.

Boger and coworkers have examined the cytotoxicity of ningalin A and B derivatives against various cancer cell lines [10, 46] and have found significant activity. Of even greater interest has been the impressive ability of these ningalins (A and B) to function as MDR reversal agents [10, 46, 47] at non-cytotoxic concentrations as reported by Boger in several papers. As presented earlier in the discussion of the lukianols, Boger and his group have very successfully utilized [10, 46] highly functionalized diazines as precursors to tetrasubstituted pyrroles and the synthesis of Ningalin A is presented in the Scheme 15.

The Boger group synthesis commences with a Stille coupling of a stannyl acetylene with two equivalents of a highly oxygenated bromobenzene to yield a symmetrical diarylalkyne (82). The subsequent reaction of this alkyne (82) with 3,6-dicabomethoxytetrazine under Diels–Alder/retrograde Diels–Alder

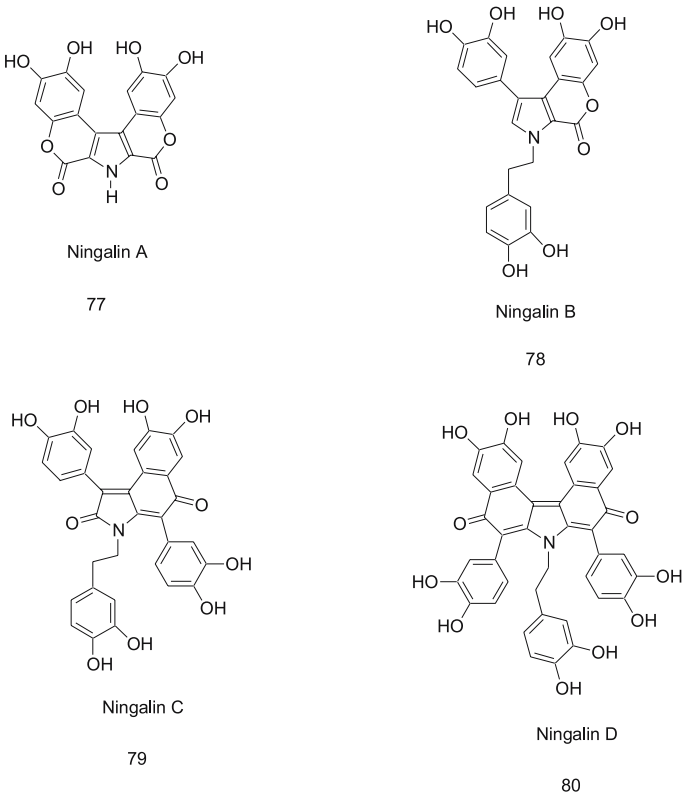
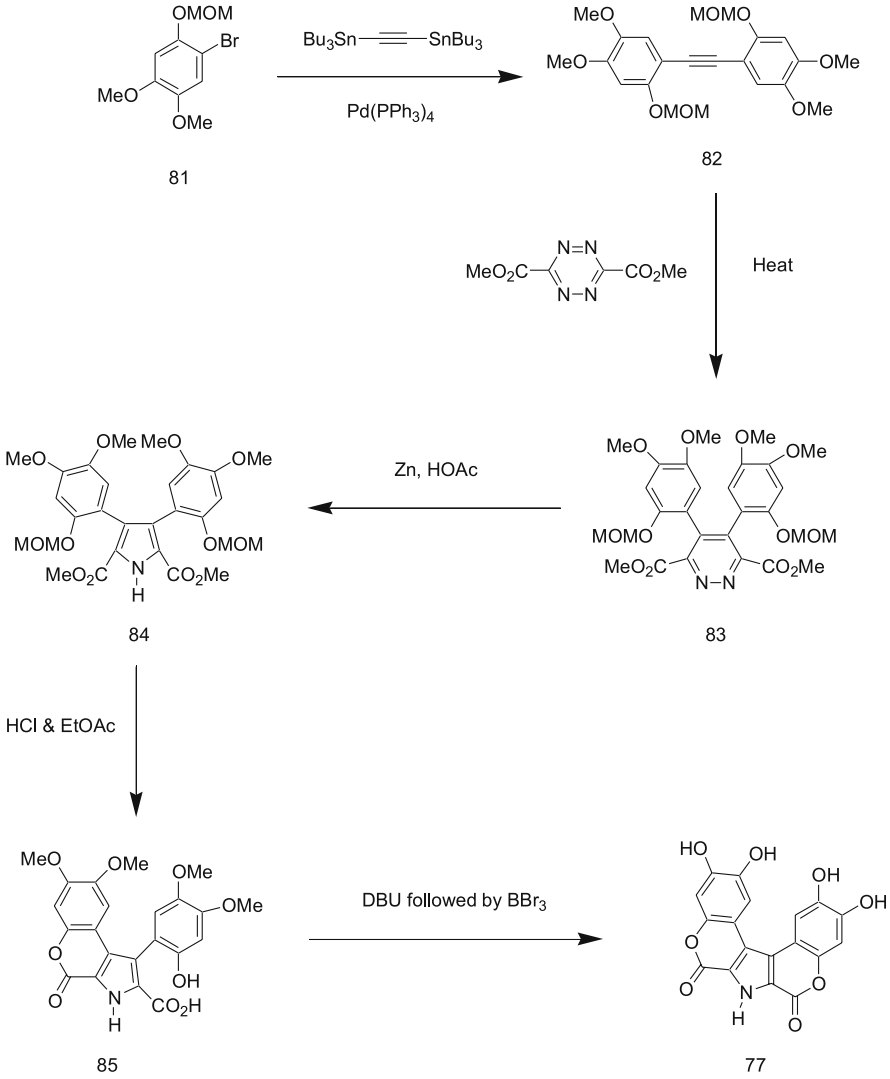


Fig. 5 General Structure of Ningalin Natural Products

conditions produces a 3,4,5,6-tetrasubstituted diazine (83). Reduction with Zn/HOAc converts the tetrazine to the pyrrole (84) and mild acid hydrolysis produces the monolactonized derivative (85). Treatment of this compound (85) with DBU followed by boron tribromide yields ningalin A (77) in good overall yield.

Our research group [48] has also been interested in the synthesis of ningalin B and our route to ningalin B hexamethyl ether is presented in Scheme 16.

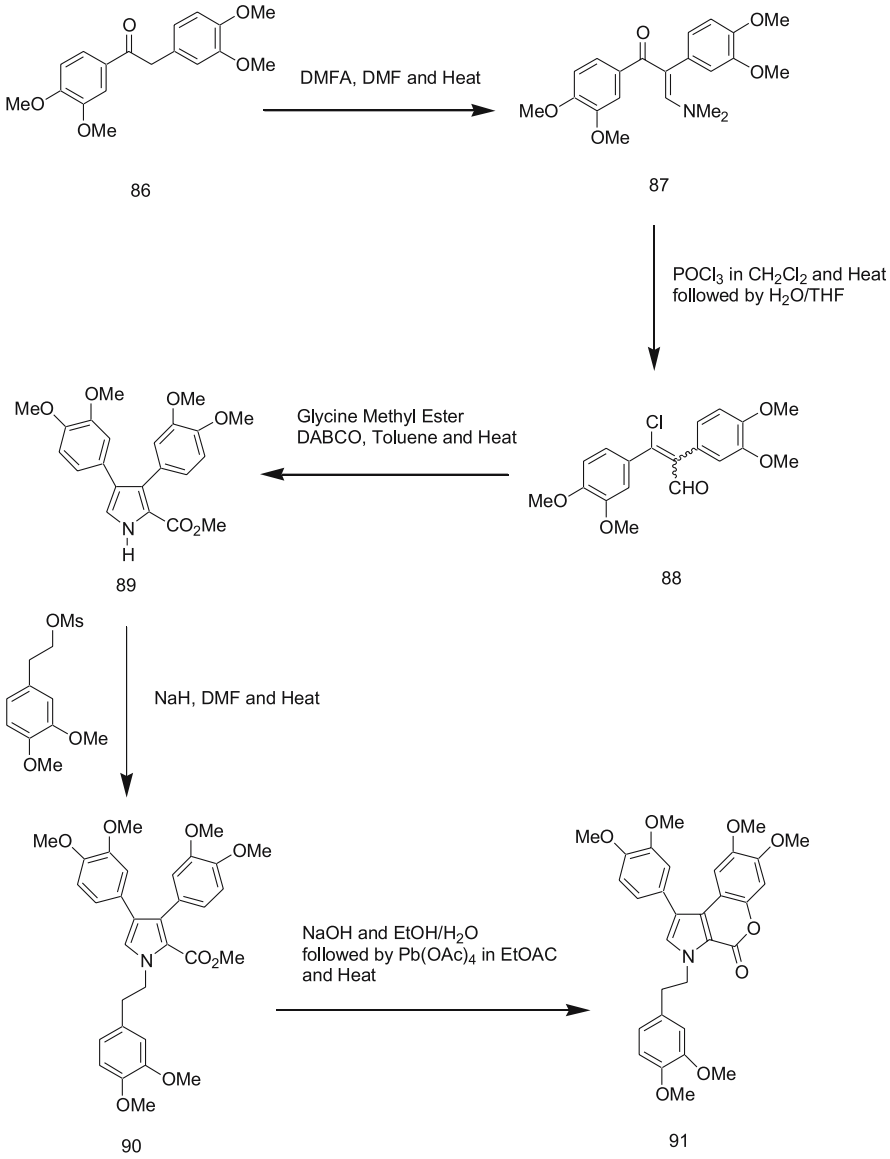
An aromatic ketone (86) is prepared by Friedel-Crafts acylation and is treated with DMF acetal to produce the corresponding vinylogous amide (87). The vinylogous amide is transformed in high yield to a mixture of β-chloroenal isomers (88) with the E-isomer predominating. Condensation of the β-chloroenal isomers (88) with glycine methyl ester produces the 2,3,4-trisubstituted pyrrole (89) in good yield. Interestingly, both β-chloroenal isomers are converted to the desired product. The pyrrole nitrogen is alkylated under basic conditions with the mesylate ester of an aryl ethanol to produce the desired pyrrole (90). Hydrolysis of the ester group of the pyrrole



Scheme 15 First Boger Group Synthesis of Ningalin Natural Products

(90) to the corresponding carboxylic acid followed by oxidative lactonization with lead tetraacetate yields ningalin B hexamethyl ether (91), which had been previously demethylated by Boger to ningalin B.

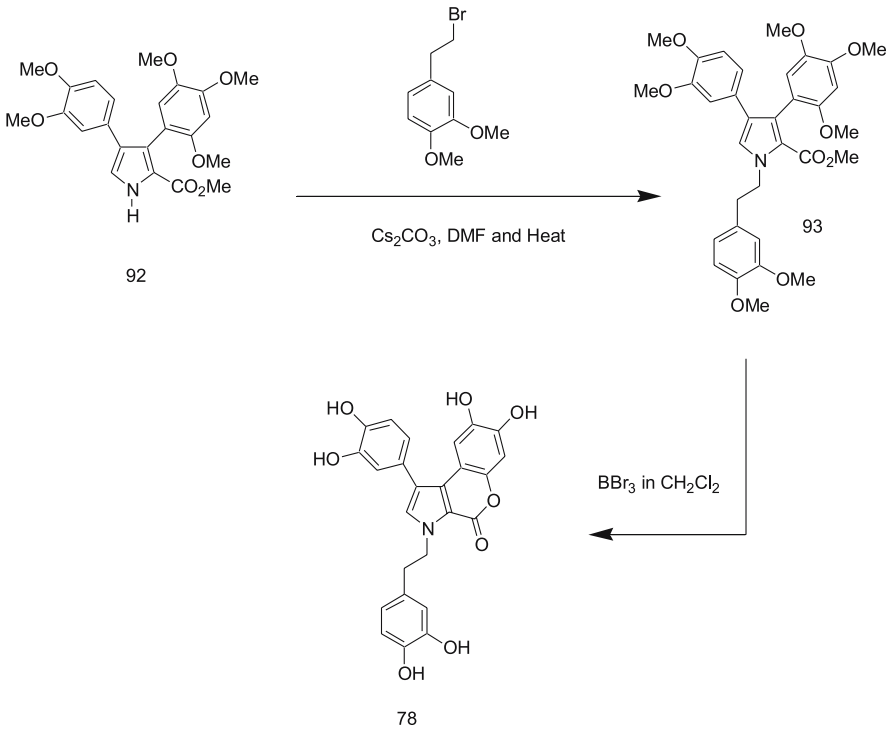
In Scheme 5 under the lukianol section, the Bullington synthesis [17] of 2,3,4-trisubstituted pyrroles was presented. Bullington has used this strategy to prepare ningalin B and the resulting steps in this route are presented in Scheme 17. This route also constitutes a very efficient method for the construction of the ningalin A and B scaffold.



Scheme 16 Gupton Group Synthesis of Ningalin Natural Products

Iwao and Ishibashi [37] have utilized their methodology depicted in Scheme 13 for the preparation of ningalin B hexamethyl ether (91) with compound 71 functioning as the key precursor for this relay synthesis. This strategy appears to be very flexible for a wide variety of pyrrole natural products.

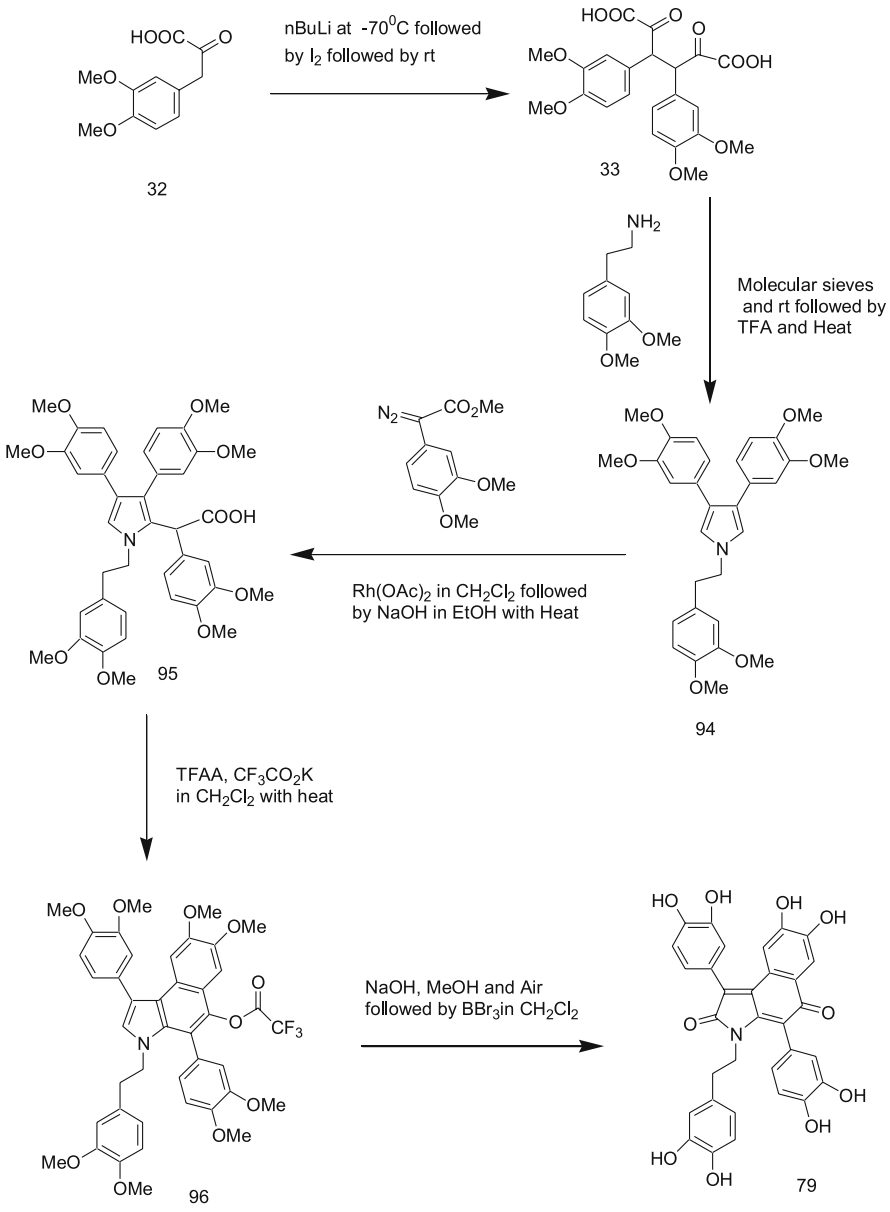
Steglich has developed [49] a synthesis (Scheme 18) of ningalin C based upon the route described in Scheme 7, which relies upon a biosynthetic pro-



Scheme 17 Bullington Group Synthesis of Ningalin Natural Products

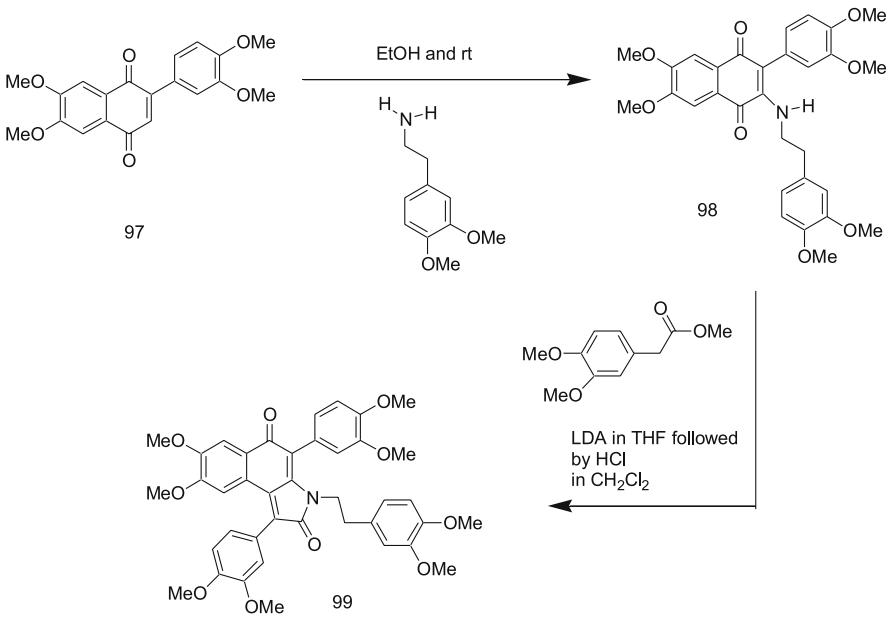
posal. A 1,4-diketone (33) is cyclized to a tetrasubstituted pyrrole (94) in the usual manner (see Scheme 7) and this compound undergoes bis decarboxylation under acidic conditions to give a 1,3,4-trisubstituted pyrrole (94). A Rhodium catalyzed insertion reaction is utilized to install a benzyl ester group at C-2 of the pyrrole (94), which is then hydrolyzed in base to the corresponding acid. Heating with trifluoroacetic anhydride/potassium trifluoroacetate effects a cyclization to give an acetate (96). Base-mediated hydrolysis of this ester (96) in the presence of air produces a double bond isomerization and oxidation to generate the 2,5-pyrroledione core. Demethylation with boron tribromide completes the synthesis of ningalin C (79).

Ruchirawat and coworkers [50] have also contributed a new methodology (Scheme 19) to the synthesis of ningalin C. Ruchirawat prepares a key benzoquinone (97) in three steps from readily available materials and this material is reacted with an arylethylamine, which undergoes Michael addition followed by air oxidation to give an aminobenzoquinone (98). This amine is then deprotonated with LDA at low temperature, trapped with an arylacetic acid ester and subjected to mild acidic dehydration conditions. The resulting product is permethyl ningalin C (99), which had been converted to ningalin C by Steglich as described in Scheme 18.



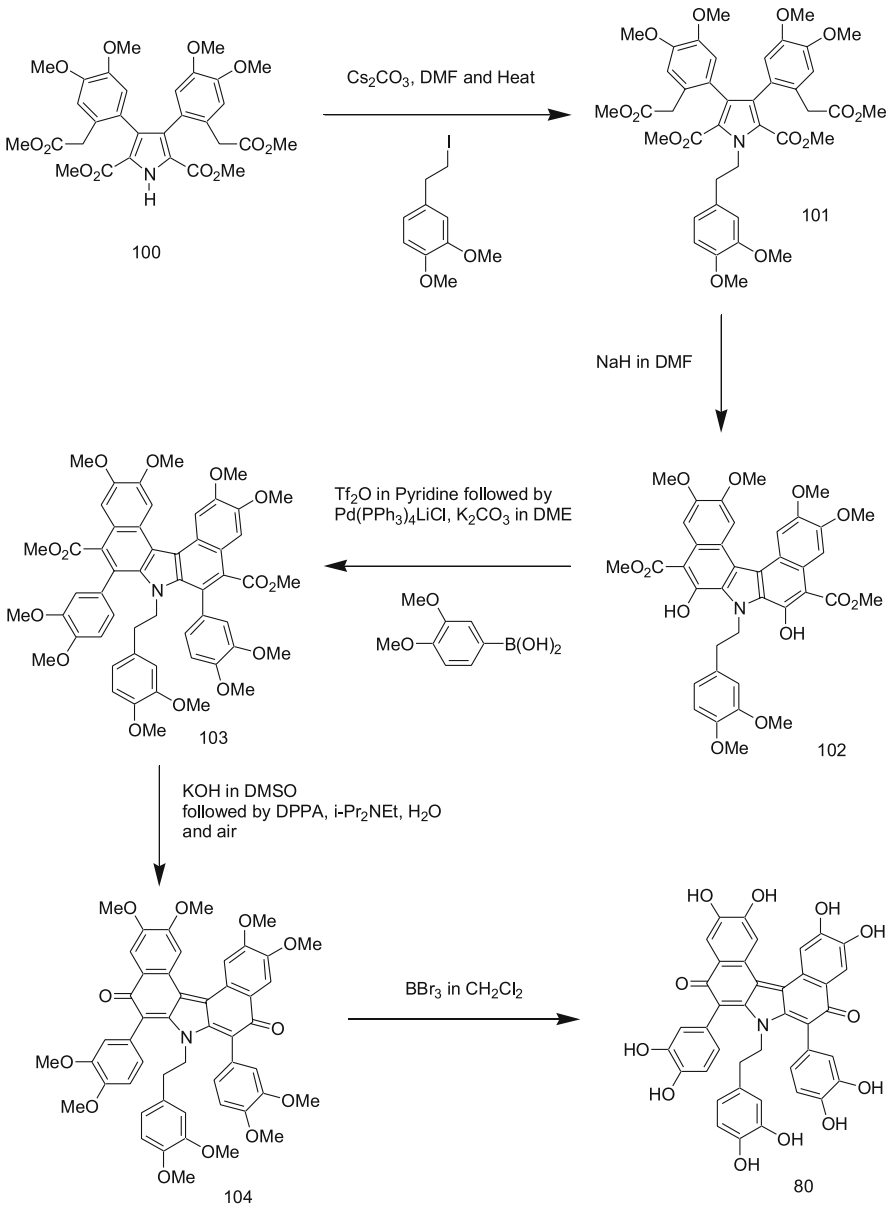
Scheme 18 Steglich Group Synthesis of Ningalin Natural Products

Boger and coworkers [51] have also extended their very useful pyrrole methodology to the preparation of ningalin D (Scheme 20). A tetrasubstituted pyrrole (100) is prepared in the usual manner (see Scheme 3) via Diels–Alder/retrograde Diels–Alder chemistry involving azines that undergo



Scheme 19 Ruchirawat Group Synthesis of Ningalin Natural Products

subsequent reductive cleavage. This pyrrole is subsequently N-alkylated and is then subjected to base-mediated Diekmann condensation to yield the desired highly functionalized pyrrole (102). The symmetrical pyrrole precursor (101) has been carefully constructed to allow the double Diekmann condensation to occur via proximate esters group attached to the aryl and pyrrole rings. The Diekmann product (102) is triflated at both phenolic sites and subjected to Suzuki cross-coupling in the presence of LiCl, which is apparently crucial for effecting a high-yield reaction, thereby producing the anticipated polycyclic framework (103). This material (103) is hydrolyzed to the corresponding diacid, which is then subjected to Curtius rearrangement conditions and the resulting isocyanate is trapped with water in the presence of air to yield permethyl ningalin D (104). Conversion to the natural product (80) is accomplished by dealkylation with boron tribromide. In this paper, Boger continues to report very interesting cytotoxicity to cancer cell lines and MDR reversal activity for the compounds, which were prepared as part of his synthetic route.

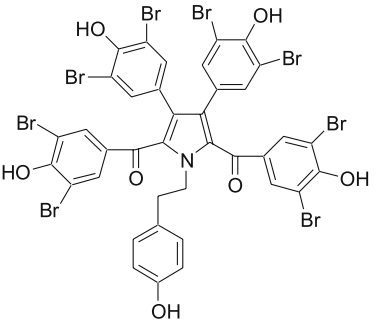


Scheme 20 Second Boger Group Synthesis of Ningalin Natural Products

5
Polycitones

The isolation from a marine ascidian and subsequent structure determination of polycitone A (105) (Fig. 6) was first reported [52] by Kashman and coworkers in 1994. In this paper, the penta-O-methyl derivative was reported to inhibit the growth of SV40 transformed fibroblast cells at a concentration of 10 μg/mL. Loya, Hizi and Kashman published [53] an extensive account of the biological activity of polycitone A in 1999 in which case inhibition of retroviral reverse transcriptases and cellular DNA polymerases was described. The isolation from an ascidian and structure determination of polycitone B (106) (Fig. 4) was subsequently reported [54] by Kashman and coworkers in 2000. Obviously, the presence of extensive bromination in both polycitone A and B make this family of compounds unique among the 3,4-diarylpyrrole natural products.

Steglich and coworkers reported [55] the first synthesis of polycitone A and B in 2002 by using their biomimetic pyrrole strategy as depicted in Scheme 7. A tetrasubstituted pyrrole synthon (3,4-diarylpyrrole-2,5-dicarboxylic acid, 107) is prepared in the usual manner (see Scheme 7). A Friedel-Crafts acylation of both carboxylic acids is accomplished to give a symmetrical diketone (108). This material is dealkylated with aluminum triiodide to give the corresponding symmetrical phenolic pyrrole (109). Treatment of this pyrrole (109) with excess bromine in acetic acid halogenates all of the phenolic groups at C-3 and C-5 to yield polycitone B, which is peracylated with acetyl chloride/triethylamine thereby producing the desired O-protected octabrominated pyrrole (110). Mitsunobu conditions allows for the alkylation of this pyrrole (110), which generates the N-phenethylated analog (111). Deprotection of this pyrrole (111) with hydrazine hydrate produces polycitone A (105).



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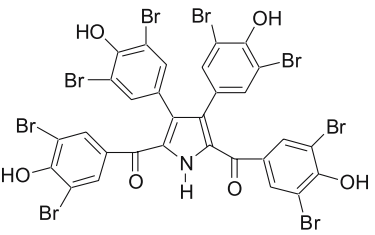
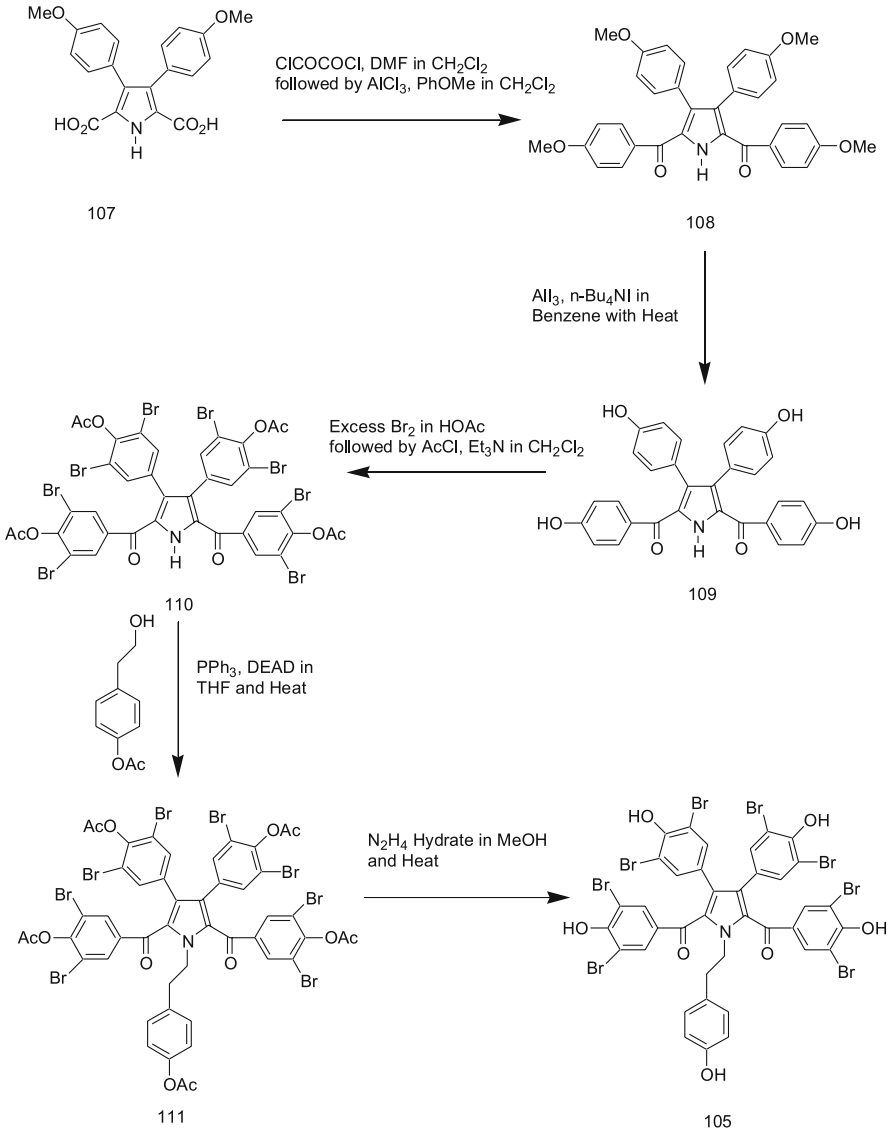


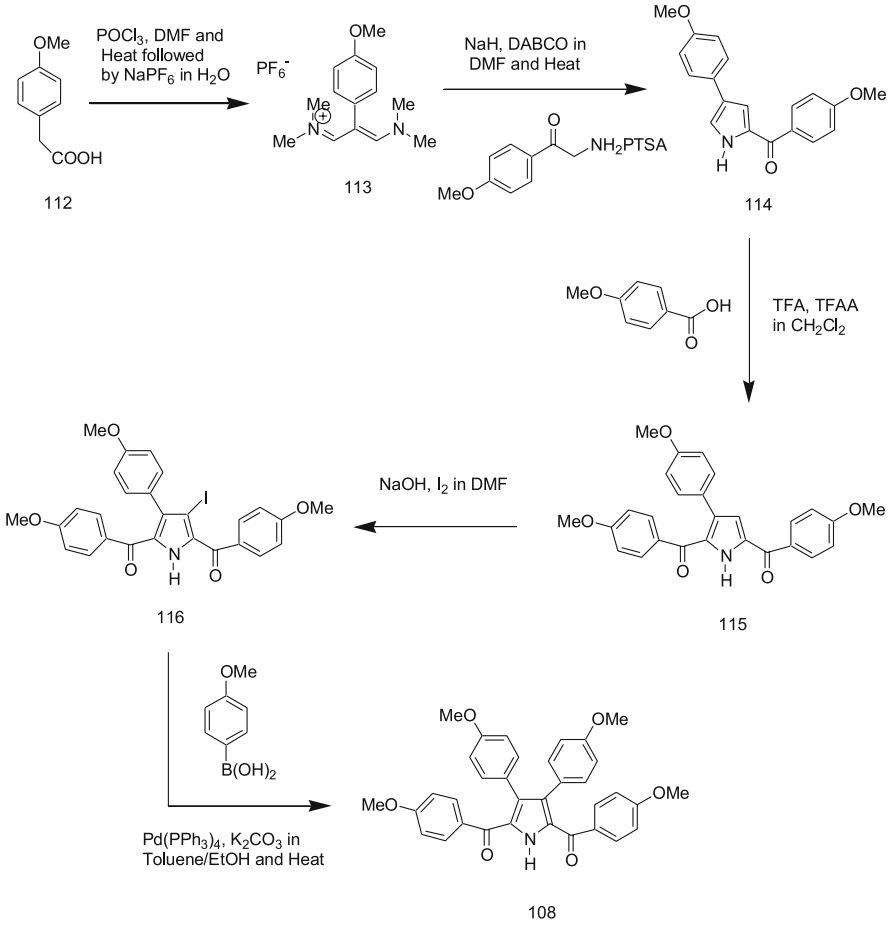
106

Fig. 6 General Structure of Polycitone Natural Products

Our research group [56] has also been interested in developing an efficient strategy (Scheme 22) for the preparation of the polycitones by utilizing vinylogous iminium salt starting materials. We quickly recognized that the symmetrical pyrrole (108) prepared by Steglich in Scheme 21 is an important synthon for any polycitone synthesis. This key intermediate (108) is prepared by formation of a vinamidinium salt (113) in high yield from 4-methoxyphenyl-



Scheme 21 Steglich Group Synthesis of Polycitone Natural Products



Scheme 22 Gupton Group Synthesis of Polycitone Natural Products

acetic acid under Vilsmeier conditions. Reaction of the vinamidinium salt (113) with the PTSA salt of α-amino-4-methoxyacetophenone produces a 2,4-disubstituted pyrrole (114) in high yield, which is acylated at the 5 position with 4-methoxybenzoic acid to give a trisubstituted pyrrole (115). Iodination of this pyrrole (115) is accomplished at position 4 and is followed by a Suzuki cross-coupling reaction to yield the Steglich synthon (108). Interestingly, the cross-coupling reaction only works well when the reaction is accelerated by microwave heating. We believe that stepwise introduction of the various aryl groups offers significant flexibility for analog synthesis and for subsequent SAR studies.

6
Storniamide

The structure determination of the storniamides was first reported [57] in 1996 by Palermo and coworkers as part of a bioassay driven extraction of a Patagonian sponge from the waters off the coast of Argentina. The structures of the storniamides (Fig. 7) differ only in the type of oxygenation of the phenyl groups and storniamide A (117) typifies this family of pyrrole-containing natural products. Initially, these compounds were found to exhibit antibiotic properties but Boger has reported [10] biological activity against an L1210 (leukemia) cell line and very significant activity as an MDR reversal agent. Furstner and coworkers [58] have reported similar results for such compounds relative to their MDR reversal behavior but he also noted that significant DNA-cleaving properties of storniamide A precursors were also observed.

Boger's group has successfully prepared [10] permethyl storniamide A (118) via a route (Scheme 23) such as the one already presented in Scheme 3.

A key pentasubstituted pyrrole (118) was prepared in the usual manner (see Scheme 3) via the Diels–Alder/retrograde Diels–Alder process followed by reduction, ring closure, N-alkylation and ester hydrolysis. This pyrrole diacid (118) was converted to the corresponding diamide (119) by use of the PyBrOP reagent. Oxidation of the thiols to sulfoxides was accomplished via sodium periodate and thermal elimination conditions produced a mixture of olefinic stereoisomers with the desired E,E-isomer predominating thereby yielding permethyl storniamide A (120). Steglich and coworkers have also prepared permethyl storniamide A (120) by a route analogous to the one presented in Scheme 7 and this will not be presented in any greater detail. Steglich's strategy for such natural products continues to be very important by virtue of its biomimetic approach.

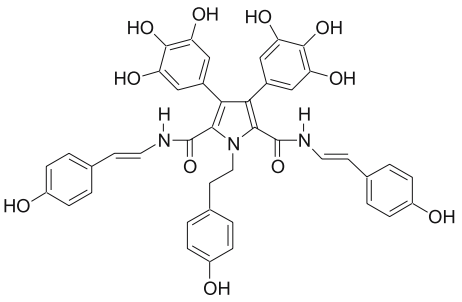


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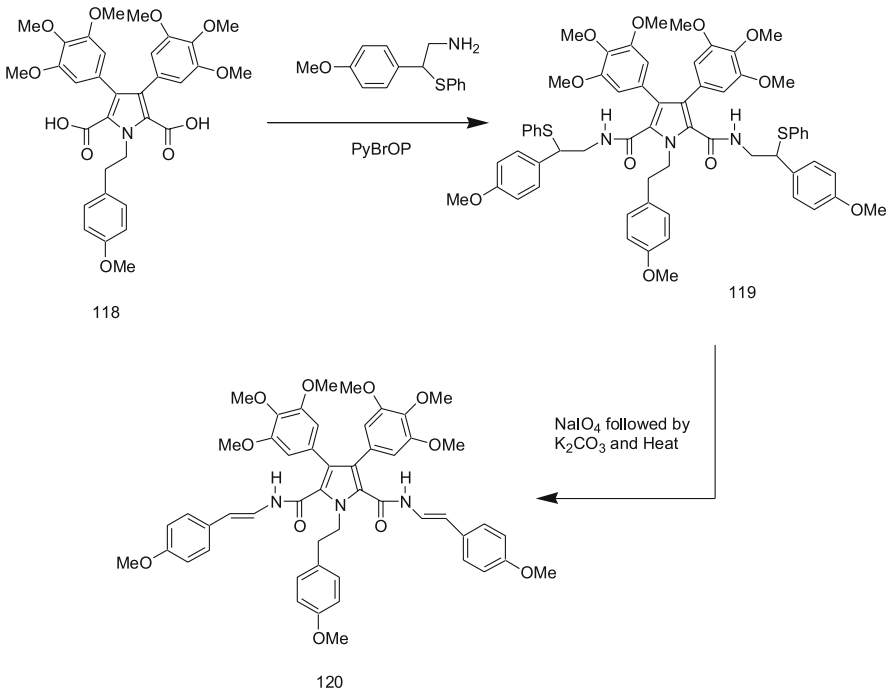
Fig. 7 General Structure of Storniamide Natural Products



118 119 120

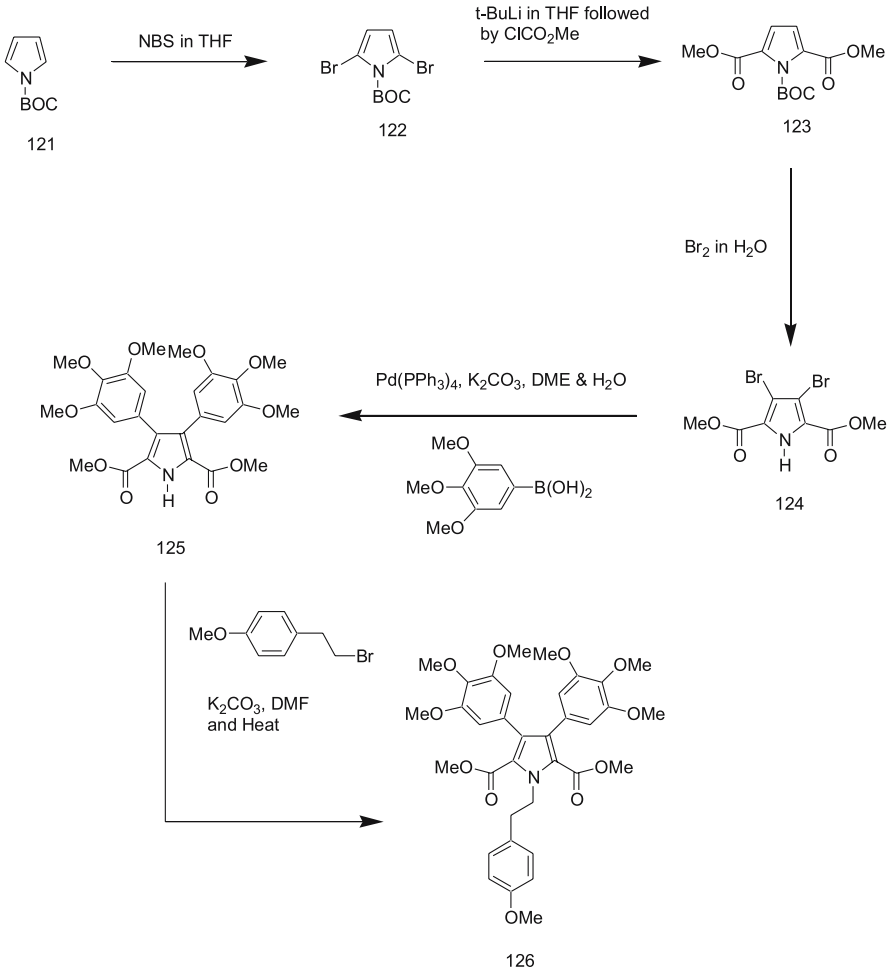
Scheme 23 Boger Group Synthesis of Storniamide Natural Products

Furstner's group has also developed [58] a very practical strategy (Scheme 24) for the relay synthesis of permethyl storniamide A (120) via an important diester (126), which was the precursor to the Boger diacid (118) presented in Scheme 23.

In this method, Furstner converts N-BOC protected pyrrole to the 2,5-dibromo compound (122) with NBS and this is followed by metalation and carbomethoxylation with t-butyl lithium in THF and subsequent trapping of the metalated species with methyl chloroformate to yield a pyrrole diester (123). Bromination of this diester at positions 3 and 4 with bromine in water followed by Suzuki cross-coupling with 3,4,5-trimethoxyphenyl boronic acid yields the symmetrical tetrasubstituted pyrrole (125). Base-mediated N-alkylation of this pyrrole with 4-methoxyphenethyl bromide produces the key Boger diester (126) and thereby constitutes a relay synthesis of permethyl storniamide A (120).

Iwao and Ishibashi [37] have also utilized their methodology presented in Scheme 13 to accomplish a relay synthesis of permethylstorniamide A and a brief representation of this route is given in Scheme 25.

The preparation of a pentasubstituted pyrrole (130) is accomplished by base-mediated condensation of an aminodiester (128) with dimethyl glyoxylate to give a 3,4-dihydroxylated pyrrole (129), which is reacted with triflic

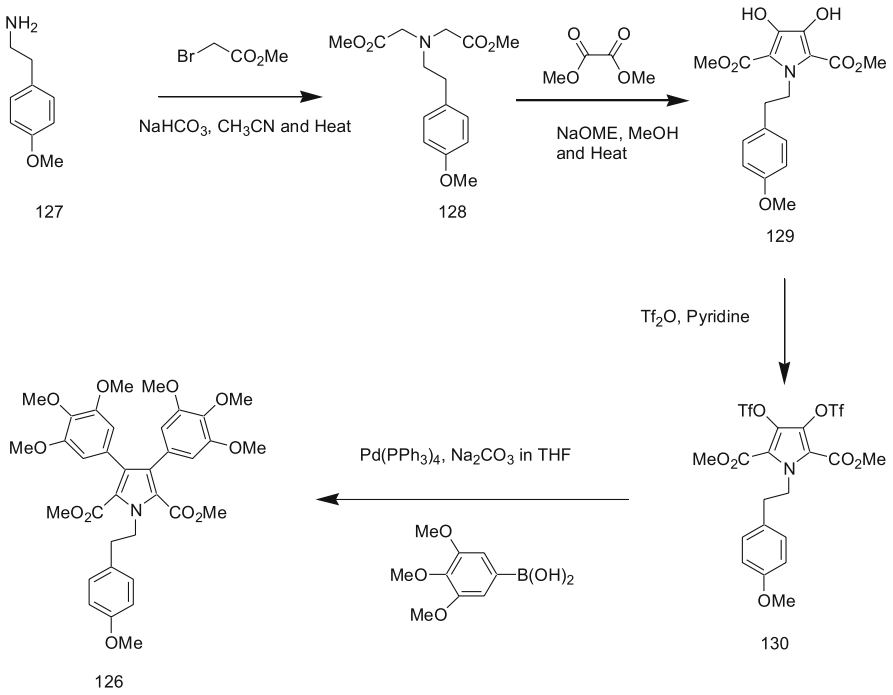


Scheme 24 Furstner Group Synthesis of Storniamide Natural Products

anhydride to produce a key pyrrole synthon (130). Application of Suzuki cross-coupling conditions to this pyrrole (130) generates the Boger diester (126) and completes the relay synthesis of permethylstorniamide A (120).

7 Lycogalic Acid and Halitulin

Lycogalic acid (131) and halitulin (132) represent unique analogs (Fig. 8) to the 3,4-diaryl pyrrole scaffold of the natural products previously discussed. Lycogalic acid (131) contains indole rings attached at both C-3 and C-4 of



Scheme 25 Iwao and Ishibashi Group Synthesis of Storniamide Natural Products

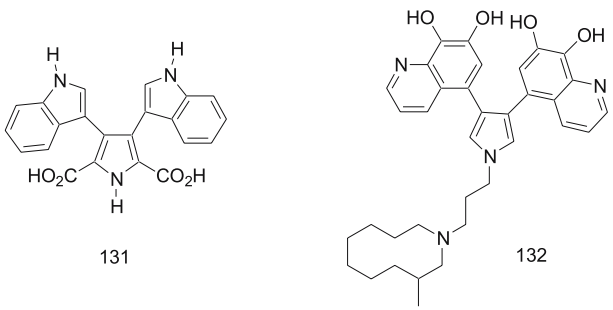


Fig. 8 General Structure of Lycogalic Acid and Halitulin Natural Products

the pyrrole system whereas halitulin contains oxygenated quinoline groups at these positions plus a cycloazadecane system attached to the pyrrole nitrogen by way of a three-carbon tether. Lycolgalic acid was isolated by Steglich [59] and coworkers in 1994 from fruit bodies making them unique in their origin. Prior to their work, Hoshino and coworkers [60] had isolated lycogalic acid in 1993 from a mutant of the bacterium *Chromobacterium violaceum*. No biological activity has yet to be reported for this pyrrole class. Kashman and coworkers reported [61] the isolation of halitulin from the marine sponge

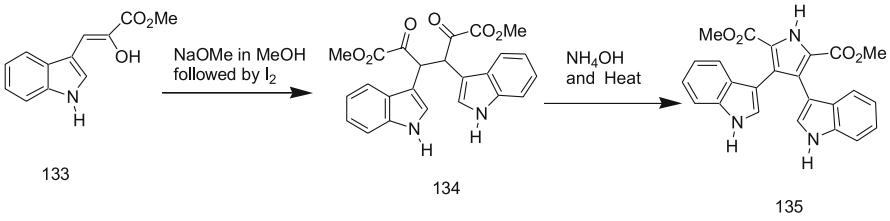
Haliclona tulearensis in 1999. In this paper, it was also reported that halitulin exhibited significant cytotoxicity against P-388 (leukemia), A-549 (lung), HT-29 (colon) and MEL-28 (melanoma) tumor cell lines.

In the report by Steglich for the isolation of lycogalic acid he also described his synthesis of lycogalic acid dimethyl ester (135) by his biomimetic approach (Scheme 26) as previously presented in Scheme 18.

Furstner has also used his methodology [58] as presented in Scheme 24 for the preparation of lycogalic acid dimethyl ester (135) and this is presented in Scheme 27. The ability to access indole boronic acids is an important consideration for this particular route.

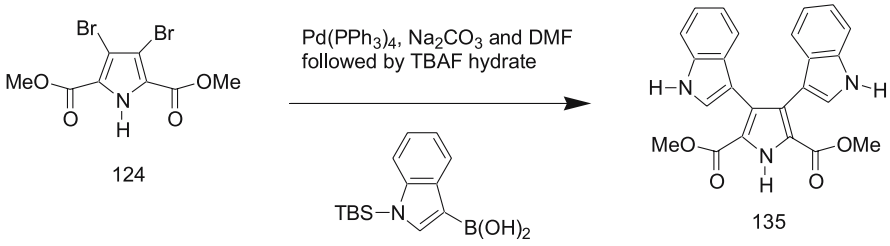
Furstner has extended this strategy to the halitulin core by using Negishi coupling conditions and this is presented in Scheme 28.

Banwell, Steglich and Kashman [62, 63] have combined their expertise to complete a total synthesis of halitulin and this is described in Scheme 29.



133 134 135

Scheme 26 Steglich Group Synthesis of Lycogalic Acid Natural Products



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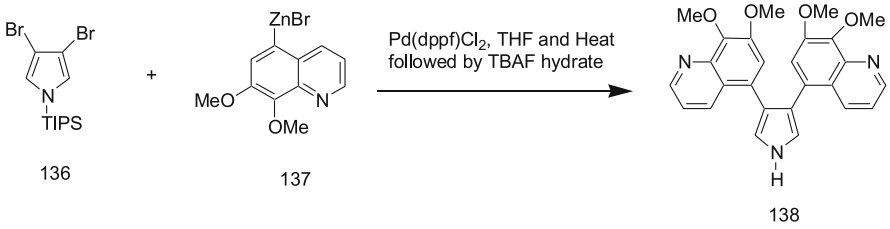
Scheme 27 Furstner Group Synthesis of Lycogalic Acid Natural Products



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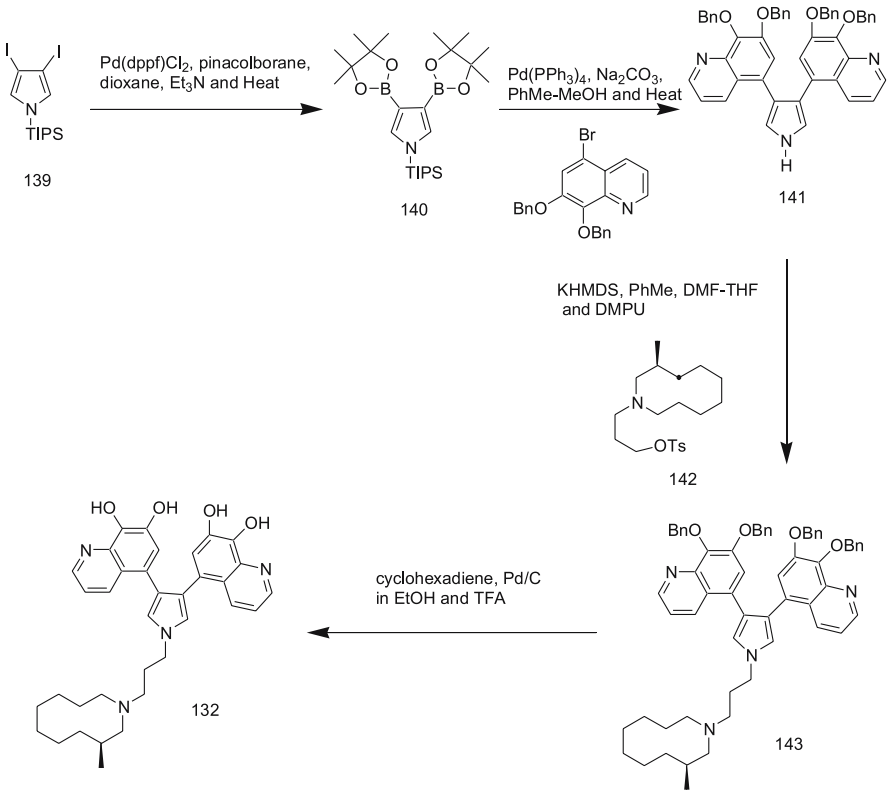
Scheme 28 Furstner Group Synthesis of the Halitulin Core



Scheme 29 Banwell, Steglich and Kashman Synthesis of Halitulin Natural Products

This route involves the conversion of a 3,4-diiodopyrrole (139) to the corresponding 3,4-diboronate ester (140) followed by a bis Suzuki cross-coupling reaction with a bromoquinoline, which generates the halitulin core (141). This pyrrole (141) is then alkylated with a tosylated cycloazadecane to generate a pentasubstituted pyrrole (143), which is converted to halitulin by debenzylation under hydrogenolysis conditions.

8 Dicytodendrins

The dicytodendrins A-E (Fig. 9) are one of the most recent 3,4-diarylpyrrole natural products to be reported [64]. Their isolation by Fusetani and coworkers from a Japanese marine sponge was reported in 2003. These substances (144–148) have been shown to inhibit telomerase at a concentration of 50 μg/mL thereby making such compounds potentially useful as antitumor agents given that telomerase activity is found in 90% of cancer cells but

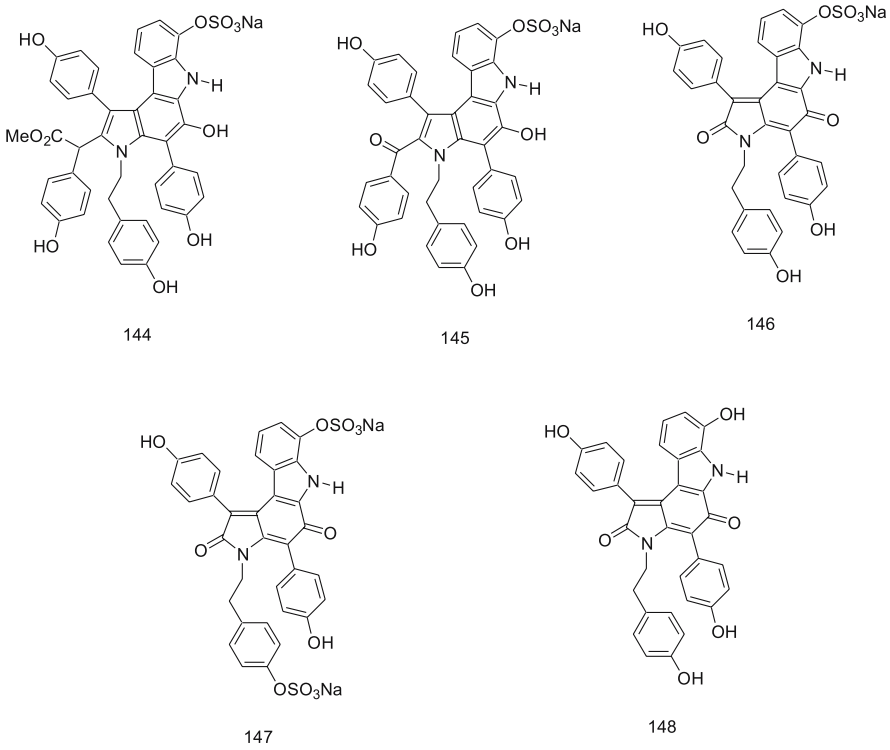


Fig. 9 General Structure of Dicytodendrin Natural Products

not in normal cells. At the writing of this review, these natural products have not yielded to total synthesis, but given the current state of synthetic methodology reported in this review, it is only a matter of time before synthetic material will be available for further biological investigations.

9 Conclusions

In summary, the isolation, structure determination, and synthesis of 3,4-diaryl pyrrole natural products has become a prominent focus of organic chemists as a consequence of the biological properties of these substances and their potential to be used as lead compounds for the discovery of novel anticancer or anti-HIV drugs or to function as MDR reversal agents for the respective diseases. A variety of synthetic methodologies have demonstrated significant value in the construction of these pyrrole-containing scaffolds. It is now possible to prepare analogs, conduct SAR studies, and thereby optimize desirable pharmacological properties and minimize the undesirable side ef-

fects. Perhaps in the very near future some analogs or synthetic precursors of these pyrrole-containing natural products will make it to the pharmaceutical market place given the rapid developments in this area of organic/medicinal chemistry.

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Synthesis of Carbolines Possessing Antitumor Activity

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Abstract Synthetic approaches to a number of carboline derivatives having antitumor activity are described. Representative examples of molecules with different substitution patterns were chosen for discussion, as well as those that illustrated both "classic" and novel synthetic methods. The majority of the compounds discussed are β-carbolines, as these have been the most widely studied. Representative examples of isomeric carbolines are also described, however. Several carboline derivatives show activity at nanomolar concentrations, and so are of keen interest for possible development into clinically useful antitumor agents.

Keywords Carboline · Antitumor · Synthesis

Abbreviations

Boc	*tert*-butoxycarbonyl
bp	Boiling point
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DIBAL	Diisobutylaluminum hydride
DMAP	4-(dimethylamino)pyridine
DME	1,2-dimethoxyethane

DPPA	Diphenylphosphoryl azide
dppf	Bis(diphenylphosphino)ferrocene
GI_{50}	50% growth inhibition
IC_{50}	50% inhibitory concentration
LAH	Lithium aluminum hydride
LDA	Lithium diisopropylamide
LiHMDS	Lithium hexamethyldisilazide
m-CPBA	*m*-chloroperoxybenzoic acid
MOM	Methoxymethyl
PPA	Poly(phosphoric acid)
SEM	(2-(trimethylsilyl)ethoxymethyl)
TFA	Trifluoroacetic acid
TFAA	Trifluoroacetic anhydride
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl

1 Introduction

Carbolines are pyridoindoles, and are classified as α, β, γ or δ according to the mode of ring fusion, as illustrated below (Fig. 1). The most abundant are β-carbolines, which are biologically derived from tryptophan. Compounds containing the β-carboline ring system have been found to exhibit a wide range of biological activity, although are perhaps best known for their ability to bind to $GABA_A$ receptors, often as effectively as clinically active benzodiazepines [1, 2]. Members of this class of compounds have also been found to bind to 5-HT_2 serotonin receptors [3] as well as imidazoline receptors [4, 5], in addition to acting as inhibitors of IκB kinase [6] and PDE5 [7–9]. This review, however, will focus on synthetic methods used to prepare β-carbolines that have been shown to possess antitumor properties. Synthesis and activity of other carbolines will also be discussed briefly. The coverage is not intended to be comprehensive – instead, representative compounds containing the carboline structure as well as the synthetic routes leading to such compounds will be discussed. Emphasis will be placed on more recent synthetic methods, and will focus primarily on strategies for constructing the carboline ring system with various substitution patterns, rather than synthetic steps required for the preparation of the substituents themselves (A review of general methods of synthesizing β-carbolines has appeared [10]).

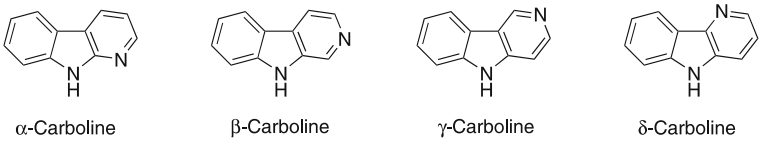


Fig. 1 General structures of carbolines

2
Syntheses of Selected β-Carbolines

2.1
Syntheses of β-Carbolines Substituted Only in the Pyridine Ring

2.1.1
β-Carbolines Substituted Only at C-1

Since tryptophan (and its decarboxylation product, tryptamine) serve as precursors in many synthetic and biosynthetic routes to β-carbolines, it is not surprising that C-1 of the β-carboline ring is the most common site of substitution (as it is the only ring atom of the β-carboline ring system not derived from tryptophan). Indeed, this is the only site of substitution for many β-carboline natural products. Two examples of naturally occurring β-carbolines substituted only at C-1 which possess antitumor activity are manzamine A and manzamine C (Fig. 2). Owing to its greater simplicity and nearly equal antitumor activity, most initial synthetic efforts were directed toward manzamine C [11, 12].

The first two reported syntheses of manzamine C utilized the two most common approaches to the β-carboline ring system—the Pictet–Spengler reaction [13, 14] and the Bischler–Napieralski reaction [15]. These two reactions are illustrated in Fig. 3.

In the Pictet–Spengler approach, a tryptamine derivative is condensed (typically using acid catalysis) with an aldehyde to yield the tetrahydro-β-carboline **1**. The reaction proceeds through formation of an iminium ion **4**, which adds initially to C-3 of the indole ring to give the spiroindolenium ion **5** (Fig. 4). An alkyl shift followed by proton loss gives the observed tetrahydro-β-carboline. Oxidation to the fully aromatic β-carboline can be accomplished using a variety of reagents, though on many occasions such oxidations prove to be problematic [10].

The Bischler–Napieralski approach to β-carboline synthesis is very similar to the Pictet–Spengler one, except that the tryptamine derivative is first

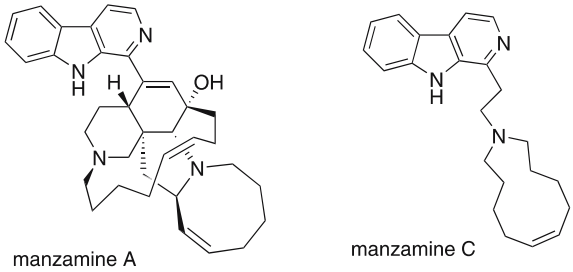


Fig. 2 Structures of manzamine A and manzamine C

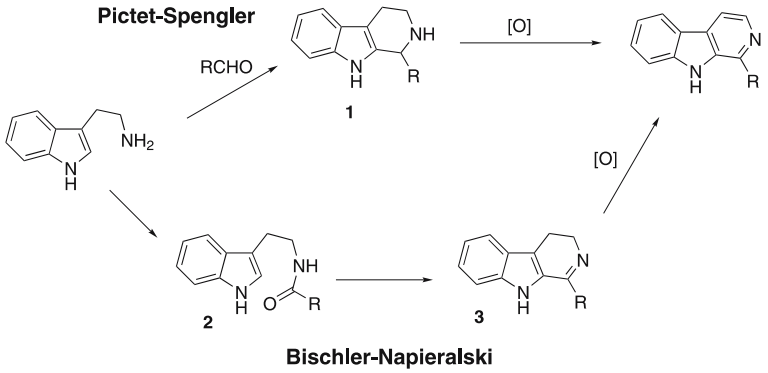


Fig. 3 Classic synthetic approaches to β-carbolines

acylated to give amide **2**. Dehydration of this amide (typically with $POCl_3$) leads to an active iminium species which cyclizes and eventually produces dihydro-β-carboline **3**. Once again, an oxidation step is required to prepare the fully aromatic β-carboline. While oxidation of dihydro-β-carbolines **3** is generally more facile than that of tetrahydro-β-carbolines **1**, a drawback to the Bischler–Napieralski approach is that the dehydration reaction leading to **3** often requires vigorous reaction conditions.

The first synthesis of manzamine C, reported by Nakagawa and Hino [16, 17], initially envisioned β-carboline **6** as a key intermediate (Fig. 5). Synthesis of this compound using the Pictet–Spengler approach, however, proceeded in fairly low yield (60% for the first step; 10–20% for the oxidation/desilylation step), and thus other routes to this compound were investigated. Though conversion of **7** to **8** under Bischler–Napieralski conditions proceeded in approximately the same yield as obtained via the Pictet–Spengler approach, oxidation of **8** to yield **9**, followed by reduction with LAH, gave alcohol **6** in significantly higher yield. Unfortunately, mesylation of **6** followed by treatment with 6-(*Z*)-azacycloundecene **10** only provided a 10% yield of manzamine C **12** after "tedious chromatography". Thus, acylation of **10** using ester **9** was inves-

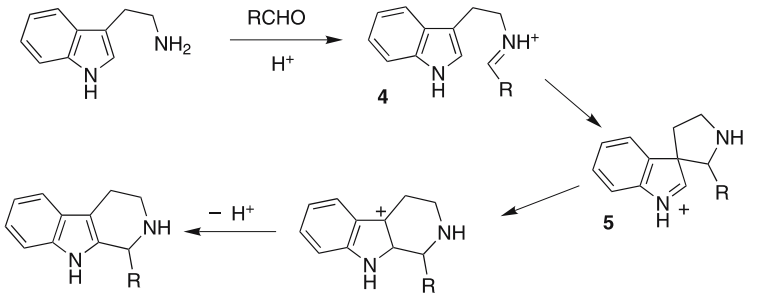


Fig. 4 Intermediates in the Pictet–Spengler reaction

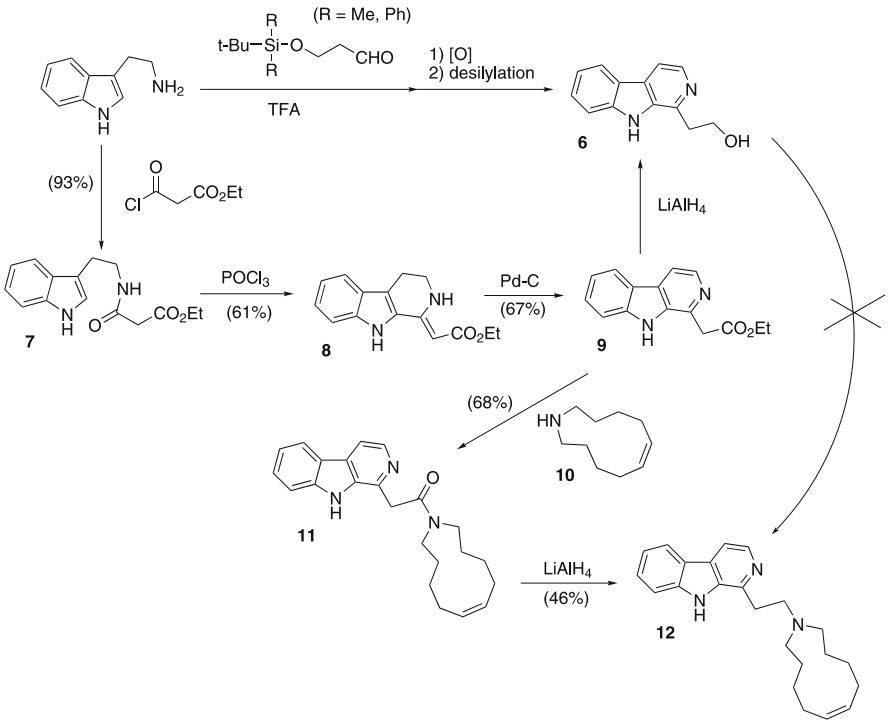


Fig. 5 Synthesis of manzamine C by Nakagawa and Hino

tigated, this reaction providing amide **11** in 68% yield. (Compound **11** could be obtained in 87% yield by conversion of ester **9** to the potassium salt of the corresponding carboxylic acid, followed by coupling with **10** mediated by diphenylphosphoryl azide). Reduction of **11** with LAH provided manzamine C **12** as colorless prisms after two recrystallizations.

A little over a year later, Gerlach reported a successful synthesis of β-carboline ester **17** via a Pictet–Spengler approach (Fig. 6) [18]. Treatment of N_b-benzyltryptamine **13** with protected formylacetic acid derivative **14** in refluxing benzene gave tetrahydro-β-carboline **15** in 89% yield. Treatment with palladium on carbon for 4.5 h in refluxing mesitylene gave aromatized β-carboline **16** in 90% yield, and methanolysis of the protecting group provided ester **17** also in 90% yield. From here, the synthesis of manzamine C was much the same as Nakagawa and Hino's. DMAP-catalyzed acylation of **10** by **17** gave amide **11**, which, after treatment with LAH, gave manzamine C **12** in 56% yield, along with a 42% yield of recovered **11** after column chromatography.

The synthesis reported by MaGee and Beck [19] also used ester **17** as a key intermediate, though the natural product harmane **18** was used as the starting material instead of tryptamine (Fig. 7). Treatment of **18** with four equivalents

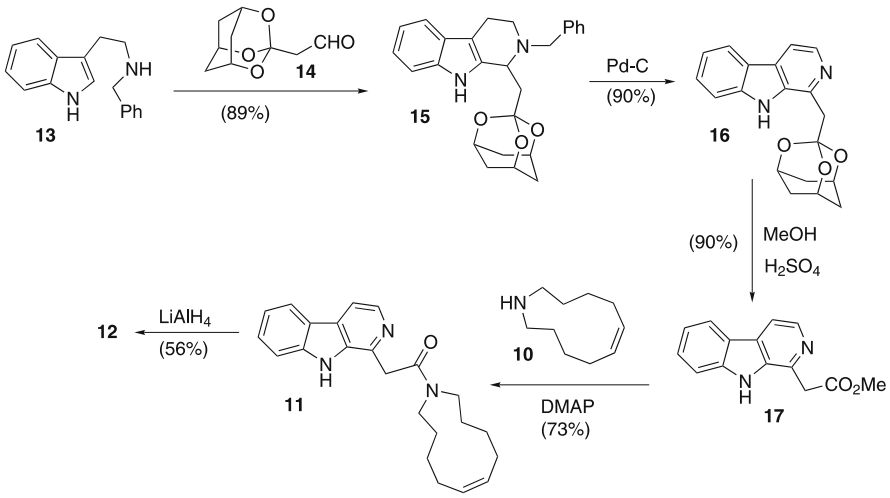


Fig. 6 Synthesis of manzamine C by Gerlach

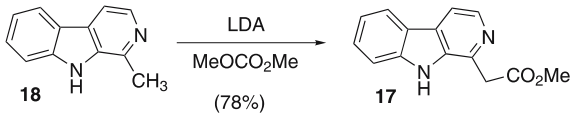


Fig. 7 Synthesis of ester **17** by MaGee and Beck

of LDA followed by an excess of dimethyl carbonate allowed isolation of ester **17** in 78% yield. From here, the synthesis of manzamine C essentially followed that of Nakagawa and Hino.

All three of the syntheses described above required multi-step procedures for the synthesis of amine component **10**. In both Nakagawa's and Gerlach's syntheses, introduction of the double bond was achieved by reduction of alkyne derivatives, while MaGee's synthesis used a Ramberg–Bäckland rearrangement to generate the alkene. New syntheses of this key intermediate and its derivatives continue to be published [20, 21]. It appears, however, that neither the geometry nor the presence of the double bond in the azaundecene ring are necessary for the antitumor activity of manzamine C. Nagakawa and coworkers have prepared manzamine C analogs (by a route directly analogous to that shown in Fig. 5) in which the 6-(*Z*)-azacycloundecene moiety has been replaced by the corresponding 6-(*E*)-azacycloundecene group, and also by the saturated dihydro derivative. The β-carbolines constructed using these modified amines showed equal or slightly better activity based on in vitro assays with several cancer cell lines, with IC_{50} (50% inhibitory concentration) values generally ranging from 2–6 μg/mL [22].

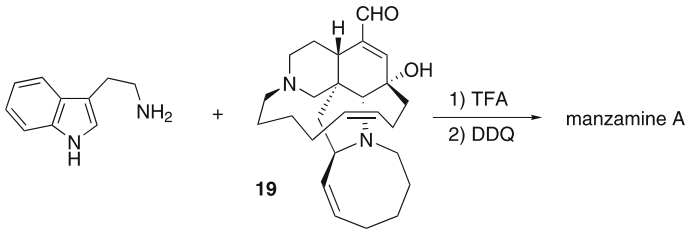


Fig. 8 Pictet–Spengler approach to manzamine A

Challenges faced in the preparation of 6-(*Z*)-azacycloundecene, however, pale in significance compared to those encountered in the preparation of the polycyclic amine required for the synthesis of manzamine A. The first [23] and subsequent [24, 25] syntheses of manzamine A all prepare the natural product ircinal A **19** and then condense this aldehyde with tryptamine via a Pictet–Spengler reaction using trifluoroacetic acid to give the corresponding tetrahydro-β-carboline (Fig. 8). Oxidation to the fully aromatic β-carboline is then achieved using DDQ, following the procedure of Kobayashi and coworkers [26].

2.1.2 β-Carbolines also Substituted at C-3 and C-4

Though C-1 is a common site of substitution in β-carbolines, the presence of carboxylic acids (or their derivatives) at C-3 is also frequently encountered, since such functionality can be derived from a tryptophan precursor. Less common is substitution at C-4, although there are a number of natural products in which the β-carboline ring is substituted at this position as well. An example of such a compound is the antitumor antibiotic lavendamycin **26**, isolated by Doyle and coworkers [27]. Not surprisingly, initial syntheses of this compound followed the classic Pictet–Spengler and Bischler–Napieralski approaches [28]. The first total synthesis of lavendamycin (as its methyl ester) was reported by Kende and Ebetino [29] and involved coupling of quinoline **20** with β-methyltryptophan methyl ester **21** using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide to give amide **22** in 90% yield (Fig. 9). This amide underwent a Bischler–Napieralski reaction upon treatment with polyphosphoric ester with concomitant oxidation to give β-carboline **23** in 31% yield. (A few years later, a similar synthesis was reported by Rao and coworkers in which $POCl_3$ was used as the condensation agent for the Bischler–Napieralski reaction, which increased the yield of this step to 85%) [30]. Further modification of the quinoline ring eventually led to the isolation of lavendamycin methyl ester **25**. Attempts to hydrolyze **25** were not successful, only producing small amounts of lavendamycin as part of a mixture.

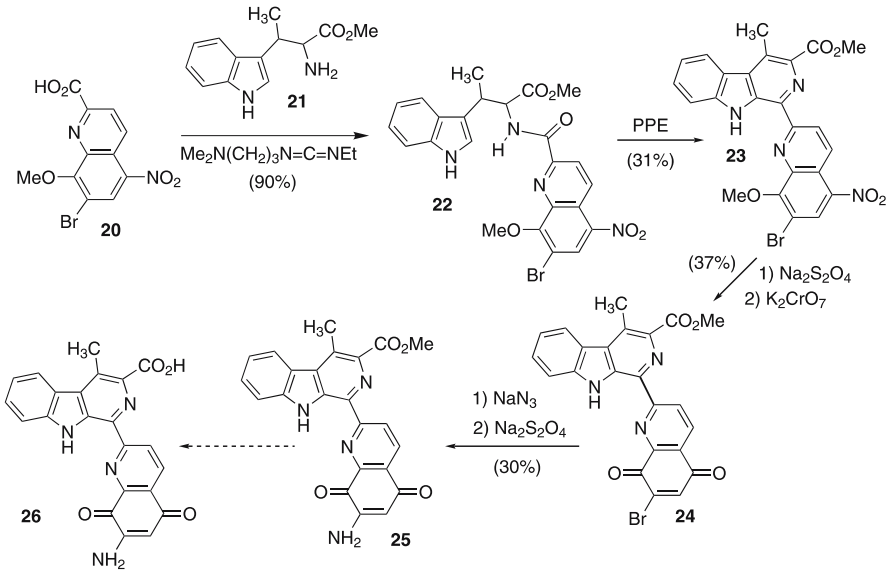


Fig. 9 Synthesis of lavendamycin methyl ester by Kende and Ebetino

The Pictet–Spengler reaction has also seen much use in the synthesis of lavendamycin methyl ester. Both Hibino [31] and Behforouz [32] used it as a key step in their syntheses of this molecule (Fig. 10). In Hibino's synthesis, β-methyltryptophan ethyl ester **27** was condensed with quinoline aldehyde **28** to give the corresponding tetrahydro-β-carboline, which was aromatized by heating with palladium on carbon in xylenes, giving β-carboline **29** in 75% yield. A five-step sequence (which included conversion to the methyl ester for easier comparison with known compounds) yielded bromoquinone **24** in 27% yield for the five steps. This completed Hibino's formal total synthesis of lavendamycin methyl ester, since this was the same intermediate used in Kende's synthesis.

Behforouz's synthesis employed more highly substituted quinoline aldehyde **30**, which, when condensed with ester **21**, produced β-carboline **31** without need of a separate oxidation step. Selective hydrolysis of the acetamide group then provided lavendamycin methyl ester in high yield. A few years later, Behforouz and coworkers reported an improved synthesis of **30**, thus boosting the overall yield of their lavendamycin synthesis [33].

Other routes to the key imine intermediate of the Pictet–Spengler reaction have also been reported. For example, Molina and coworkers [34] generated imine intermediate **34** by reaction of azide **32** with aldehyde **33** in the presence of tributylphosphine, via the corresponding iminophosphorane (Fig. 11). Use of triphenylphosphine was unsuccessful in the formation of an iminophosphorane. Without isolation, the intermediate was heated at 165 °C in a sealed tube with palladium on carbon, giving β-carboline **35** in 45% yield. This com-

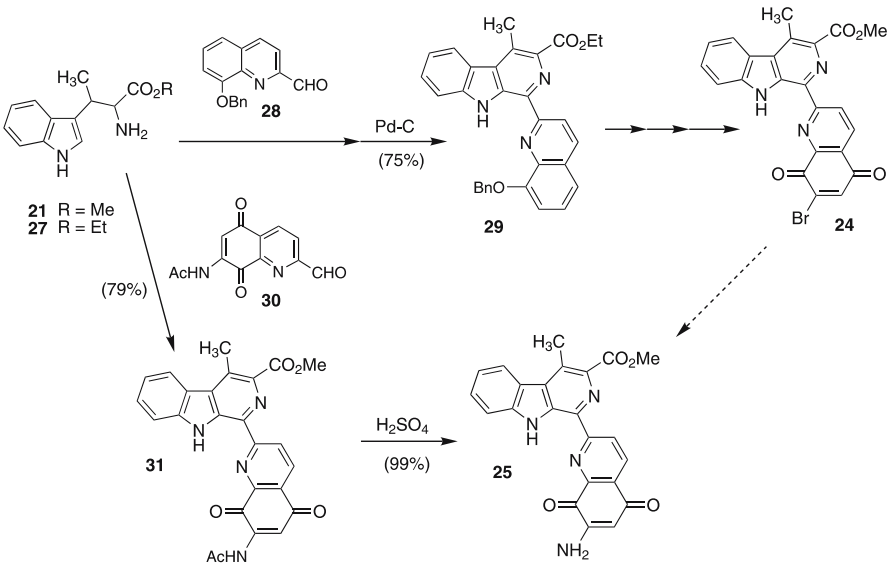


Fig. 10 Syntheses of lavendamycin methyl ester and intermediate by Behforouz and Hibino

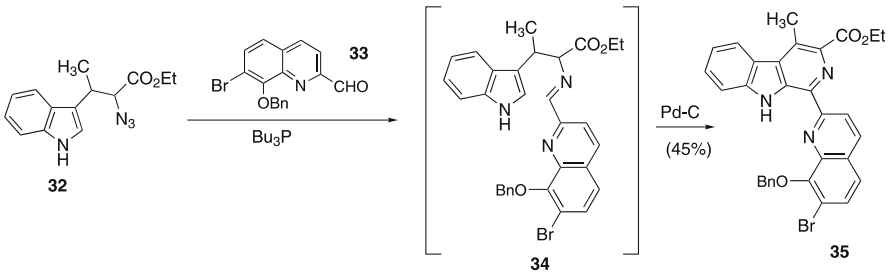


Fig. 11 Synthesis of lavendamycin intermediate by Molina

pleted Molina's formal total synthesis, as the corresponding methyl ester had previously been used in Boger's synthesis of lavendamycin methyl ester (see below).

One of the earliest syntheses of lavendamycin methyl ester, however, did not employ either the Pictet–Spengler or the Bischler–Napieralski reactions for construction of the β-carboline ring system. Instead, a palladium-promoted ring closure of aryl pyridine **36** (Fig. 12) was used to prepare β-carboline **37** by Boger and coworkers [35]. Unfortunately, stoichiometric palladium was found to be necessary, in this case 1.5 equivalents of the tetrakis(triphenylphosphine)palladium(0) being used. Friedlander condensation with aldehyde **38** in the presence of benzyltrimethylammonium hydrox-

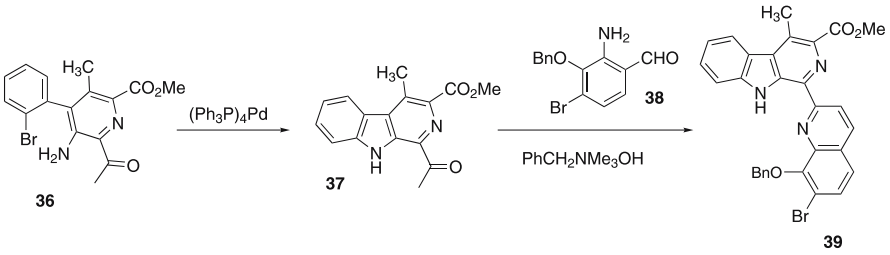


Fig. 12 Synthesis of lavendamycin methyl ester by Boger

ide (Triton B) gave **39** in 52–58% yield, and intermediate **39** was then carried on to lavendamycin methyl ester under carefully controlled conditions.

Yet another route to intermediate **39** was reported by Ciufolini and Bishop [36]. In their synthesis, heating azide **41** in refluxing *o*-dichlorobenzene produced β-carboline **42** in 83% yield (Fig. 13). Oxidation with $NaClO_2$ followed by esterification with diazomethane produced **39** in 97% yield. Since intermediate **41** could be prepared in just four steps in 74% overall yield from quinoline **40** and *o*-azidobenzaldehyde, this approach represented a fairly quick and efficient route to **39**.

Although lavendamycin was not used clinically due to solubility and toxicity problems [37], interest in lavendamycin analogues continues today. For

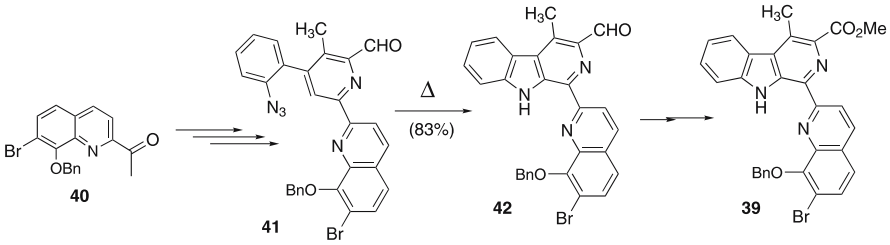


Fig. 13 Synthesis of lavendamycin intermediate by Ciufolini and Bishop

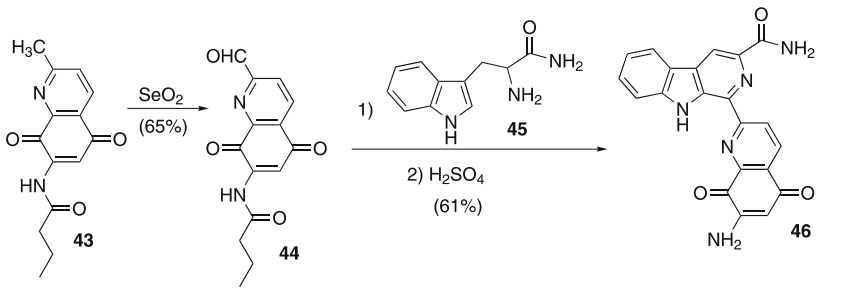


Fig. 14 Synthesis of lavendamycin analog

example, Behforouz and coworkers have prepared a number of variants on the lavendamycin structure [38], many of which show promising antitumor activity according to several assays, with GI_{50} (50% growth inhibition) values in the 200 nM range [37]. The most active compound was amide derivative **46**, prepared from quinone **44**, which was itself synthesized from the corresponding methyl compound **43** in 65% yield by treatment with selenium dioxide (Fig. 14) [39]. Condensation of **44** with amide **45** in refluxing anisole gave a 63% yield of the corresponding β-carboline by a Pictet–Spengler reaction, and hydrolysis of the butyramide with sulfuric acid proceeded in 96.5% yield, completing the synthesis of **46**.

2.1.3 β-Carbolines Substituted with Heteroatoms on the Pyridine Ring

A few β-carbolines in which heteroatoms are directly attached to the pyridine ring have also been found to exhibit antitumor activity. For example, Coldham and Boursereau have prepared a series of 1-amino-β-carbolines as simple analogs of the manzamine alkaloids [40]. Of those studied, the most active was **49**, prepared in 54% yield from chloro-β-carboline **48**, which itself was prepared from tryptamine by treatment with triphosgene, followed by oxidation with palladium on carbon, and finally, chlorination using phosphorus oxychloride (Fig. 15). Though no yields were reported for the synthesis of **48**, the authors in the literature cited reported an overall yield of 49% for the three steps [41]. Amino-β-carboline **49** was found to be active against a number of cancer cell lines, with a GI_{50} value against HOP-92 non-small cell lung cancer cells nearly as good as that obtained for manzamine A (0.38 μM for **49**, compared to 0.25 μM for manzamine A). GI_{50} values against several other cell lines ranged from 1.6 to 5.2 μM [40].

Substituted analogues of β-carbolin-1-one **47** have also been found to inhibit tumor cell proliferation. For example, Hu and coworkers have reported that β-carbolin-1-one **51** inhibited cell proliferation of HeLa cells with an

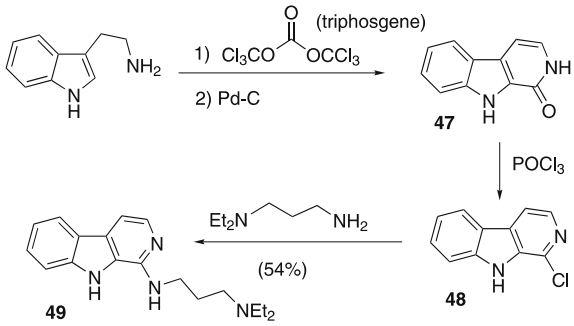


Fig. 15 Synthesis of amino-substituted β-carboline

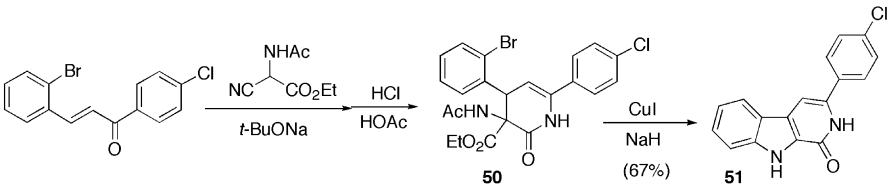


Fig. 16 Synthesis of 3-aryl-β-carboline-1-one

IC_{50} of 1.17 μM [42]. Unsubstituted β-carbolin-1-one **47** showed no such activity, and 3-aryl β-carbolin-1-ones substituted with oxygenated groups at positions 6 and 7 generally had greatly reduced activity.

The key step in Hu's synthesis of **51** was cyclization of **50** by heating with copper(I) iodide and sodium hydride in DME, followed by a 10% aqueous ammonia work-up. Intermediate **50** was prepared via Michael addition of ethyl acetamidocyanoacetate to the appropriate chalcone followed by acid-catalyzed ring closure [42, 43].

2.2 Syntheses of β-Carbolines Substituted in Both the Pyridine and Indole Rings

Although ring C (the pyridine ring) is the most common site of substitution in β-carbolines, there are a number of both natural and synthetic β-carbolines substituted in the indole ring as well that show antitumor activity. One of the simplest such compounds is the natural product harmine **52** (Fig. 17), which has shown cytotoxicity to a number of human tumor cell lines with ED_{50} values [44] in the range of 1.6–2.4 μg/mL [45]. The *trans* complex of harmine with $PdCl_2$ (fourth coordination site taken up by DMSO) has also been studied as an antitumor agent, with activity against several cell lines being better than either cisplatin, carboplatin, or 5-FU [46]. Harmine derivative **53** was found to be active against a wide variety of tumor cell lines, with ED_{50} values typically in the range of 1 μg/mL [45]. Since **53** was purchased for the study, no synthesis was reported, but presumably it can be made by base-catalyzed condensation of harmine (or an *N*-protected derivative) with *p*-nitrobenzaldehyde.

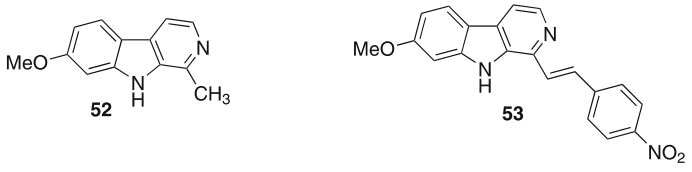


Fig. 17 Harmine and a derivative

Although harmine **52** is frequently obtained by isolation (or purchase order), a synthesis of this compound as well as a number of analogs has recently appeared [47, 48]. The key step to this synthesis was the thermal electrocyclization of oxime intermediate **55**, which was prepared by acylation of vinylindole derivative **54** followed by treatment with hydroxylamine hydrochloride. Neither oxime **55** nor its ketone precursor were isolated—instead, the crude reaction mixture was heated at reflux in *o*-dichlorobenzene to ultimately yield harmine in 56% yield overall starting from **54** (Fig. 18).

Harmine derivatives alkylated at N-9 (the indole nitrogen) have been shown to have even better antitumor activity than their non-alkylated counterparts [49]. For example, *N*-ethylharmine **56** (Fig. 19) showed IC_{50} values in the 14–45 μM range with regard to cytotoxicity to five different cancer cell lines, while the corresponding values for harmine **52** were between 45 and 68 micromolar. Only with colon carcinoma cells (Lovo) was **56** less effective than **52**. Significantly better activity has been shown by the analogous *N*-benzylharmine **57**, with an IC_{50} value of 0.01 μM against human HepG2 cells [50].

Tumor inhibition rates in mice with Lewis lung carcinoma as well as sarcoma 180 have also been studied. While **52** displayed inhibition rates of 34.1% and 15.3%, respectively, against these two types of tumors, **56** showed inhibition rates of 42.0% and 37.6% respectively [49]. Nevertheless, such inhibition rates are still less than those observed for known antitumor agent cytophosphane, which had inhibition rates of 88.6% and 87.5% against these same two tumors. Additionally, both **56** and **57** have been found to be neurotoxic [50].

The *N*-alkylated harmine derivatives were prepared by simple alkylation of harmine anions (generated using sodium hydride) with alkyl halides and bromides. For example, **56** was prepared in 83% yield in this manner, and the *N*-methyl analog (which had similar antitumor activity to that of **56**) was obtained in 80% yield using methyl iodide.

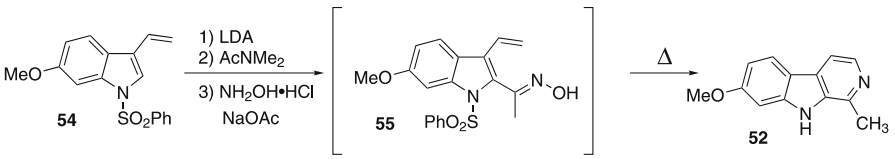


Fig. 18 Synthesis of harmine

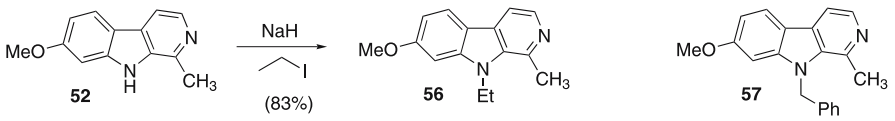


Fig. 19 Alkylated harmine derivatives

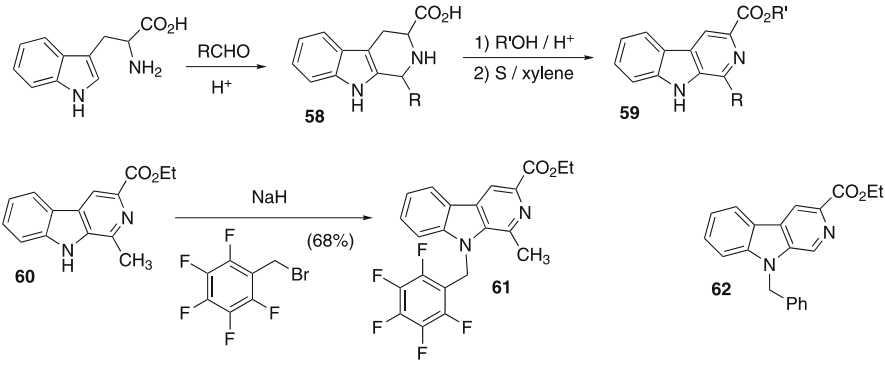


Fig. 20 Synthesis of β-carboline esters

Good antitumor activity was also observed for a series of *N*-alkylated β-carbolines lacking any substituents in the A ring [51]. These compounds were prepared by initial treatment of tryptophan with a series of aldehydes, yielding tetrahydro-β-carbolines **58** via the classic Pictet–Spengler reaction (Fig. 20). None of the tetrahydro-β-carbolines **58** showed antitumor activity, but Fisher esterification followed by oxidation with sulfur in refluxing xylenes produced aromatic β-carboline esters **59**, some of which showed antitumor activity, as did the free carboxylic acids derived from them. Most of the activity, however, was confined to compounds in which the C-1 substituent was a methyl group (those derived from initial reaction of tryptophan with acetaldehyde). As was found with harmine, alkylation of N-9 appeared to improve cytotoxicity. Of the many compounds investigated, **61**, prepared by alkylation of **60** with pentafluorobenzyl bromide, showed the best activity, with IC_{50} values ranging from 4–47 μM for six different tumor cell lines. As part of another study, **62** was prepared and found to have high antitumor activity, yet did not show the neurotoxicity exhibited by **56** and **57** [50].

2.3 Syntheses of β-Carbolines Fused with Other Rings

Tetracyclic systems in which another ring has been fused to the β-carboline nucleus have also shown promising antitumor activity. Of these, the best known are the derivatives of canthin-6-one **63** (numbered as shown in Fig. 21). Though perhaps better known for their antileukemic activity, canthin-6-one and several of its methoxy derivatives (particularly 1-methoxycanthin-6-one and 9-methoxycanthin-6-one) have also been shown to possess antitumor properties [52–56]. For example, 9-methoxycanthin-6-one had ED_{50} values [44] of < 2.5 and 4.5 μg/mL against A-549 (human lung cancer) and MCF-7 (human breast cancer) cell lines, respectively, while canthin-6-one had ED_{50} values of 3.6 and 7.3 μg/mL against the same cell lines [55].

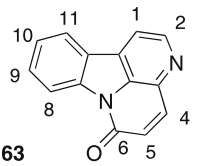


Fig. 21 Canthin-6-one

A recent synthesis of canthin-6-one **63** relies upon the classic Pictet–Spengler reaction for construction of the β-carboline framework [57]. Czerwinski and coworkers allowed N_b-benzyltryptamine **13** (prepared by reaction of tryptamine with benzoyl chloride followed by LAH reduction) to react with 2-ketoglutaric acid **64** in a refluxing mixture of benzene and dioxane in a flask equipped with a Dean–Stark trap (Fig. 22). This produced hexahydrocanthin-6-one derivative **65** in 80% yield. Removal of the benzyl group using ammonium formate and palladium on carbon followed by oxidation with freshly prepared manganese dioxide produced canthin-6-one **63** in 65% yield for the two steps. This completed an exceptionally short synthesis of this compound, proceeding in 48% overall yield from tryptamine.

Snyder and coworkers followed a completely different path to canthin-6-one (Fig. 23). Earlier they had shown that indole-substituted 1,24-triazine **66** could be heated in refluxing triisopropylbenzene (bp = 232 °C) to give β-carboline **67** via an intramolecular cycloaddition/cycloreversion reaction [58]. Selective oxidation of **67** at C-6 was achieved through the use of triethylbenzylammonium permanganate [59]. Success of the reaction proved to be very sensitive to the solvent chosen. Heating **67** for 4 h at 70 °C in a 5 : 1 mixture of dichloromethane and acetic acid gave a 65% yield of **63**, yet use of increasing amounts of dichloromethane slowed the reaction down (no reaction occurred in pure dichloromethane), while use of pure acetic acid led to an intractable mixture.

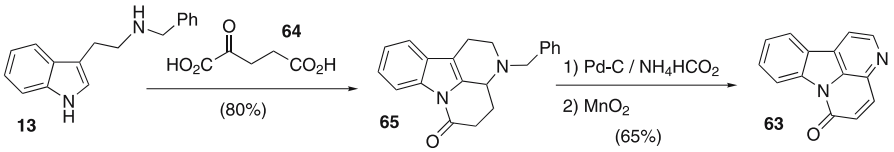


Fig. 22 Synthesis of canthin-6-one by Czerwinski

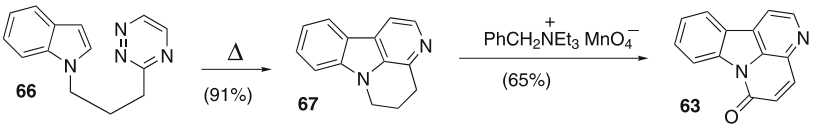


Fig. 23 Synthesis of canthin-6-one by Snyder

Blechert and coworkers investigated another cycloaddition route to canthin-6-one **63** which proceeded through a single electron transfer mechanism [60]. Their synthesis began with natural product harmalane **68**, recognizable as the product of a Bischler–Napieralski reaction conducted on N_b-acetyltryptamine (Fig. 24). (In fact, this precise reaction has been reported previously, proceeding in 70–74% yield) [61, 62]. Treatment with triflic anhydride produced sulfonamide **69**, which was allowed to react with methyl (*E*)-3-*N*,*N*-dimethylaminoacrylate **70** at a potential of 400 mV in an electrochemical cell. Use of various ratios of **69** : **70** ranging from 5 : 1 to 1 : 5 were investigated, with **71** being produced in yields ranging from 57% to 87%. The best yield was obtained when a 5 : 1 ratio of **69** : **70** was employed. Reductive removal of the triflyl group with sodium and naphthalene followed by oxidation with manganese dioxide gave **72** in 44% yield for the two steps. Acidic hydrolysis of the ester followed by decarboxylation using copper and pyridine produced canthin-6-one **63** in 50% yield.

As alluded to earlier, methoxy-substituted canthin-6-ones have also shown antitumor activity, so not surprisingly, there has been interest in the synthesis

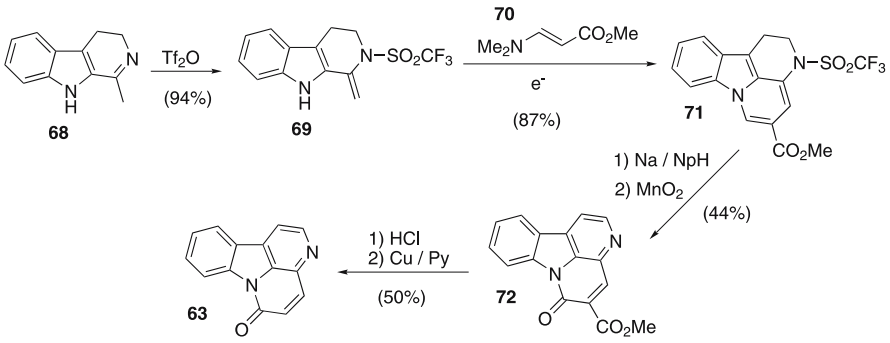


Fig. 24 Synthesis of canthin-6-one by Blechert

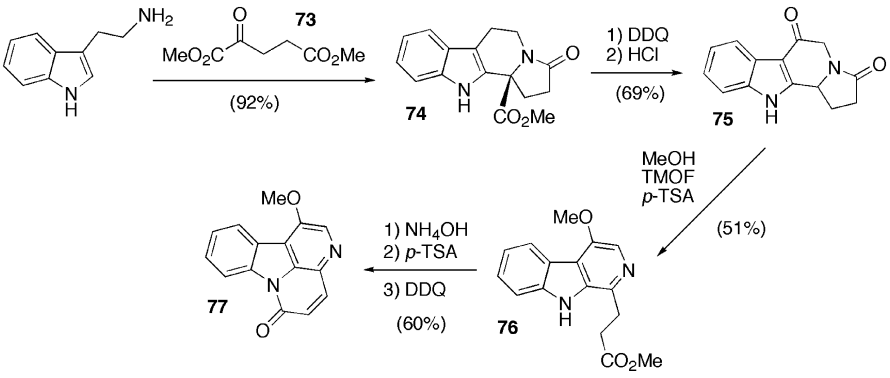


Fig. 25 Synthesis of 1-methoxycanthin-6-one by Cook and Hagen

of these compounds as well. The first synthesis of 1-methoxycanthin-6-one **77** was reported by Cook and Hagen [63, 64] and began with a Pictet–Spengler reaction between tryptamine hydrochloride and dimethyl α-ketoglutarate **73** (Fig. 25). In contrast to Czerwinski's later synthesis of canthin-6-one (Fig. 22), since unprotected tryptamine was employed, tetracyclic intermediate **74** was obtained in high yield rather than a product bearing the canthin-6-one skeleton. Treatment of **74** with seven equivalents of DDQ at room temperature for 3 days resulted in selective oxidation at C-1 in 78% yield. The methyl ester was then hydrolyzed and decarboxylated in 88% yield, giving intermediate **75**. Heating **75** in methanol and trimethyl orthoformate with *p*-toluenesulfonic acid resulted in concomitant enol ether formation, oxidative aromatization, and methanolysis of the lactam to give ester **76** in 51% yield. Hydrolysis of the ester with ammonium hydroxide followed by acid-catalyzed lactamization gave 4,5-dihydro-1-methoxycanthin-6-one in 86% yield, which could then be oxidized to the final product **77** in 70% yield by treatment with DDQ.

Recently, Suzuki and coworkers [65] have reported another synthesis of 1-methoxycanthin-6-one **77** (Fig. 26). Starting from indole-2-carboxaldehyde **78**, reductive amination with ethyl glycinate and sodium cyanoborohydride, followed by formylation with ethyl formate and formic acid gave amide ester **79** in 79% overall yield (the formylation step was nearly quantitative) [66]. Acid-catalyzed ring closure could be effected using polyphosphoric acid, or, in somewhat higher yields, using methanesulfonic acid, to give tetrahydro-β-carboline-4-one **80**. Ketalization, deprotection and oxidative aromatization

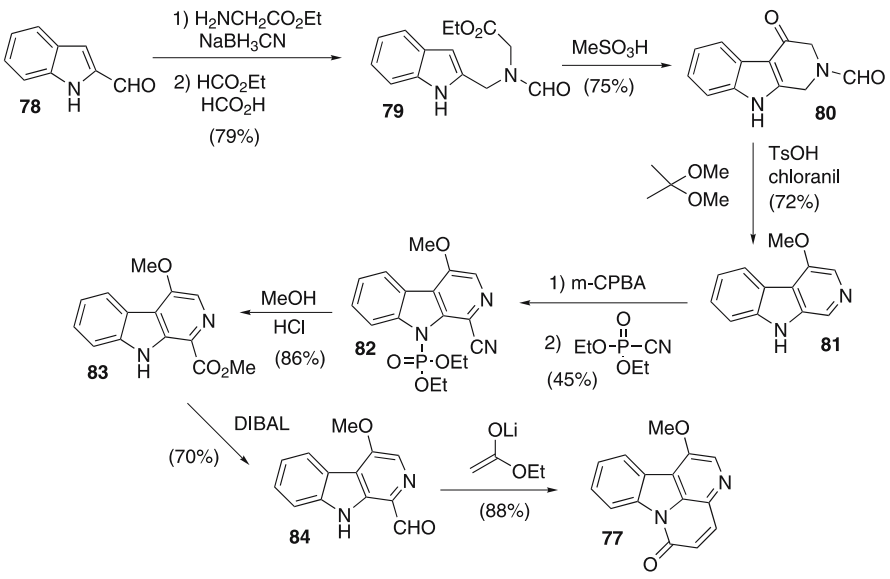
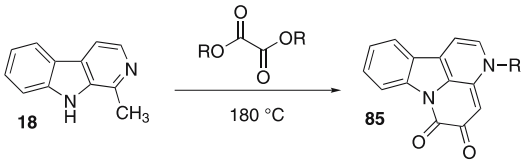


Fig. 26 Synthesis of 1-methoxycanthin-6-one by Suzuki

were all accomplished in one pot and in 72% yield by treatment with 2,2-dimethoxypropane and chloranil in the presence of *p*-toluenesulfonic acid. Functionalization at C-1 of β-carboline **81** was accomplished by *N*-oxidation with *m*-CPBA (74% yield), followed by reaction with diethyl phosphorocyanidate and triethylamine to give **82** in 61% yield. The phosphoryl group could be cleaved from N-9 either in a separate step, or, more conveniently, as part of the methanolysis of nitrile **82** to ester **83**. Reduction of the ester with diisobutylaluminum hydride provided β-carboline aldehyde **84** in 70% yield. Treatment of ethyl acetate with LiHMDS generated the corresponding enolate, to which was added aldehyde **84**. Aldol condensation and intramolecular acylation provided 1-methoxycanthin-6-one **77** in 88% yield after appropriate work-up conditions. Unsubstituted canthin-6-one **63** was also made in an analogous manner in 83% yield from the corresponding aldehyde [65].

Synthesis and evaluation of a series of canthin-5,6-diones **85** (Fig. 27) has also been reported [67]. The synthesis started with 1-methyl-β-carboline (harmane) **18**, which had been prepared by classic Pictet–Spengler methodology. Heating **18** at 180 °C with a series of oxalate esters resulted in alkylation of *N*-2, as well as annulation of the fourth ring, producing the corresponding series of canthindiones **85** which included the natural product picrasidine L **85a**. Activity against PC-6 human lung carcinoma cells was measured, and was found to increase with increasing length of the alkyl group. Unfortunately, yields of **85** were found to drop dramatically with increasing length of this same alkyl group. Even for **85c**, which was the most active compound, GI_{50} values were significantly higher than those obtained for cisplatin (0.28 μg/mL). Similar results were obtained using harmine **52** as the starting material instead of harmane **18**. Yields once again decreased with increasing length of R, while antitumor activity increased. GI_{50} values, however, were worse than those obtained for **85**.

Another tetracyclic β-carboline that has been evaluated for antitumor activity is indolizino[8,7-*b*]indole **88** (Fig. 28). Alkylation of 1-ethyl-β-carboline



	R	yield (%)	GI_{50} (μg/mL)
a	Me	75.0	27.2
b	Et	28.2	16.2
c	Bu	6.3	8.5

Fig. 27 Synthesis of canthin-5,6-diones

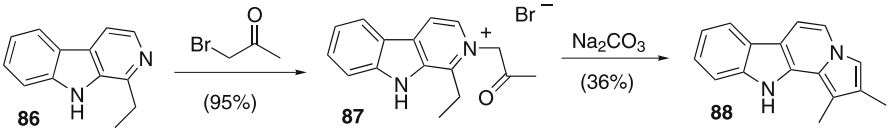


Fig. 28 Synthesis of pyrrole-fused β-carboline

86 with bromoacetone gave β-carbolinium salt **87** in excellent yield [68]. Cyclization under basic conditions then produced **88** in 36% yield after column chromatography and recrystallization. When tested against PC-6 cells (see above), a GI_{50} of 0.3 μg/mL was obtained, essentially the same as that of cisplatin. The analogous compound prepared from harmane **18** was also prepared, in somewhat higher yield, and was found to have essentially the same GI_{50} value against PC-6 cells (0.5 μg/mL). Pyrrole-fused β-carbolines were also prepared starting from **18** and **86** using phenacyl bromide as the alkylating agent, but these were found to have significantly lower activity.

2.4 Syntheses of Selected Dihydro- and Tetrahydro-β-Carbolines

β-Carbolines in various reduced states have also been found to possess antitumor properties. For example, dihydro-β-carboline **91** (Fig. 29), known as "Mana-Hox", has been tested against a large number of human cancer cell lines, and found to be quite effective [69]. IC_{50} values for **91** were between 1.3 and 6.2 μM for 16 of the 17 cell lines tested. Synthesis of **91** began with condensation of tryptamine with aldehyde **89** in the presence of TFA to give tetrahydro-β-carboline **90** [70]. The exact yield for this reaction was not reported, but was in the range of 30–50%. Oxidation with DDQ produced **91**, once again in unspecified yield, though a series of compounds were prepared by this method, and yields were reported as being between 30 and 75%. IC_{50} values for both **90** and **91** were determined for several tumor cell lines. While those for **90** fell between 0.5 and 0.8 μg/mL, IC_{50} values for **91** were all reported as < 0.001 μg/mL, as were the values for paclitaxel.

Tetrahydro-β-carbolines have also been investigated as antitumor agents. For example, there has been much interest in azatoxin **95**, a rationally designed inhibitor of DNA topoisomerase II [71]. Reaction of L-tryptophanol **92** with diethyl carbonate allowed isolation of oxazolidinone **93** in 79% yield after recrystallization (Fig. 30). Pictet–Spengler reaction with syringaldehyde dimethyl acetal **94** produced azatoxin **95** in 91% yield after flash chromatography [72]. A similar approach, utilizing 3,4,5-trimethoxybenzaldehyde and regioselective demethylation with HBr had been reported earlier [73]. The

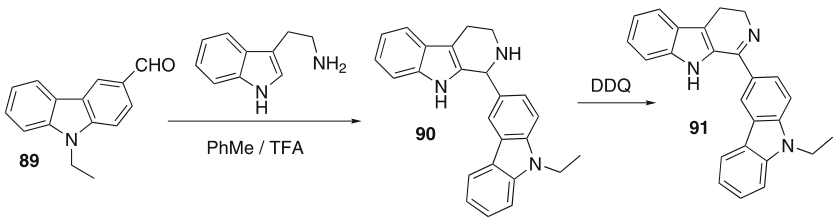


Fig. 29 Synthesis of "Mana-Hox"

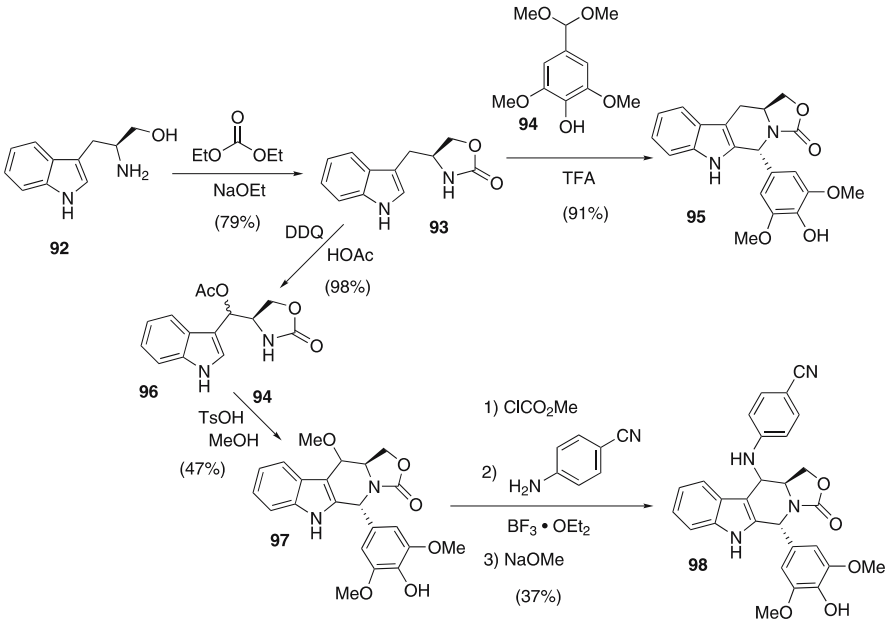


Fig. 30 Synthesis of azatoxin and analog

stereochemistry of the product proved to be important, as the enantiomer of **95** was inactive, as was the diastereomeric compound corresponding to preparation from D-tryptophanol, yet retaining the (R) configuration at C-1 [72].

A large number of derivatives of **95** have been prepared, and through this work it has become apparent that both the planarity of the tetracyclic ring system, as well as the hydroxyl group at the 4′ position of the pendant aromatic ring, are important for activity [74]. A fair amount of variation is tolerated at C-4 with retention of activity. For example, oxidation of **93** with DDQ in acetic acid produced **96**, which was unstable, but allowed the introduction of substituents at C-4 during the cyclization process as shown for the synthesis of **97**. Displacement of the 4-methoxy group by *p*-aminobenzonitrile (between protection and deprotection of the 4′-hydroxyl as a methyl carbonate) gave analog **98**, which was approximately nine times more active than **95** [71]. Unfortunately, solubility and other problems with these compounds have prevented their clinical use [75].

Synthetic analogs of the natural product rutaecarpine **99** (Fig. 31) have also been investigated [76, 77]. Diazotization of aniline and coupling with lactam **100** produced **101** in unspecified yield via a Japp–Klingemann reaction [78]. Cyclization via Fischer indole synthesis gave β-carbolinone **102**, again in unspecified yield. Condensation of **102** with a series of anthranilic acid derivatives utilizing phosphorus oxychloride gave β-carbolines such as **103** in 80–90% yield [77]. Of the derivatives tested, the most promising was

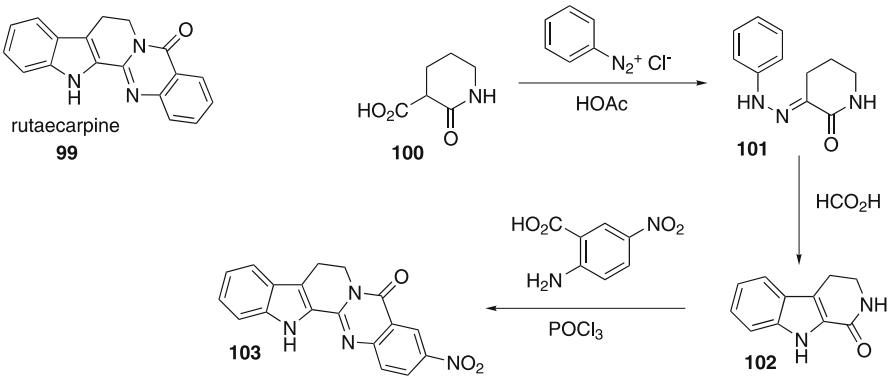


Fig. 31 Synthesis of rutaecarpine analog

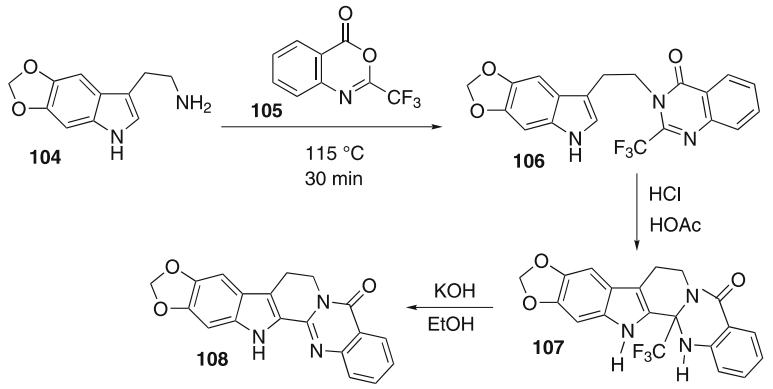


Fig. 32 Synthesis of rutaecarpine analog

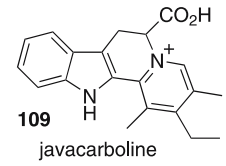
103, prepared from 5-nitroanthranilic acid. In vitro studies against a number of cancer cell lines gave GI_{50} values of 2–3 μM for almost all of the cell lines tested, but in vivo studies were disappointing, perhaps due to low bioavailability of the compound.

A different approach to rutaecarpine and its analogs was reported by Yang and coworkers [79]. For example, heating tryptamine derivative **104** (prepared in six steps from 6-nitropiperonal) with oxazinone **105** (prepared by treatment of isatoic anhydride with trifluoroacetic anhydride) at 115 °C for 30 min produced quinazolinone **106**. Yields for this and subsequent transformations were not reported. Heating at reflux in acetic acid in the presence of hydrochloric acid gave **107**, which eliminated trifluoromethane upon treatment with ethanolic KOH, yielding rutaecarpine analog **108**. This compound (the most active of the ones examined) had GI_{50} values in the 1.08–1.55 μM range against several cell lines, though it was found ineffective against others.

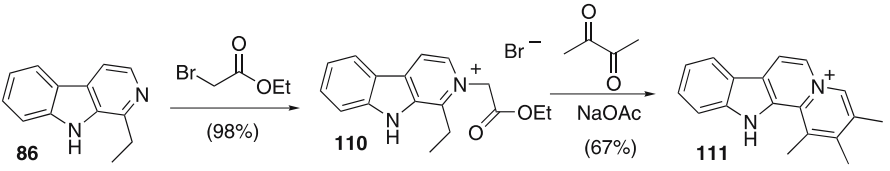
2.5 Syntheses of Selected β-Carbolinium Salts

β-carbolines that exist as positively charged β-carbolinium ions have also been isolated from natural sources and found to have antitumor properties. For example, javacarboline **109** (Fig. 33) has been isolated from an Indonesian medicinal plant and found to have mild antitumor activity (GI_{50} value of 35.9 μg/mL against PC-6 human lung carcinoma cells) [68]. Analogs of this compound have been prepared, however, which show increased activity. For example, indolo[2,3-*a*]quinolizine **111** has a GI_{50} value of 0.2 μg/mL against this same cell line, which is comparable to cisplatin, which has a value of 0.3 μg/mL. This salt has been prepared starting from 1-ethyl-β-carboline **86**, which itself was prepared from tryptamine via a Pictet–Spengler reaction followed by oxidation. Alkylation of **86** with ethyl bromoacetate gave **110** in 98% yield, which was then condensed with 2,3-butanedione by refluxing in anhydrous acetone for 6 h, giving **111** in 67% yield. Other salts were prepared in an analogous manner and displayed similar activity [68].

A similar β-carbolinium natural product that has shown promise as an antitumor agent is flavopereirine **117** (Fig. 34). This compound has shown reasonable activity against a number of cancer cell lines, without showing significant toxicity to healthy cells [80]. An early approach to this system was reported by Gribble [81] and utilized an indole synthesis reported by Smith [82]. Treatment of *N*-trimethylsilyl-*o*-toluidine **112** with 2.2 equivalents of *n*-butyllithium generated the corresponding dianion, which was condensed with ethyl 5-ethylpicolinate **113** to give indole **114** in 67% yield. Protection of the indole nitrogen with a phenylsulfonyl group then allowed the pyridine nitrogen to direct lithiation at C-3 of the indole ring upon treatment with *n*-butyllithium. This lithiated indole was then trapped with bromoacetaldehyde to give intermediate **115**, which upon acidic workup cyclized to give **116** in



109
javacarboline



86 **110** **111**

Fig. 33 Synthesis of javacarboline analog

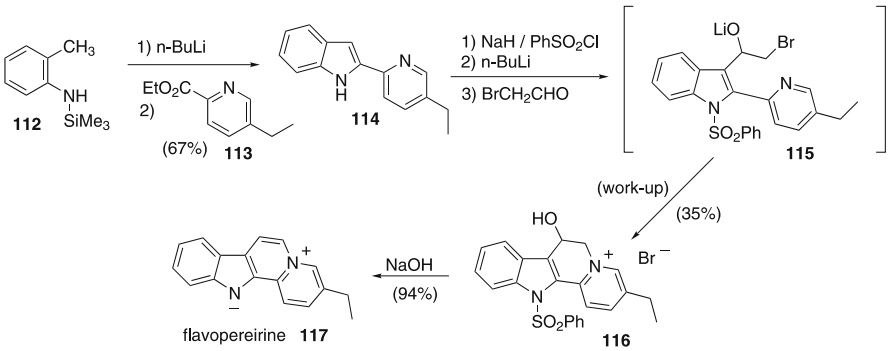


Fig. 34 Synthesis of flavopereirine by Gribble

35% yield starting from **114.** Deprotection with methanolic NaOH resulted in concomitant dehydration to give flavopereirine **117** in 94% yield.

A different approach to the synthesis of flavopereirine has been reported by Fürstner, which utilizes reductive coupling of ketoamides using low-valent titanium as the indole-forming step [83]. Palladium-catalyzed coupling of *o*-iodoaniline **118** with methyl propargyl ether produced alkyne **119** in 96% yield (Fig. 35). Hydration of the alkyne proceeded in 53% yield, and was followed by acylation of the amine with 5-ethylpicolinyl chloride in 85% yield to give ketoamide **120.** During the hydration step, use of methanol as a cosolvent was found to suppress the formation of the corresponding unsaturated ketone, the generation of which would lead to polymerization. The low valent titanium species needed for the reductive cyclization of **120** was prepared by combining potassium graphite (C_8K) with $TiCl_3$ in a 2 : 1 (C_8K : $TiCl_3$)

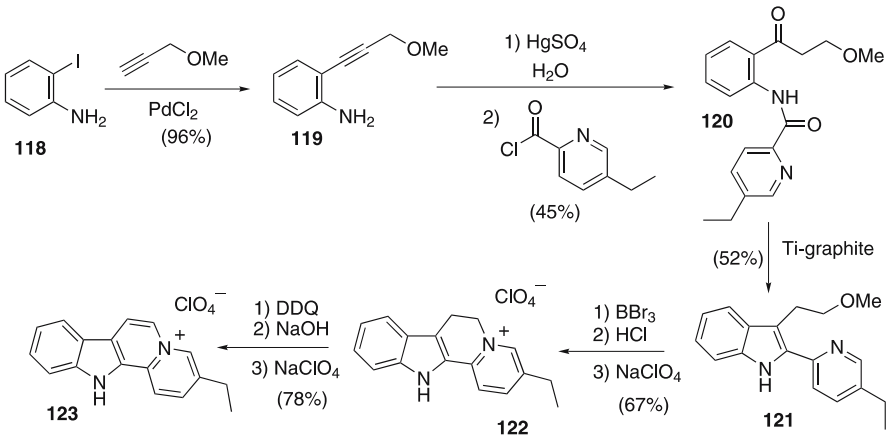


Fig. 35 Synthesis of flavopereirine by Fürstner

ratio. Slow addition of **120** to a refluxing suspension of this reagent in THF then afforded indole **121** in 52% yield. Demethylation of the methyl ether and cyclization was achieved in the same step through the use of boron tribromide, though the product was most easily isolated by conversion to the perchlorate salt **122**. DDQ oxidation gave flavopereirine in good yield, again isolated as the perchlorate salt **123**.

Lounasmaa and coworkers [84] reported an interesting approach to flavopereirine starting from alcohol **124** (Fig. 36), which had been prepared as part of another synthesis [85]. Acetylation of **124** was reported to proceed in 80% yield, though the reagent used was not specified. Oxidation of this acetate with *m*-CPBA produced *N*-oxide **125** in 52% yield. Treatment of this *N*-oxide with trifluoroacetic anhydride initially gave iminium ion **126** via a Potier-Polonovski reaction [86], though under the reaction conditions (not specified) elimination of acetate and several proton rearrangements led to di-

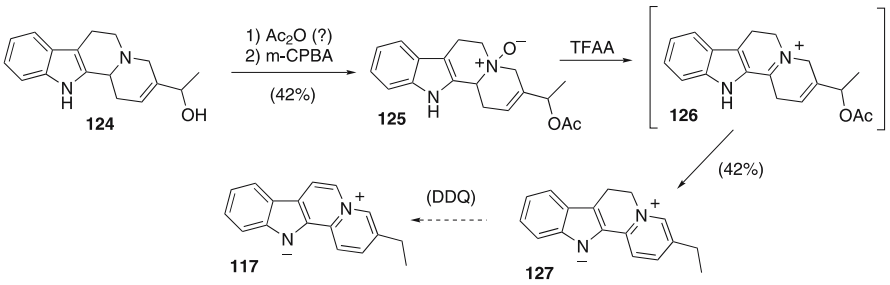


Fig. 36 Synthesis of flavopereirine by Lounasmaa

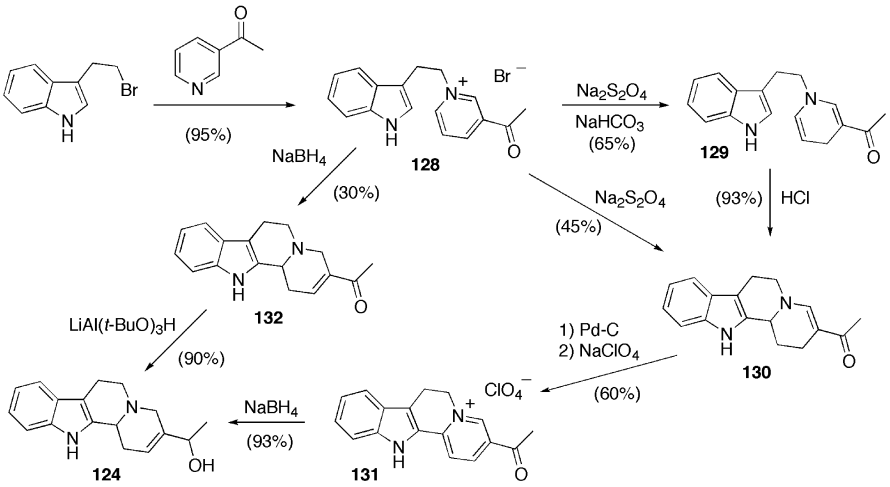


Fig. 37 Synthesis of intermediate in flavopereirine synthesis

hydroflavopereirine **127**. As this intermediate had previously been converted to flavopereirine **117** (as its perchlorate salt) using DDQ, this completed Lounasmaa's formal total synthesis of flavopereirine.

The key intermediate **124** was prepared starting with tryptophyl bromide alkylation of 3-acetylpyridine, to give **128** in 95% yield (Fig. 37) [87]. Reduction of **128** with sodium dithionite under buffered (sodium bicarbonate) conditions lead to dihydropyridine **129**, which could be cyclized to **130** upon treatment with methanolic HCl. Alternatively, **128** could be converted directly to **130** by sodium dithionite if the sodium bicarbonate was omitted. Oxidation with palladium on carbon produced pyridinium salt **131**, which could then be reduced to **124** (as a mixture of isomers) upon reaction with sodium borohydride. Alternatively, direct reduction of **128** with sodium borohydride gave a mixture of compounds, from which cyclized derivative **132** could be isolated in 30% yield after column chromatography [88]. Reduction of **132** with lithium tri-*t*-butoxyaluminum hydride then gave **124** (once again as a mixture of isomers) in 90% yield.

3 Syntheses of Selected α-Carbolines

Although α-carbolines (in particular, 2-amino-α-carboline) are perhaps better known as mutagens [89], some α-carbolines have been found to possess antitumor properties. For example, there has been much interest in the synthesis of natural products grossularine-1 **133** and grossularine-2 **134** (Fig. 38) since the report of their activity as antitumor agents at the 10 ng/mL level [90].

The first total syntheses of these compounds were by Hibino and coworkers [91, 92] and utilized palladium(0)-catalyzed coupling of **135** and **136** to produce indole-substituted imidazole **137** in 82% yield (Fig. 39). (A similar approach to closely related compounds was reported by Achab and coworkers at about the same time as the first report by Hibino) [93]. Ester **137** was hydrolyzed in near quantitative yield by reaction with sodium car-

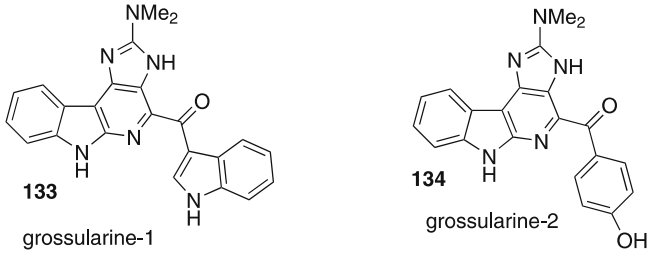


Fig. 38 Structures of grossularines

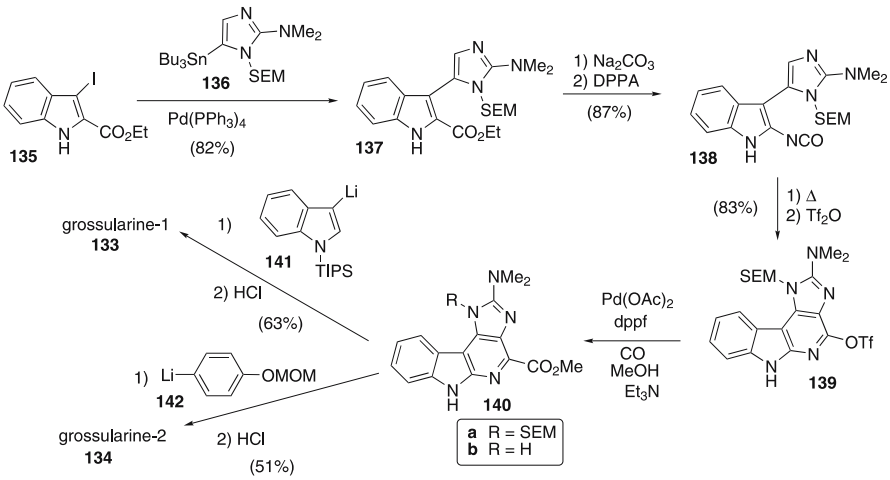


Fig. 39 Synthesis of grossularines by Hibino

bonate in ethanol, and the resulting acid converted to isocyanate **138** via a Curtius rearrangement by treatment with diphenylphosphoryl azide and triethylamine. Heating **138** in *o*-dichlorobenzene at 170 °C for five minutes effected an electrocyclic ring closure to the corresponding α-carbolinone, which was transformed into triflate **139** by treatment with triflic anhydride. Palladium-catalyzed methoxycarbonylation produced key intermediate **140a** in 77% yield (though under slightly different conditions the deprotected product **140b** could be obtained in 93% yield).

Ester **140a** served as the immediate precursor for both grassularines, and was allowed to react with the appropriate aryl lithium (each prepared from the corresponding bromide by treatment with *t*-butyllithium), giving the desired grassularines after acidic work-up in the yields indicated. Use of **140b** in place of **140a** produced a slightly higher yield of **134** (61%), but a lower yield of **133** (37%).

An alternative route to an intermediate similar to **139** has been reported by Molina and coworkers [94]. Indole **143** (Fig. 40) was prepared in several steps starting from 3-acetyl-2-chloroindole, the nitrogen at C-2 being introduced

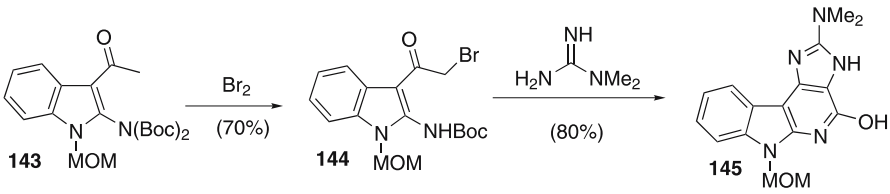


Fig. 40 Synthesis of grossularine intermediate by Molina

via the azide. Bromination of **143** in methanol/DMF followed by monodeprotection of the exocyclic amine with trifluoroacetic acid afforded **144** in 68% yield for the two steps. (Alternatively, **144** could be obtained directly from **143** in 70% yield by treatment with bromine in chloroform). Reaction of **144** with *N*,*N*-dimethylguanidine at room temperature for 3 hours provided **145** in 80% yield, although the precise mechanism of this transformation was not determined. The authors concluded that **145** could then be converted into grassularines by a process analogous to that reported by Hibino [92].

An exceptionally short synthesis of grassularine-1 **133** was reported recently by Horne and coworkers [95]. This approach utilized intermediate oxotryptamine **146**, which had been prepared previously as part of another synthesis [96]. Condensation of **146** with dimethylcyanamide produced **147**, which was prone to oxidation, and was only stable as its hydrochloride salt (Fig. 41). This sensitivity to oxidation was utilized in the key reaction step in which oxidative dimerization to give **148** was accomplished by stirring **147** in methanolic ammonia solution at room temperature for 1 day. Continued stirring under these conditions for another 5 days eventually resulted in the production of **149** in 60% yield directly from **147**. Hydrolysis of **149**, which required forcing conditions (12-h reflux in a mixture of ethanol and 6M HCl), but nevertheless proceeded in 95% yield, completed this synthesis of grassularine-1 **133**.

The proposed pathway for the conversion of **148** into **149** is shown in Fig. 42. A proton shift converts **148** into **150**, which then undergoes electrocyclic ring closure, yielding **151** after another proton shift. Oxidation of **151** gives iminium ion **152**, which undergoes ring-opening aminolysis, ultimately yielding the observed product **149**. As oxidation products of indolylimidazole **147** have been isolated along with **133** from the same source, the authors make a good case for this approach to the synthesis of **133** being biomimetic.

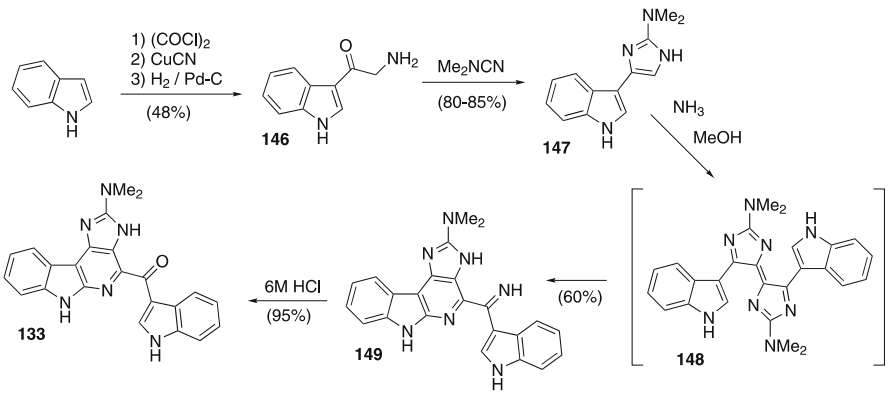


Fig. 41 Synthesis of grossularine-1 by Horne

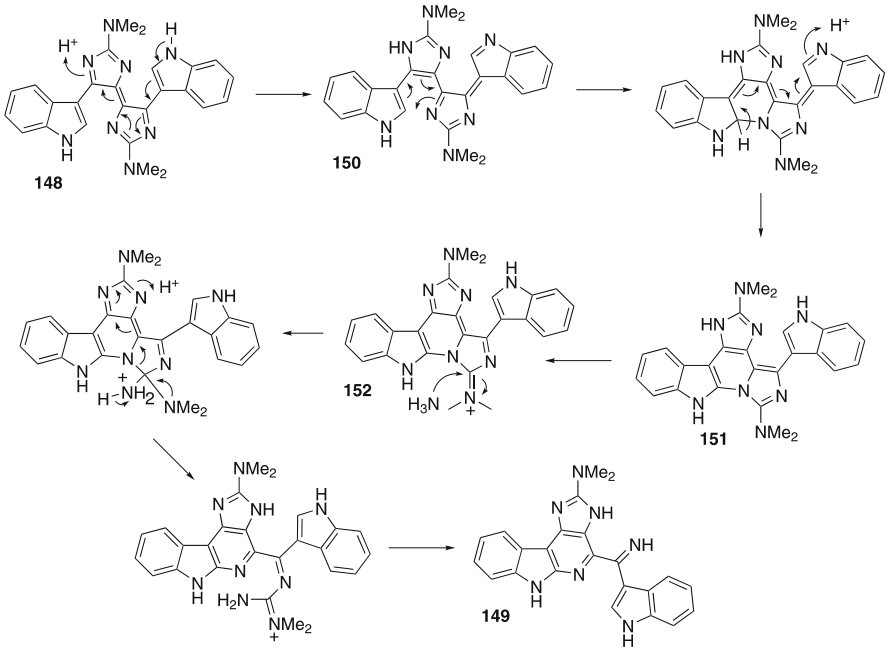


Fig. 42 Pathway for conversion of **148** into **149**

Synthetic α-carbolines have also attracted interest as antitumor agents. For example, Chen and coworkers [97] prepared a series of indolo[2,3-*b*]quinoline derivatives, the most active of which was **154**, which had a mean GI_{50} value against three cancer cell lines of 0.78 μM. This compound was prepared by methylation of **153** with dimethyl sulfate (Fig. 43), and was isolated in 12% yield, along with isomeric **155**, which was isolated in 40% yield, but had significantly lower cytotoxicity. Precursor **153** itself was found to be inactive.

Synthesis of **153** began with 4-hydroxy-1H-quinolin-2-one **156** which was heated in refluxing diphenyl ether (boiling point = 259 °C) with *p*-anisidine

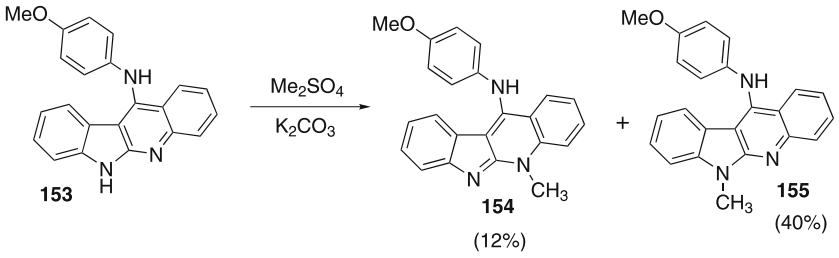


Fig. 43 Methylation of α-carboline **153**

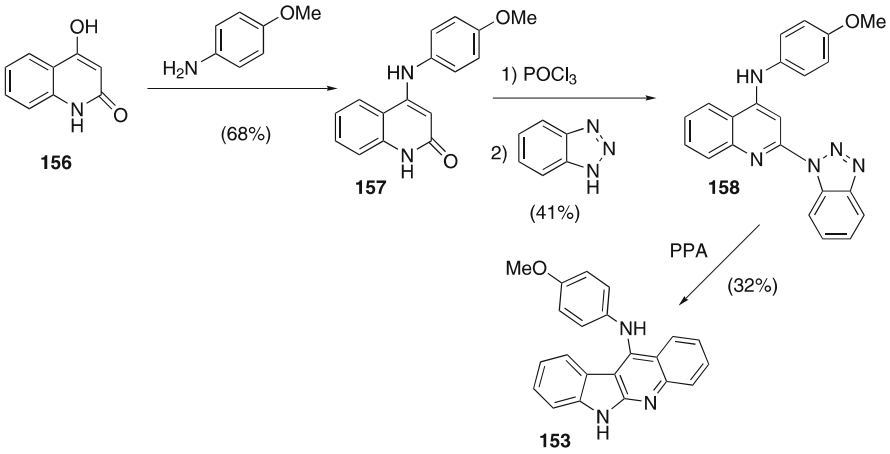


Fig. 44 Synthesis of α-carboline **153**

to give **157** in 68% yield (Fig. 44). Chlorination with phosphorus oxychloride followed by heating with benzotriazole gave intermediate **158**, which was heated at 140–150 °C in PPA for 2 h, producing **153** in 32% yield.

4
Syntheses of Selected γ-Carbolines

The isomeric indolo[3,2-*c*]quinolines (which formally are γ-carbolines) have also been investigated as antitumor agents. For example, He and Cheng [98] prepared a series of such compounds, the most active of which was **161**, starting with a Fischer indole synthesis between phenylhydrazine and 2,3-dihydroquinolin-4-one **159** to give indolo[3,2-*c*]quinoline **160** in 53% yield (Fig. 45). Demethylation with hydrobromic acid gave a 97% yield of the corresponding phenol, which was then alkylated with (dimethylamino)ethyl chloride to give **161** in 65% yield after recrystallization from ethanol. This compound exhibited an IC_{50} value of 0.05 μM against small-cell lung cancer cells, which is comparable to, and in some cases better than, that observed for anticancer agents in clinical use [98].

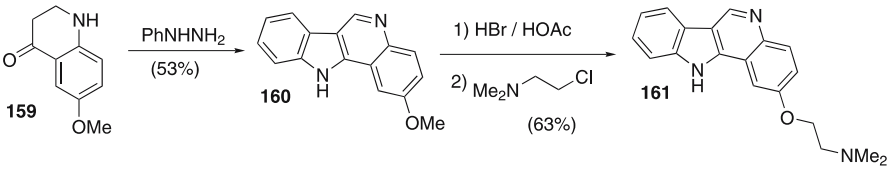


Fig. 45 Synthesis of γ-carboline **161**

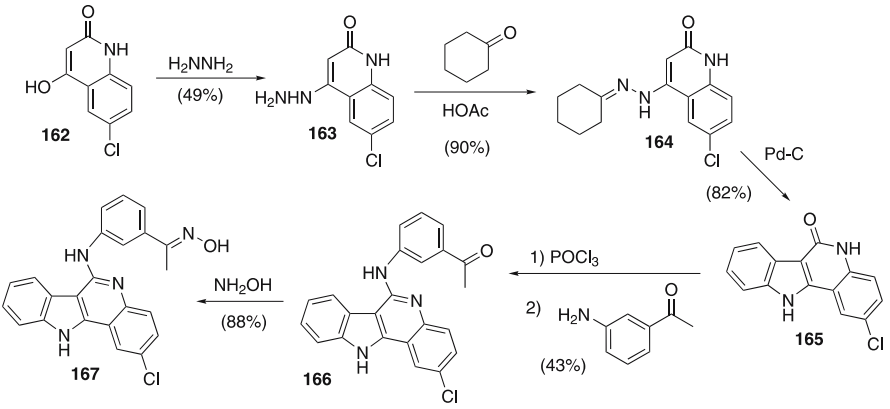


Fig. 46 Synthesis of γ-carboline **167**

Chen and coworkers [99] used a Fischer indole synthesis in a different sense to prepare indolo[3,2-*c*]quinoline **167** (Fig. 46). Heating quinolin-2-one **162** at reflux in ethoxyethanol for 2 h with 40% hydrazine hydrate gave **163** in 49% yield. Stirring **163** with cyclohexanone at room temperature for 36 h gave a 90% yield of hydrazone **164** which was then heated with palladium on carbon at 250 °C to effect both the Fischer indolization as well as aromatization of the cyclohexanone ring, resulting in the formation of benzo-fused γ-carbolinone **165** in 82% yield. Chlorination with phosphorus oxychloride proceeded in 63% yield, which was followed by heating at reflux in 2-butanol with 3-aminoacetophenone to give **166** in 69% yield. Though **166** showed good cytotoxicity toward a panel of cancer cell lines (mean GI_{50} of 4.26 μM), the corresponding oxime **167** was even better (mean GI_{50} of 1.35 μM against the same panel). A number of other derivatives were prepared, including the non-chlorinated version of **167**, which was found to be almost as active as **167**.

5
Syntheses of Selected δ-Carbolines

Compounds containing the δ-carboline structure have also been studied. For example, Deady and coworkers prepared a series of (10*H*)-indolo[3,2-*b*]quinolines, (also known as "quindolines") which are formally benzo-δ-carbolines [100]. Of those tested, the most active was **171**, which was prepared via condensation of indole derivative **168** with methyl 2-amino-3-formylbenzoate **169** to give δ-carboline **170** (Fig. 47) [101]. Treatment of **170** with methyl iodide and potassium hydroxide in DMSO resulted in *N*-alkylation of the indole nitrogen as well as conversion of the carboxylic acid to the corresponding methyl ester, this reaction proceeding in 55% yield. Heating the resulting ester with *N*,*N*-dimethylethylenediamine in dioxane at reflux for

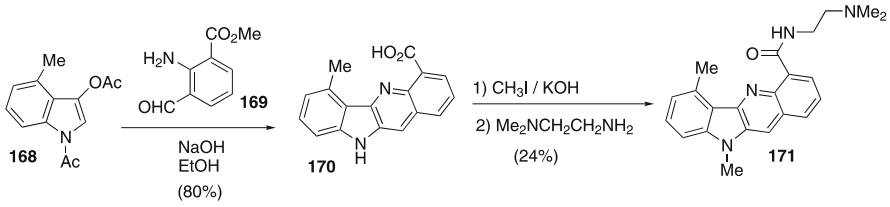


Fig. 47 Synthesis of δ-carboline **171**

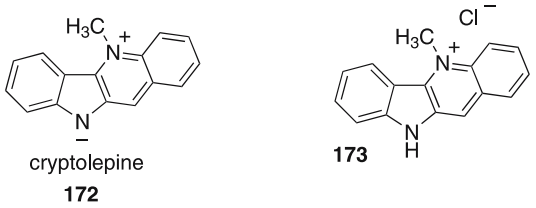


Fig. 48 Structure of cryptolepine and its hydrochloride salt

7 days resulted in a 44% yield of amide **171.** This compound exhibited an IC_{50} value of 18 nM when tested against murine Lewis lung carcinoma cells [100].

Since natural product cryptolepine hydrochloride **173** (Fig. 48) has been found to be cytotoxic to B16 melanoma cells with an IC_{50} of 0.3 μg/mL (1.3 μM) [102], there has also been interest in cationic δ-carboline-based compounds as antitumor agents. For example, Wright and coworkers synthesized a series of these compounds and evaluated their activity against MAC15 cells (murine adenocarcinomas of the colon) [103]. Of the compounds prepared, the most active was **179** which had an IC_{50} value of 1.03 μM.

Synthesis of this compound began with the condensation of *O*,*N*-acetylindoxyl **174** with 5-bromoisatin **175** to give indolo[3,2-*b*]quinoline **176** after stirring at room temperature for 10 days (Fig. 49) [104]. This product was then decarboxylated by heating at reflux for 6 h in diphenyl ether. Yields were

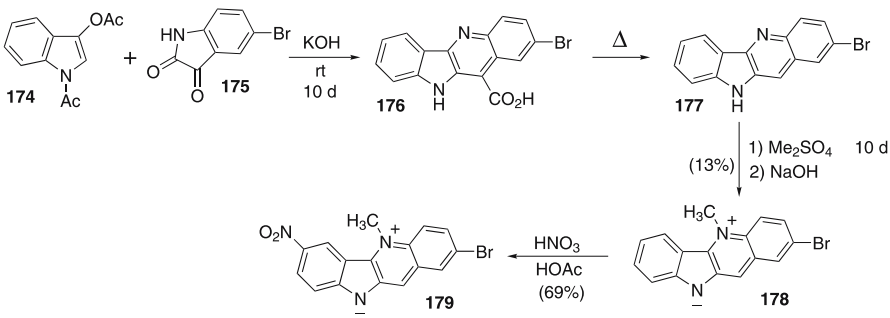


Fig. 49 Synthesis of cryptolepine analog **179**

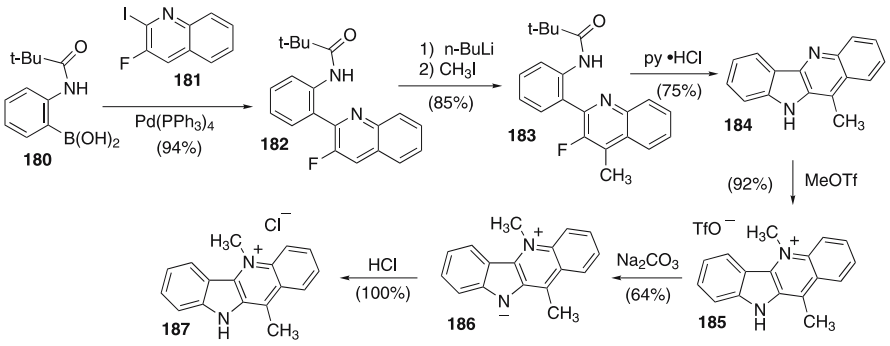


Fig. 50 Synthesis of cryptolepine analog **186**

not reported for either of these reactions. Heating a solution of **177** with ten equivalents of dimethyl sulfate at reflux in chloroform for 10 days followed by treatment with base gave **178** in low yield. The authors stated that significantly higher yields could be obtained if **177** were heated overnight in a sealed tube at 50 °C with a large excess of methyl iodide in tetramethylenesulfone, but no details were provided. Bromocryptolepine **178** was then nitrated by stirring at room temperature for 24 h in a 1 : 1 mixture of nitric and acetic acids, giving **179** in 69% yield [103].

Rocca and coworkers have described a completely different route to 11-substituted cryptolepines, and have evaluated the cytotoxicity of such compounds against KB (mouth epidermoid carcinoma) cells [105]. Of the compounds tested, the most active were **186**, its hydrochloride salt **187**, and its triflate salt **185**, which had IC_{50} values of 0.53, 0.42 and 0.57 μM, respectively (Fig. 50). Their synthesis began with a Suzuki coupling of boronic acid **180** with quinoline derivative **181** which proceeded in 94% yield to give **182.** Lithiation with 2.8 equivalents of *n*-butyllithium followed by quenching with methyl iodide gave methylated derivative **183** (other derivatives were prepared by introducing other electrophiles at this point). Adding boiling hot anhydrous pyridinium hydrochloride to **183** followed by heating this mixture at reflux (*bp* = 215 °C) for 30 min afforded a 92% yield of cyclized indolo[3,2-*b*]quinoline **184.** Methylation with methyl triflate gave triflate salt **185**, which could be deprotonated to give cryptolepine derivative **186** which in turn could be re-protonated with HCl to yield hydrochloride salt **187**. As mentioned above, all three of these compounds showed significant activity.

6 Summary

A number of carbolines have promise as lead compounds for antitumor agents. These compounds include natural products, synthetic derivatives of

such products, and wholly synthetic compounds. Though most of the compounds investigated have been β-carbolines, other isomeric carbolines show promise as well.

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Diazo and Diazonium DNA Cleavage Agents: Studies on Model Systems and Natural Product Mechanisms of Action

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Abstract Diazonium salts have been previously used to cleave DNA via generation of carbon centered radicals and cations. Efforts have been made in the past decade or so to develop diazo compounds and α-diazoketones for physiologically relevant DNA cleavage. These efforts, coupled with their relevance to the mechanism of action of kinamycin and lomaiviticin antibiotics and other naturally occurring diazo compounds, will be discussed.

Keywords Alkylation · Diazo · Diazonium · DNA cleavage · Kinamycin antibiotics · Lomaiviticin antibiotics

1
Diazonium and Diazo Compounds: An Introduction

Diazonium salts have been known for more than a hundred years [1–3] and their synthetic potential as reactive intermediates in organic chemistry is well established [4]. While aromatic diazonium compounds have firm roots as intermediates for the synthesis of important azo dye compounds [5], aliphatic diazonium salts have been relatively less emphasized due to their inherent instability [6]. In order to understand the role that diazonium salts play as reactive intermediates, it is important to look at the reaction processes where the diazo group is eliminated to give a cationic intermediate via a heterolytic dissociation pathway (Fig. 1, route a) or a radical intermediate via a homolytic bond fission in the presence of a reducing agent (Fig. 1, route b) [7]. These dediazoniation reactions of diazonium salts have been broadly responsible for their applicability and potential drug use. While aromatic diazonium salts have been known to decompose via radical and cation pathways due to their greater stability and longer lifetimes, aliphatic diazonium salts undergo rapid loss of nitrogen to give the corresponding carbocation.

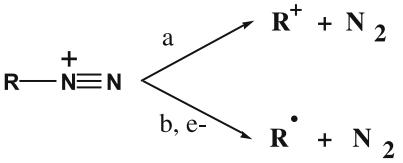


Fig. 1 Modes of diazonium ion fragmentation

1.1
Alkyl Diazonium Salts: DNA Alkylation

While diazonium salts are mostly formed by diazotization of the corresponding amines, they have also been postulated as intermediates in the hydrolysis of other reactive intermediates (triazenes, alkanediazoates) with biological relevance. Triazenes [8] (Fig. 2) are open chain compounds of the general structure $RN_1 = N_2N_3R'R''$. They have attracted significant attention because of their mutagenic and carcinogenic properties [9, 10]. Extensive studies involving the decomposition of arylalkyltriazenes have shown the intermediacy of alkyl diazonium salts consistent with a mechanism involving protonation of the triazene followed by release of the alkyl cation. Evidence supporting different decomposition mechanisms involving buffer catalyzed, general acid catalyzed (A – S_E2), and uncatalyzed unimolecular *N-N* heterolysis, has also been reported [11, 12].

The bicyclic imidazotetrazinone temozolomide [13, 14] (Fig. 3) is an example of an antitumor drug that acts via decomposition of the triazene inter-

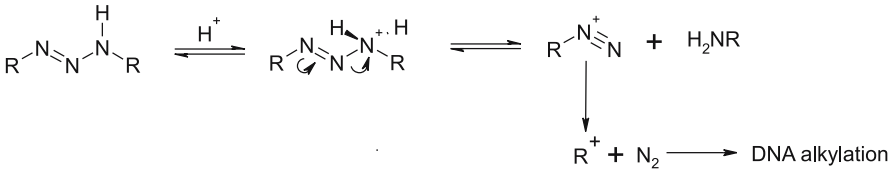


Fig. 2 Acid-catalyzed decomposition of triazenes

mediate to give an aliphatic diazonium salt that loses nitrogen and alkylates nucleophilic sites in DNA [15]. The drug temozolomide and its analogous tetrazinones have undergone clinical trials against a variety of tumors.

Alkanediazoates [16] (Fig. 4) are reactive intermediates responsible for DNA-alkylating activities of compounds that contain the *N*-alkyl-*N*-nitroso functionality. They are mutagenic and carcinogenic, as well as potential cancer chemotherapeutic agents. The mechanism of general acid catalysis of the decomposition of diazoates involves proton transfer to oxygen followed by N – O bond heterolysis to yield the diazonium ion [17–21]. The generation of alkanediazoates and their decomposition is of extreme importance due to their intermediacy in cancer chemotherapeutic agents like bis(choroethyl)nitrosourea, which contains the chloroethane moiety [22]. Other (haloethyl)nitrosoureas, especially (E) chloroethanediazoate have also been shown to have antileukemic activity in vivo [23].

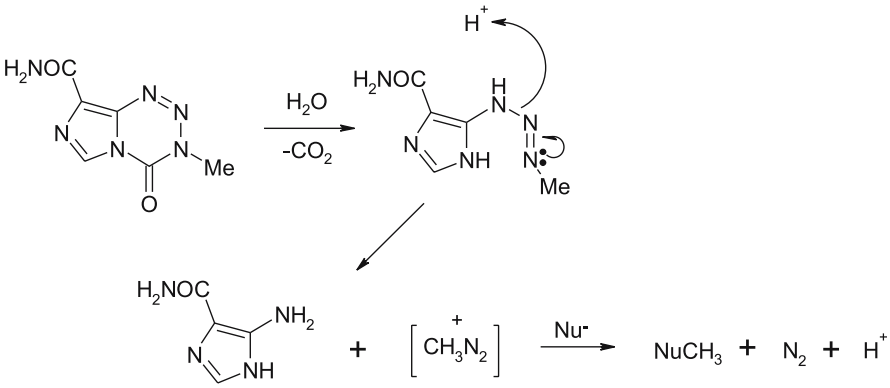


Fig. 3 Decomposition of antitumor drug temozolomide

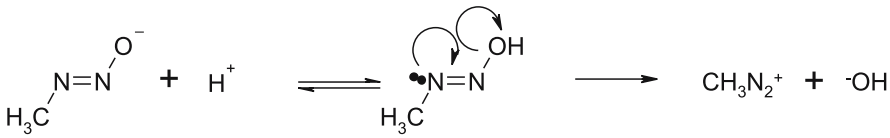


Fig. 4 Acid catalyzed decomposition of alkanediazoates

1.2 Carbon Centered Radicals for DNA Cleavage

Before the advent of enediyne antitumor antibiotics, there had been few examples of carbon radicals believed to mediate DNA cleavage. The simplest of all, the methyl radical has been shown to effect DNA cleavage under enzymatic as well as chemical conditions [24]. Oxidation of methylhydrazine by horseradish peroxidase or ferricyanide (Fig. 5) gave high yields of methyl radicals, which were shown to cleave DNA by purine ring alkylation.

Of wider significance was the generation of 2-phenylethyl radical by oxyhemoglobin-mediated oxidation of phenelzine (2-phenylethylhydrazine), which was shown to be more efficient in promoting alkali-labile sites than in producing direct DNA strand scission (Fig. 6) [25].

The generation of trimethylenemethane diyls [26] has been shown to effect DNA cleavage. Attachment of this group to a DNA binding molecule (Fig. 7) made the intramolecular hydrogen atom abstraction (DNA–drug being considered as one molecule) more efficient than the competitive dimerization of diyls.

While enediyne natural products are efficient DNA cleaving agents, synthetic enediynes have been utilized widely to study the mechanistic details and obtain more efficient DNA cleavage activity. Simple enediynes (Fig. 8), with no binding units, show DNA cleavage albeit at very high concentra-

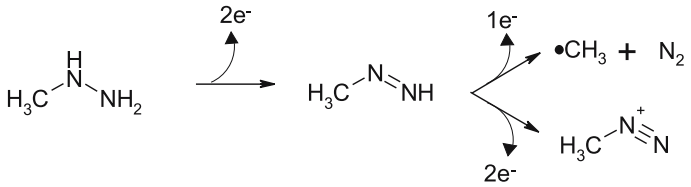


Fig. 5 Generation of methyl radicals via oxidation of methylhydrazine

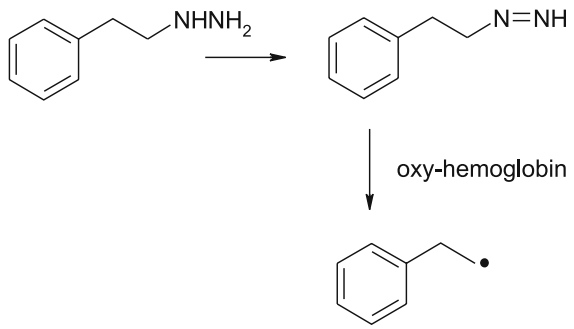


Fig. 6 Oxidation of phenelzine to carbon centered 2-phenylethyl radical

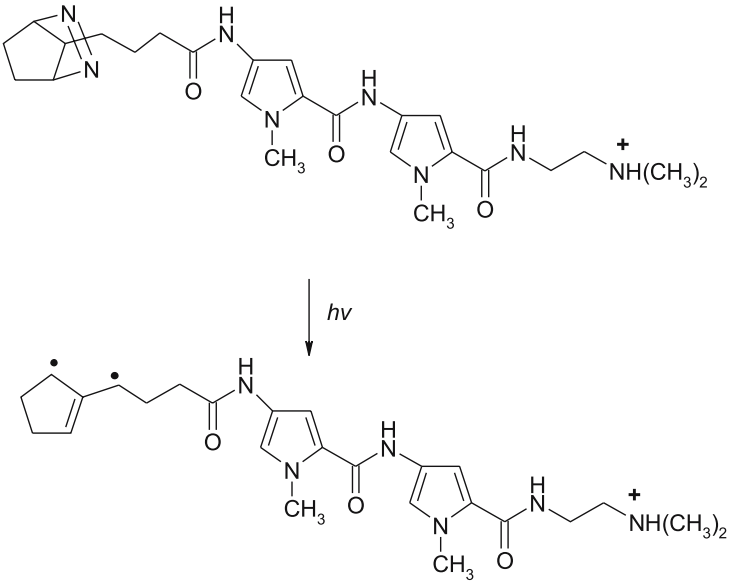


Fig. 7 Photogeneration of distamycin-bound trimethylenemethane diyls generated from a diazene precursor

tions. The cyclodecaenediyne diol [27] (**1**, Fig. 8) is one such example. Among others, the propargylic sulfones [28] were shown to mediate DNA cleavage by a mechanism different from enediyne compounds (Fig. 8, pathways a and b). The mechanism inferred was similar to the interaction of cumulene intermediate with the nucleophilic nitrogens of DNA, analogous to the Maxam–Gilbert chemistry. The allenylphosphine oxide [29] (**3**, Fig. 8) was shown to cleave DNA, presumably by the formation of a diradical intermediate after undergoing cyclization at moderate temperatures compatible with DNA cleavage. For a current review on designed enediynes, the reader is advised to look at recent reviews on the subject [30].

4-Alkynyl-3-methoxy-4-hydroxycyclobutenones [31] have also been shown to cleave supercoiled DNA, and DNA damage was believed to be mediated by diradicals (Fig. 9) arising from the thermal decomposition of cyclobutenones at 49 °C.

1.3 Aryl Diazonium Salts: Rationale and Development

An alternative approach to developing new reagents for DNA cleavage was founded in the conversion of aryl diazonium salts to aryl halides by the well-known Sandmeyer reaction. The reaction (Eq. 1) is thought to proceed via aryl radicals [7]. These reactions are high yielding and catalytic with respect

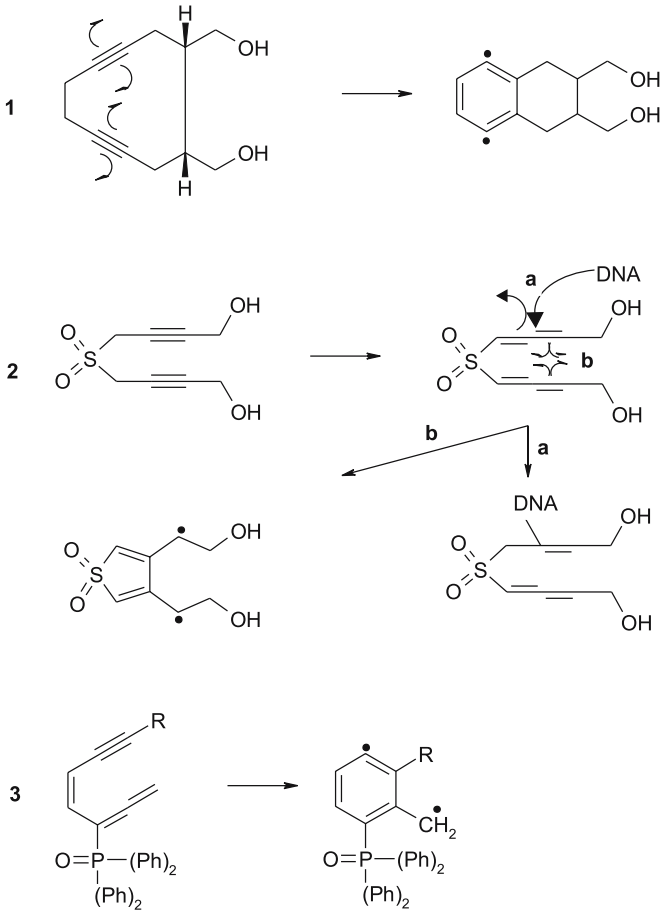


Fig. 8 Structures and proposed mechanisms of synthetic enediynes: *1* cyclodecaenediyne diol, *2* propargylic sulfones, *3* allenylphosphine oxide

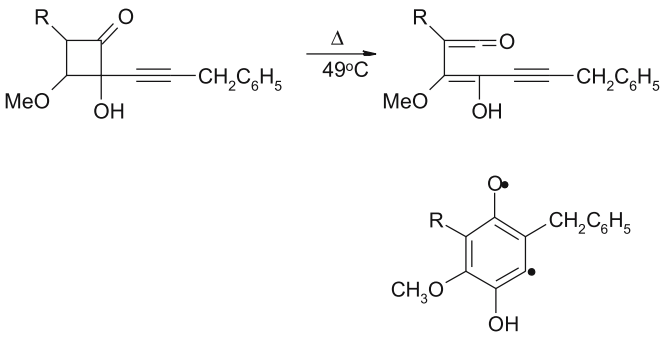
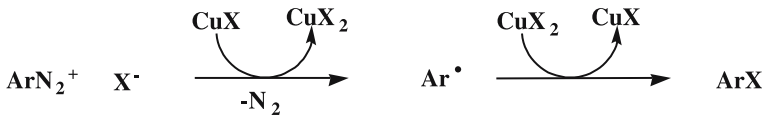


Fig. 9 Generation of DNA-cleaving diradicals from 4-alkynyl-3-methoxy-4-hydroxycyclobutenones

to the cuprous salts.



(1)

It was therefore envisioned that compounds of the type described in Fig. 10, wherein two diazonium units (or their aryl amine precursors) are attached to a DNA binding molecule, should deliver high concentrations of nondiffusible 1,*n*-aryl diradicals ($n > 4$) along the strands of duplex DNAs. The recognition element could be designed to provide sequence specificity and stronger binding to DNA. The interaction between the positively charged diazonium moieties and the negatively charged phosphodiester backbone was expected to further increase the affinity of these molecules to DNA. In addition, the positive charges on the diazonium units should render water solubility.

The d(A.T)-specific, minor groove binding drug **NSC-101327** (Fig. 11) served as an important lead structure for the design of didiazonium com-

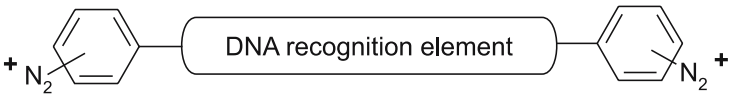


Fig. 10 DNA-binding aryl didiazonium salts

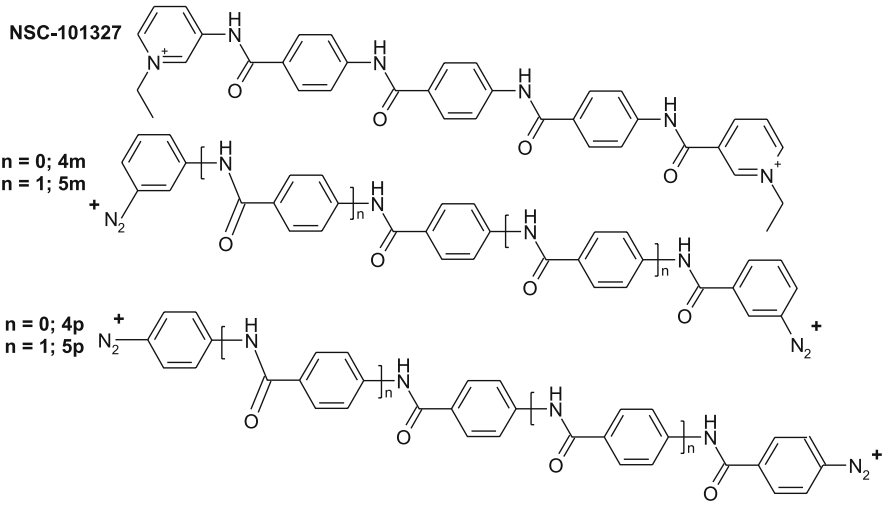


Fig. 11 The d(A.T)-specific, minor groove binding drug **NSC-101327** and the designed didiazonium compounds **4m-5p**

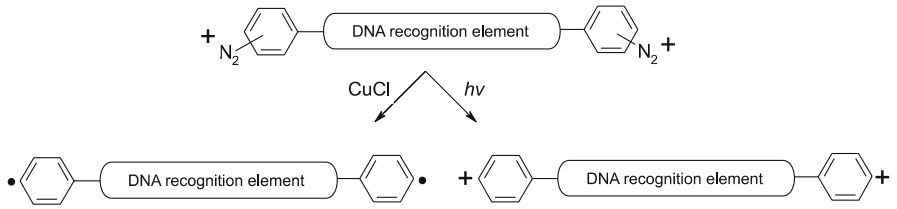


Fig. 12 Generation of cation vs. radical intermediates from aryl didiazonium salts

pounds **4m** and **5m** [32, 33]. The central part, which has been suggested to be involved in specific hydrogen bonding to dA . dT [33, 34] pairs, was retained in **4m**. However the two pyridinium units, which are believed to be responsible for water solubility and interaction with the negatively charged phosphodiester backbone, were replaced by two diazonium units capable of functioning in the same way.

The photolytic activation of **5m** was also shown to lead to DNA cleavage [33, 35–38]. This reaction appeared to be faster and more efficient than the Cu^{+}-catalyzed cleavage conditions. The mechanism(s) of DNA cleavage should be different because aryl cations (*not* aryl radicals) are believed to be produced under photolytic conditions (Fig. 12) [7]. Such electrophiles should target the nucleic acid bases and/or the positively charged phosphodiester backbone, and both of these could lead to DNA cleavage.

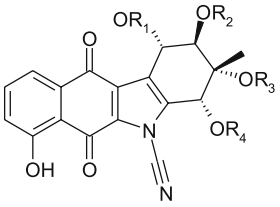
1.4 DNA Cleavage with Diazonium Salts: Key Features

A key feature of DNA cleavage with diazonium salts has been the efficient cleavage at very low concentrations (sub μM). The use of phenyldiazonium tetrafluoroborate to demonstrate cleavage of DNA has also been reported [39]. The enormously high concentrations required therein (800 μM) are an elucidation of the lack of binding capability of that molecule and in retrospect is the reason for such low concentration cleavage intensity shown by these molecules. This is further confirmed by the fact that these didiazonium salt analogs (Figs. 10–12), designed after the known anticancer drug NSC-101 327, show very efficient cleavage at low concentrations.

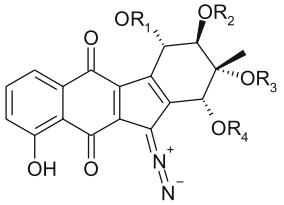
This approach was the first application of non-enediyne carbon centered radical mediated DNA cleavage agents that were not only capable of binding to DNA but could also be sequence specific. Further work is still needed to elucidate and confirm the sites of cleavage, nature of binding of these molecules and the mechanism of hydrogen abstraction from the nucleic acid backbone.

2
Kinamycin Antibiotics: Revised Structures as Diazobenzo[*b*]fluorenes

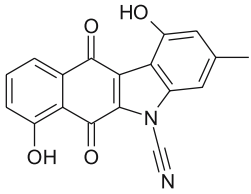
The kinamycin antibiotics were first isolated from *Streptomyces murayamaensis* [40] and originally characterized by Omura and coworkers as being benzo[*b*]carbazole cyanamides **6** (Fig. 13). They have been shown to possess activity against Gram-positive, and to a lesser extent, Gram-negative bacteria, as well as against Ehrlich ascites carcinoma and sarcoma-180 [40]. Using spectroscopic methods, the structures were later revised by Gould [41] to be 5-diazobenzo[*b*]fluorenes **7**. Additionally, the cyanocarbazole prekinamycin



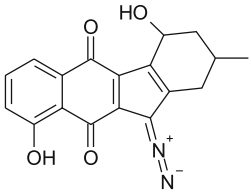
6a Cynamide Kinamycin A R_1=H, R_2=R_3=R_4=Ac
6b Cynamide Kinamycin B R_1=R_2=R_4=H, R_3=Ac
6c Cynamide Kinamycin C R_1=R_2=R_4=Ac, R_3=H
6d Cynamide Kinamycin D R_1=R_3=H, R_2=Ac, R_4=H
6e Cynamide Kinamycin E R_1=R_2=R_3=H, R_4=Ac
6f Cynamide Kinamycin E R_1=R_2=R_3=R_4=H



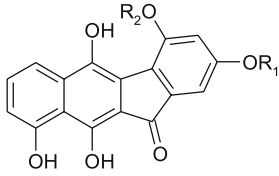
7a Kinamycin A R_1=H, R_2=R_3=R_4=Ac
7b Kinamycin B R_1=R_2=R_4=H, R_3=Ac
7c Kinamycin C R_1=R_2=R_4=Ac, R_3=H
7d Kinamycin D R_1=R_3=H, R_2=Ac, R_4=H
7e Kinamycin E R_1=R_2=R_3=H, R_4=Ac
7f Kinamycin E R_1=R_2=R_3=R_4=H



8 cyanacarbazole prekinamycin

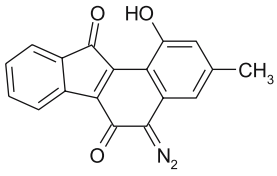


9 prekinamycin



kinafluorenone

10a R_1=R_2=Me
10b R_1=Me R_2=H
10c R_1=R_2=H



Isoprekinamycin

Fig. 13 Structures of kinamycin antibiotics

structure **8** was regioselectively synthesized by Dmitrienko [42] and used to demonstrate by IR and NMR spectroscopy that authentic kinamycins do not possess the cyanamide functionality. The 5-diazobenzo[*b*]fluorene structure remains to date the best fit to experimental data, although Hauser [43] recently completed the synthesis of prekinamycin **9** and found it not to be identical to the fraction previously characterized by Gould [44] as prekinamycin. Subsequently, Gould [45] has identified a *S. murayamaensis* metabolite identical to the compound synthesized by Hauser and named it prekinamycin, while naming the original structure isoprekinamycin.

A series of publications elucidating the correct structure of kinamycins A, B, C, and D as 5-diazobenzo[*b*]fluorenes (**7**a–f, Fig. 13), have appeared in the literature [41, 42]. The earlier assignment of these antibiotics as benzo[*b*]carbazoloquinonecynamides [40] (Fig. 14) was discarded upon mismatch of the synthesized structure with the natural products. While most of them are known to possess antibacterial activity, some have shown considerable toxicity to cancerous cells [44, 46, 47].

The kinamycin antibiotics had been characterized as benzo[*b*]carbazoloquinone cyanamides [40] (Fig. 13) on the basis of chemical, spectroscopic, and X-ray crystallographic data [48, 49]. The unusual cyanamide moiety had been assigned from the infrared absorbance (2150 cm^{-1}) and from detection of ammonia upon hydrolysis. The biosynthetic studies made the ^{13}C NMR assignment possible when the initial doubts were cast on the structural assignment of kinamycins. All other carbons were assigned satisfactorily, but the cyanamide carbon, expected to occur at δ 110–120, showed up at δ 78. The unusual chemical shift was not explained satisfactorily at that time but was attributed to possible electronic effects of the indoloquinone. The total synthesis of *N*-cyanoindoles [41, 42] gave the final answer to this 20-year old problem. The incongruous IR absorbances (2250 cm^{-1}) and the ^{13}C NMR resonance (δ 112) were sufficient reason for the reassignment of the kinamycin structures. Since the X-ray structural solution of **7e** had given R = 8.9% it was reasonable to assume that the crystallographic data set had not distin-

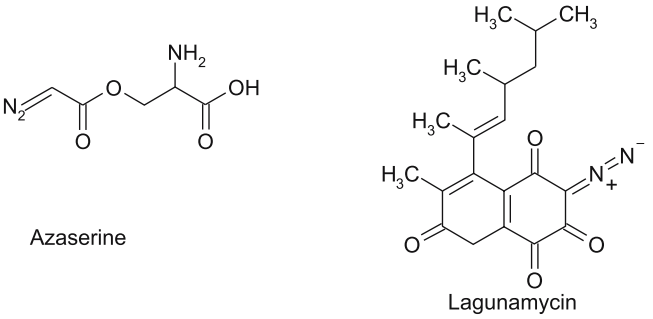


Fig. 14 Structures of azaserine and lagunamycin

guished R_2NCN from R_2CNN or R_2NNC. The structure was reassigned as diazobenzo[*b*]fluorenes since the diazo ^{13}C NMR resonance is in the range of δ 60–80 and was confirmed by total synthesis [41, 42].

3
α-Diazoketones as Natural Products: DNA as a Target?

A few other antitumor natural products containing the diazoketone moiety have been isolated earlier. Azaserine [50], 6-diazo-5-oxo-L-norleucine and lagunamycin [51] (Fig. 15) are known antibiotics isolated from *Streptomyces* and share the common structural feature of α-diazoketones. Recently, lomaiviticin antibiotics (Fig. 16) have been isolated and add to the growing, yet still small number of diazo-containing natural products [52]. While lagunamycin showed inhibitory activity against 5-lipoxygenases [51] and antibacterial activity against Gram-positive bacteria, azaserine showed effective inhibitory activity against sarcoma in mice [53, 54].

Their mode of interaction may involve DNA cleavage, and this proposal is supported by the fact that α-diazoketones have been shown to mediate efficient DNA cleavage under photolytic conditions [55]. The work involved designed α-diazoketones containing an ene-yne structure to generate the radicals responsible for DNA cleavage [55]. The key step (Fig. 16) involves the Wolff rearrangement [55] of α-diazoketone **14** to generate the ene-yne-ketene intermediate **15**, which may mimic the ene-yne-cumulene **12**, an intermediate responsible for the generation of sp^2 diradical from neocarzinostatin chromophore via Myers cyclization (i.e, **12** to **13**) [56, 57].

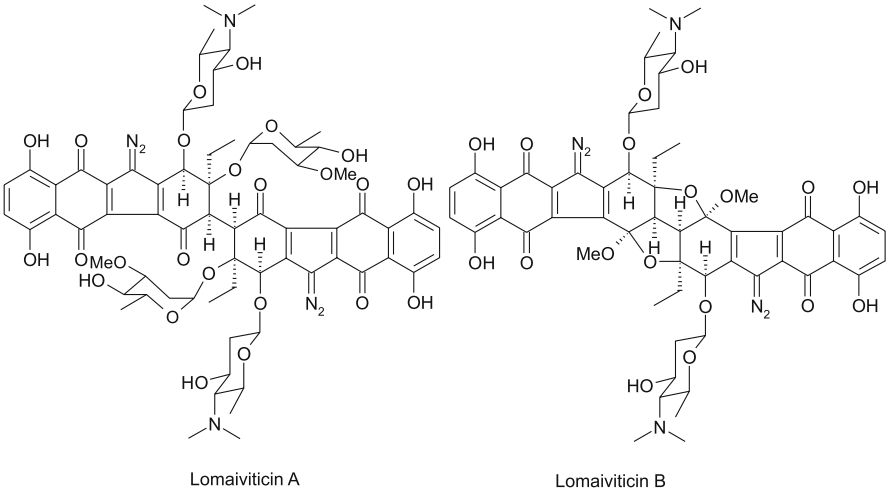


Fig. 15 Structure of lomaiviticin antibiotics

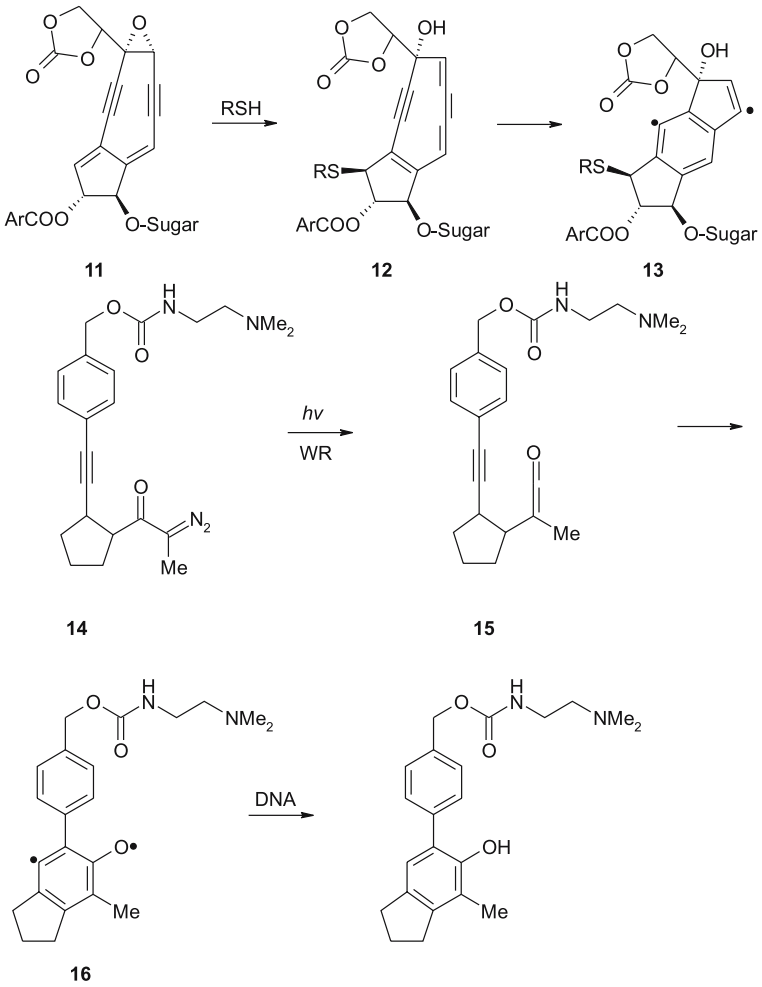


Fig. 16 α-Diazoketones for the generation of neocarzinostatin radical chromophore under photolytic conditions

4 Kinamycin and Lomaiviticin Antibiotics: Importance of Diazo Group

The presence of 9-diazofluorene groups in kinamycin antitumor natural products would lead one to think of an active role for the diazo group. The hypothesis may be substantiated by the fact that one of the precursors in kinamycin biosynthesis, kinafluorenone **10** [44], which lacks the diazo moiety, shows no antibiotic activity against *B. subtilis* ATCC 6633, known to be very sensitive to the kinamycins. However, prekinamycin (**9**) [49], which is similar to kinafluorenone but retains the diazo group, shows activity to-

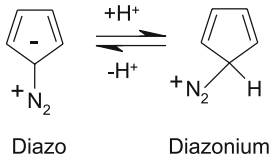


Fig. 17 Diazo–diazonium equilibrium

wards Gram-positive bacteria. The antitumor activity of **9** and **10** remains to be determined. The isolation of kinamycins and lomaiviticins [41, 58] as stable diazo-containing antitumor natural products (e.g., kinamycin C and prekinamycin) is also of interest because diazo groups can be considered as the deprotonated forms of the diazonium compounds (Fig. 17). Biosynthetic studies have revealed polyketide precursors in the kinamycin biosynthetic pathway and several intermediates, prekinamycin and kinafluorenone among others, have been isolated from *Streptomyces murayamaensis* [59].

4.1 Diazonium and Diazo Reagents for DNA Cleavage

Diazonium salts have been utilized [58, 60–62] for effective DNA cleavage. Diazonium salts, however, have limited drug potential due to problems of instability and the need for a metal to carry out the reductive process necessary for radical generation [7, 63, 64]. The protonation of kinamycins, perhaps under physiologically relevant pHs, may generate unstable diazonium ions [58, 60–62] or diazonium-like species [65]. These intermediates, after a spontaneous loss of nitrogen (N_2), could generate carbocations capable of cleaving DNA via alkylation or could be reductively activated to yield radical species capable of inflicting DNA damage.

Attempts to utilize an in situ diazotization procedure (1.2 equiv. isoamyl nitrite, acetic acid, 25 °C, 30 min) [58, 60–62] for DNA cleavage were made via generation of diazonium compound **17** directly from commercially available 9-aminofluorene (Fig. 18) [58]. However, addition of this solution to aqueous buffers (pH = 4–7) did not produce any DNA cleavage; neither did the addition of cuprous chloride, which had been demonstrated to be successful in activating diazonium compounds for DNA cleavage [60–62]. This may be explained by:

a Instability of 9-diazoniumfluorene, which if generated would tend to behave as an aliphatic diazonium salt [9, 10] and decompose before its addition to DNA
b Conversion to 9-diazofluorene **19**, due to the presence of conjugating phenyl groups, which would render the radical generation via reductive mechanisms extremely difficult; groups capable of conjugation, such as phenyl, are known to favor such transformations [63, 64]

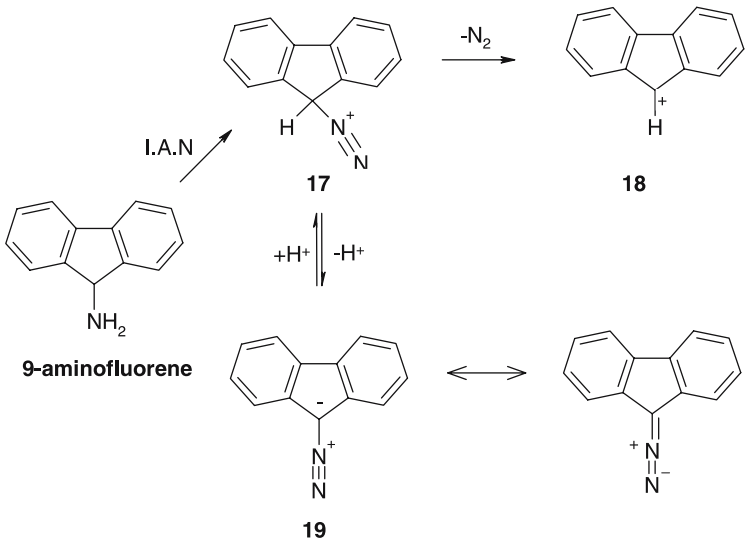


Fig. 18 Activation of 9-aminofluorene for DNA cleavage (*I.A.N.* isoamyl nitrite)

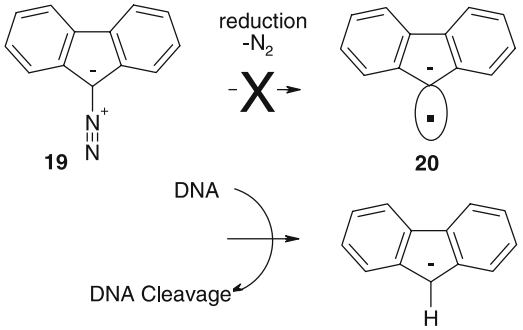


Fig. 19 Reduction of 9-diazofluorene

4.2 Diazo vs. Diazonium

A closer look at Figs. 18 and 19 helps explain the lack of DNA cleavage obtained on diazotization of 9-aminofluorene and its potential in DNA cleavage. The diazonium salt **17** (once formed) may either:

a Lose nitrogen (homolytic dediazoniation) to yield the 9-fluorenyl cation **18**

b Deprotonate to yield the 9-diazofluorene **19**

The cation **18** did not show any DNA cleavage, due probably to its inherent stability (**18** would be more stable than a benzyl cation [66], relatively stable ions that do not alkylate the heterocyclic bases in DNA). The 9-diazofluorene **19** would not undergo reduction like the corresponding diazonium salts (**17**) because of the presence of a negative charge on the fluorenyl carbon.

5
DNA Cleavage with 9-Diazofluorenes

The isolation of diazobenzo[*b*]fluorenes as stable antitumor natural products raises several questions about their mode of action. The inability to cleave DNA by diazotization of 9-aminofluorene may imply that if the diazo functionality is involved in the mode of interaction of kinamycins with DNA, its conversion to diazonium and the ensuing reduction may seem to be of negligible importance. An additional possibility, which will be discussed later, is that 9-diazofluorene may not be the ideal model for these natural products. In exploring DNA cleavage as a possible route to the kinamycins' role as a stable antitumor agent, which may supplement their speculative and as yet unconfirmed role as alkylating molecules [67], this early model seemed to suggest that the well-established activation of diazonium may not be relevant.

5.1
9-Diazofluorenes as the Key Intermediates

For the generation of DNA-cleaving moieties from 9-diazofluorenes two other mechanisms, which may not be relevant under physiological conditions, can be envisioned:

a Reduction [63] (e.g., by metal ions) followed by loss of nitrogen may give radical anions, which may be capable of inducing DNA cleavage via radical mechanisms (Fig. 20)
b Oxidation [68, 69] (e.g., by metal ions) followed by loss of nitrogen may lead to radicals, which should induce DNA cleavage via well-established radical pathways (Fig. 20) [70, 71]

These mechanistic possibilities, which give such an active role for the diazo group, are summarized in Fig. 20 using the model compound 9-diazofluorene **18**.

The synthesis of 9-diazofluorene **19** is easily accomplished from commercially available 9-fluorenonehydrazone (HgO, Et_2O followed by KOH in EtOH; yield = 98%) [72]. Diazo compounds on photolytic decomposition tend to generate carbenes [73, 74]. Fluorenylidene, generated by the photolysis of 9-diazofluorene adds to olefins with negligible amounts of hydrogen abstraction [75, 76]. Copper and its salts, however, have been shown to lead to

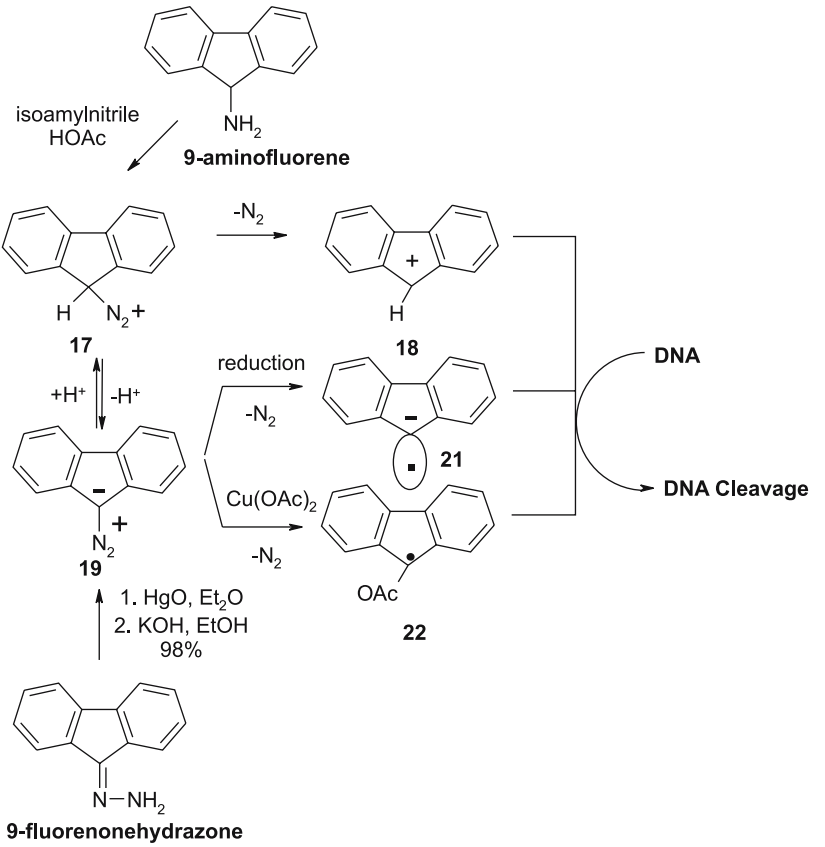


Fig. 20 Pathways for activation of diazonium and diazo compounds

modified carbenes [77]. Reports exist of the catalytic decomposition of diazo compounds utilizing copper carboxylates, leading to modified carbenes or radicals [78]. A copper carbenoid species might be involved in these reactions. The formation of radical **22** from **19** has been proposed from the one electron oxidation of the fluorenylcarbene-acetate adduct by Cu(II) ion (Fig. 21) [78], which was suggested to abstract hydrogens from the DNA backbone leading to strand cleavage.

5.2 Diazo-Mediated Mechanisms of DNA Cleavage

The involvement of carbenes has been excluded in the DNA cleavage reactions activated by cupric acetate as these experiments were conducted in the dark. However, the contribution of metal–carbenoids [79] could not be ruled out. In a series of studies dealing with the metal-catalyzed

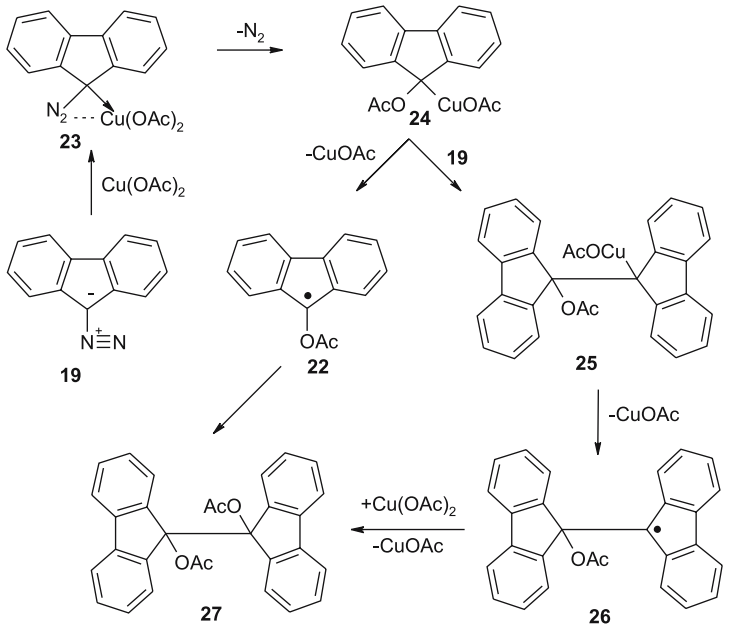


Fig. 21 Proposed mechanisms of formation of diacetates in the presence of copper(II)acetate as proposed by Nozaki [78]

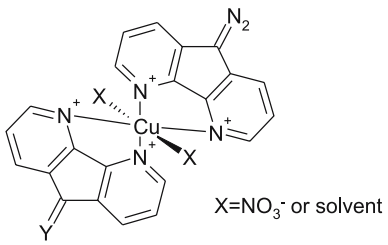


Fig. 22 Structure of bis(9-diazo-4,5-diazafluorene)copper(II)nitrate

decomposition of 9-diazofluorene **19**, Nozaki and coworkers [78] reported that cupric acetate in aqueous DMF provided the highest yield (~ 70%) of the 9-fluorenonepinacoldiacetate, the dimerization product of radical **22** (Fig. 21). It is the potential intermediacy of radical **22** that prompted the use of cupric acetate in aqueous DMF in these experiments and, as already discussed, the approach has been successful in demonstrating DNA cleavage under these conditions [58].

In addition, no DNA cleavage was observed with **19** under silver acetate activated conditions, also consistent with Nozaki's [78] observation that this metal acetate produced little of this dimerization product. The DNA cleavage observed with high concentrations of silver acetate and no cleavage with

thallium or mercuric acetates were cited as further proof of the relevance of Nozaki's observations to these studies [58].

Another interesting use of diazo functionality in DNA cleavage applications was shown by Elington and Zaleski [80]. They showed that bis(9-diazo-4,5-diazafluorene)copper(II)nitrate, shown in Fig. 22, was an effective DNA-photocleaving agent. The complex cleaved DNA under anaerobic conditions using visible light. The ability to employ metal–ligand photoredox chemistry via visible region excitation (similar to diazonium photolysis) may allow such phototriggered compounds to have applications in photomedicine.

6 Diaryldiazomethanes for Mimicking the "ACD" Ring System of the Kinamycins

The role of different ring systems present in kinamycin has also been investigated. The β-naphthylphenyldiazomethane (**29**), readily available from β-naphthylphenylketone [81] **28**, also showed DNA cleavage under conditions identical to 9-diazofluorene (Fig. 23). Additionally, consistent with results with 9-diazoniumfluorene, no DNA cleavage was observed upon treatment of β-naphthylphenyldiazomethane with buffered acid.

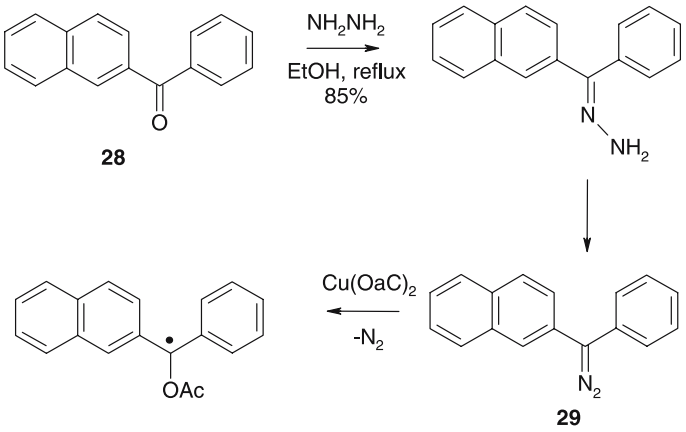


Fig. 23 Diaryldiazomethanes for mimicking the "ACD" ring system of the kinamycins

7 Kinamycin and Lomaiviticin Antibiotics: Do They Cleave DNA?

With these model studies establishing that 9-diazofluorenes can efficiently cleave DNA [58], more effort needs to be focused on other functional groups,

such as the quinone and the acetates, and the effect they may have on the functioning of kinamycin and lomaiviticin antibiotics. Lomaviticins were reported to be DNA damaging agents, although details on the mechanism of damage were not provided [52]. While the structure elucidation of kinamycin antibiotics had rested on them being recognized as carbazoles, a proposal of their mode of interaction with DNA had been made as early as in 1977 [67]. Harold Moore suggested that these indole quinones with potential leaving groups as acetates could interact with nucleophilic sites in DNA. Their function as a bioreductive alkylating agent is outlined in Fig. 24 with kinamycin C (shown as carbazoloquinone cyanamides) as the model drug. The mechanism involves the bioreduction of quinone to hydroquinone **30**, which rearranges to semiquinone **31** via loss of an acetate in ring A [67]. A similar reversion to the quinone leads to the proposed active form **32** of kinamycin C, which may act as a Michael acceptor to the nucleophilic sites in DNA. Nucleophilic (DNA) attack can occur on the Michael acceptor **32** through pathway *a* or *b* and will most likely be dependent on the mode of binding of kinamycins to DNA. This mechanism of activation and reaction with DNA should not be affected by the reassigned structures of these drugs as diazo-containing natural products.

While the successful DNA cleavage with copper(II)acetate establishes the mediacy of a similar activation mechanism with kinamycins, as shown before with 9-diazofluorenes, their mode of action may or may not involve the use of an oxidizing agent under physiological conditions. An important cor-

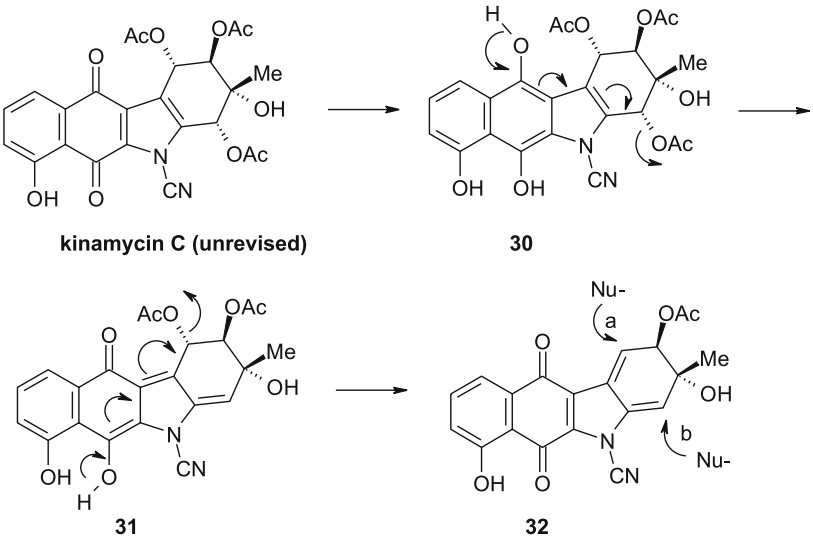


Fig. 24 Reductive alkylation mechanism of interaction of kinamycin C with DNA as originally proposed [67]. Nucleophilic (DNA) attack can occur on the Michael acceptor **32** through pathway a or b. The same mechanism should be applicable with the revised diazo structures

relation has been recently made by Dmitrienko. The degree of diazonium character in the N = N bond of compounds related to kinamycins (Fig. 25) was evaluated [65]. Based on the IR stretching frequencies of the N = N bond, it was suggested that the diazo group in the lomaiviticins has nearly as much

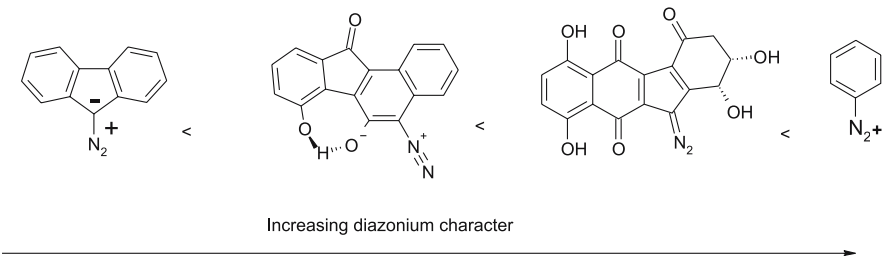


Fig. 25 Model compounds with increasing diazonium character of the N = N bond [65]

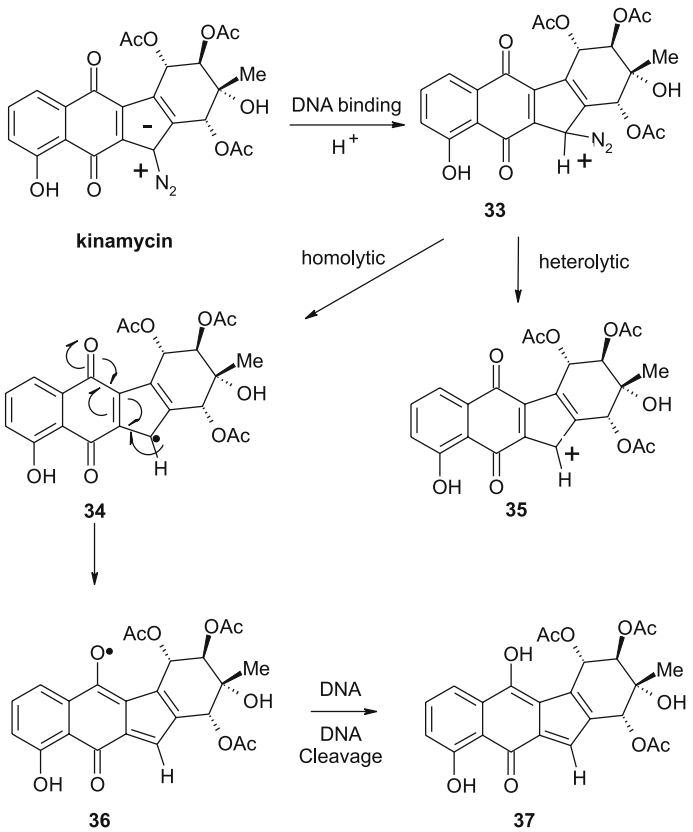


Fig. 26 DNA cleavage with kinamycin C: protonation as a trigger

diazonium character as phenyl diazonium ion (a reasonable finding if one considers the stabilization of the 6π cyclopentadienyl anion). Thus, the DNA damaging activities of kinamycin and lomaiviticins could be compared to aromatic diazonium salts rather than simply diazoalkanes, as previously envisioned [58]. 9-Diazofluorene, while opening up a new lead in diazo-mediated DNA damage, acts more as a diazoalkane.

Additionally, the presence of redox-active quinones in close proximity to the diazo functionality suggests that the natural product may use the quinone-diazo combination as an intramolecular redox switch to promote nitrogen release and activate the drug for DNA cleavage. Additional mechanisms need to be investigated. Protonation, perhaps after DNA binding (as the local pK_a are altered) of the diazo group (Fig. 26), could take us to diazonium **33**, which under conditions similar to the activation of 9-diazoniumfluorene may lose nitrogen to yield **35** (heterolytic cleavage) or could be reductively activated to yield the radical species **34** (homolytic cleavage). While a simple 9-fluorenylradical may be too stable to abstract hydrogens from the DNA backbone, its conjugation with a γ-unsaturated ketone could lead to vinyloxy radical species (**36**) similar to those produced by the Wolff rearrangement of α-diazoketones (Fig. 16). Alternatively, photolytic decomposition of diazofluorene leads to the carbene **38**, which itself may induce DNA cleavage or rearrange to more reactive radical species. However, its rearrangement to **39** leads to a vinylic carbon centered radical similar to that present in neocarzinostatin. More efforts are needed to better understand the mechanism of action of these antibiotics.

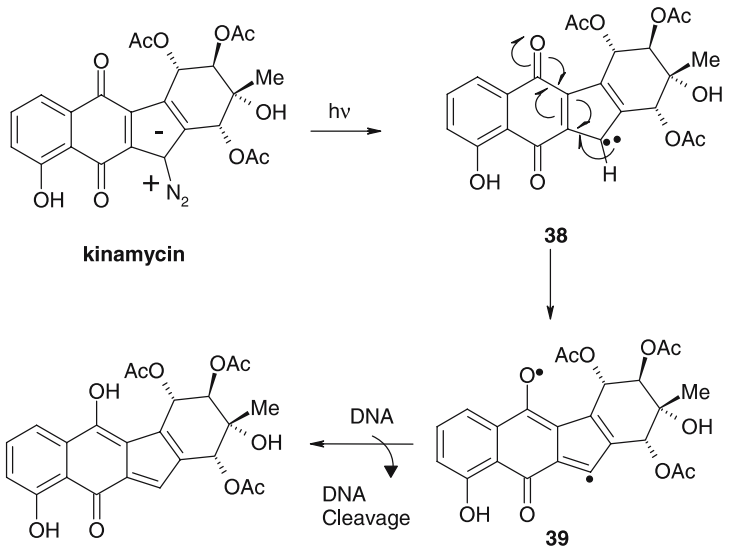


Fig. 27 Proposed photochemical DNA cleavage with kinamycin C

Nevertheless, functional groups, such as diazo and diazonium capable of inducing cleavage in DNA, hold promise in the development of DNA damaging drugs. This, together with the fact that diazobenzo[*b*]fluorene groups are stable entities in the kinamycin and lomaiviticin antibiotics, promises to introduce an exciting new arena for the development of DNA targeted reagents [58, 61].

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Novel Synthetic Antibacterial Agents

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Abstract Classical (fermentation-based) and nonclassical (nonfermentation-based) antibacterials are conventionally used for the treatment of bacterial infections. This chapter

describes the syntheses of different classes of nonfermentation-based antibacterial agents that have been reported during the past decade (1995–2005). The general trends in chemotherapy of infectious diseases and general classes of mechanism-based antibacterials are described in Sect. 1. The syntheses of novel quinolones including gemifloxacin, DQ-113, and garenoxacin, as well as of fused quinolones, is discussed in Sect. 2. In Sect. 3, the syntheses of novel oxazolidinone antibacterials, including eperezolid, linezolid, and PNU-10048, as well as that of fused oxazolidinones is described. The syntheses of antibacterial agents that inhibit bacterial peptide deformylase (PDF) including *N*-alkyl urea hydroxamic acid derivatives, proline-3-alkylsuccinyl hydroxamates, 5-arylidene-2-thioxothiazolidin-4-one-3-hexanoic acid derivatives, macrocyclic peptidomimetic PDF inhibitors, and isoxazole-3-hydroxamic acid derivatives is discussed in Sect. 4. Sect. 5 describes the syntheses of the inhibitors of bacterial fatty acid biosynthesis (LpxC) including oxazoline hydroxamates (such as L-159,692), its isoxazolone analogues, and carbohydrate-derived hydroxamic acid derivatives. The mechanism of action and rationale for the synthesis of each class of antibacterial agents is described in the corresponding section.

Keywords Antibacterials · LpxC inhibitors · Oxazolidinones · PDF inhibitors · Quinolones

Abbreviations

BOC	*t*-Butoxycarbonyl
BPO	Benzoyl peroxide
n-BuLi	*n*-Butyllithium
CbzCl	Benzyloxycarbonyl chloride
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DPPA	Diphenylphosphoryl azide
DIBALH	Diisobutylaluminum hydride
DMS	Dimethyl sulfide
DIEA	Diisopropylethylamine
DMF	Dimethylformamide
EDAC	Ethylenediamine carbonate
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDDA	Ethylenediamine *N*,*N*′-diacetate
FDA	Food and Drug Administration
HATU	2-(1*H*-9-azobenzyltriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HTS	High-throughput screening
LAH	Lithium aluminum hydride
LDA	Lithium diisopropylamide
LHMDS	Lithium hexamethyldisilazide
LPS	Lipopolysaccharide
LpxC	UDP-[3-*O*-(*R*-3-hydroxymyristoyl)]-*N*-acetylglucosamine deacetylase
MMP	Matrix metalloprotease
MRSA	Methicillin-resistant *Staphylococcus aureus*
NaH	Sodium hydride
$NaBH_4$	Sodium borohydride
NBS	*N*-Bromosuccinimide
NCS	*N*-Chlorosuccinimide
NDA	New Drug Application

NaOEt	Sodium ethoxide
PDF	Peptide deformylase
PEG	Polyethylene glycol
PRSP	Penicillin-resistant *Streptococcus pneumoniae*
PyBop	Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
SAR	Structure–activity relationships
TBDMSCl	(*tert*-Butyldimethyl)silyl chloride
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMSA	Trimethylsilyl azide
VRE	Vancomycin-resistant enterococci

1 Introduction

The never-ending battle of humankind against infections caused by microorganisms dates back millennia to before the dawn of recorded history. The crucial efforts of scientists like Robert Koch and Louis Pasteur resulted in the identification of bacteria as the cause of infections. This was followed by the herculean and global efforts of microbiologists, biochemists, and chemists which paved the road in identifying natural as well as synthetic antibacterial agents. Naturally occurring antibacterials, including those derived from plants, marine organisms, and microorganisms, have been the subject of comprehensive studies for decades starting in the early 1920s, while the first report of the synthesis of sulfonamides, the first reported synthetic antibacterials, dates back to the mid-1930s. Since then, thousands of natural, semisynthetic, and synthetic antibacterials have been introduced as chemotherapeutic agents, hundreds of which have been used clinically. The overuse of these chemotherapeutic agents in the past 50 years has resulted in the emergence of bacterial mutants resistant to these agents and, as a result, inefficiency in therapeutic application.

Different approaches have been taken to overcome the problem of bacterial resistance and to resolve the chemotherapeutic inefficiency. The first approach focuses on agents that combat the bacterial resistance mechanisms to revive the antibacterial potency of the parent compound. These include inhibitors of β-lactamases, efflux–pump inhibitors, etc. The second approach focuses on developing antibacterial agents with novel structures and mechanisms of action different from those of the currently utilized compounds [1, 2]. Although remarkable progress has been reported in the first approach and several effective compounds with bacteria-resistant inhibitory activities have been introduced during the past three decades, interest in discovering new antibacterial agents has been declining and only a few new antibacterial agents with novel structures and mechanisms of action have been introduced as potential chemotherapeutic agents during this period. Based on the

mechanism of action, the antibacterial agents are traditionally classified as: those that interfere with bacterial cell wall/membrane formation and function including β-lactam antibiotics; those that interfere with bacterial nucleic acid synthesis and replication, including rifamicin and quinolones; those that interfere with protein biosynthesis including natural products such as aminoglycosides, macrolides, tetracyclines, and synthetic products like oxazolidinones; and antimetabolites such as sulfonamides [1, 2]. Recently, inhibitors of other bacterial targets, such as peptide deformylase (PDF) [3, 4] and the enzyme required for bacterial fatty acid biosynthesis (LpxC) [5, 6], have been explored as potential antibacterial agents.

Except for the quinolones and oxazolidinones, which are synthetic products, the traditionally utilized antibacterials (antibiotics) either are isolated as fermentation products or are semisynthetic modifications of the original antibiotics. The inhibitors of PDF and LpxC were discovered via high-throughput screening as lead compounds, followed by further structural modifications using a structure-based design approach. Since the mandate of this chapter is to review the chemistry of synthetic heterocyclic antibacterials under investigation during the period 1995–2005, the syntheses of novel quinolones and oxazolidinones, from traditional antibacterials, and novel inhibitors of PDF, and bacterial fatty acid synthesis (LpxC) will be discussed in detail.

2
Novel Quinolone Antibacterials

This class of compounds comprises a series of synthetic agents patterned after nalidixic acid, a naphthyridine derivative introduced in 1963 for the treatment of urinary tract infections. Isosteric heterocyclic groupings in this category include the quinolones (e.g., norfloxacin, ciprofloxacin, lomefloxacin, gatifloxacin, sparfloxacin, moxifloxacin, and ofloxacin), the naphthyridones (e.g., nalidixic acid, enoxacin, and trovafloxacin), and the cinnolones (e.g., cinnoxacin) [2] (Fig. 1).

The bactericidal action of these compounds is known to be due to the inhibition of DNA synthesis as a result of inhibition of bacterial topoisomerase II (DNA gyrase) and topoisomerase IV [1, 7]. Different quinolones inhibit these essential enzymes to different extents. Topoisomerase IV seems to be of greater importance in Gram-positive bacteria, while DNA gyrase is of greater importance in Gram-negative bacteria. This difference explains the activity of early quinolones toward Gram-negative bacteria and the broad-spectrum activity of new quinolones, as well as the structural requirements for each activity profile. Bacterial resistance due to genetic mutations in these enzymes has been reported for the past decade [8].

Based on structure–activity studies, the 1,4-dihydro-4-oxoquinoline-3-carboxylic acid moiety is essential for antibacterial activity, due to its

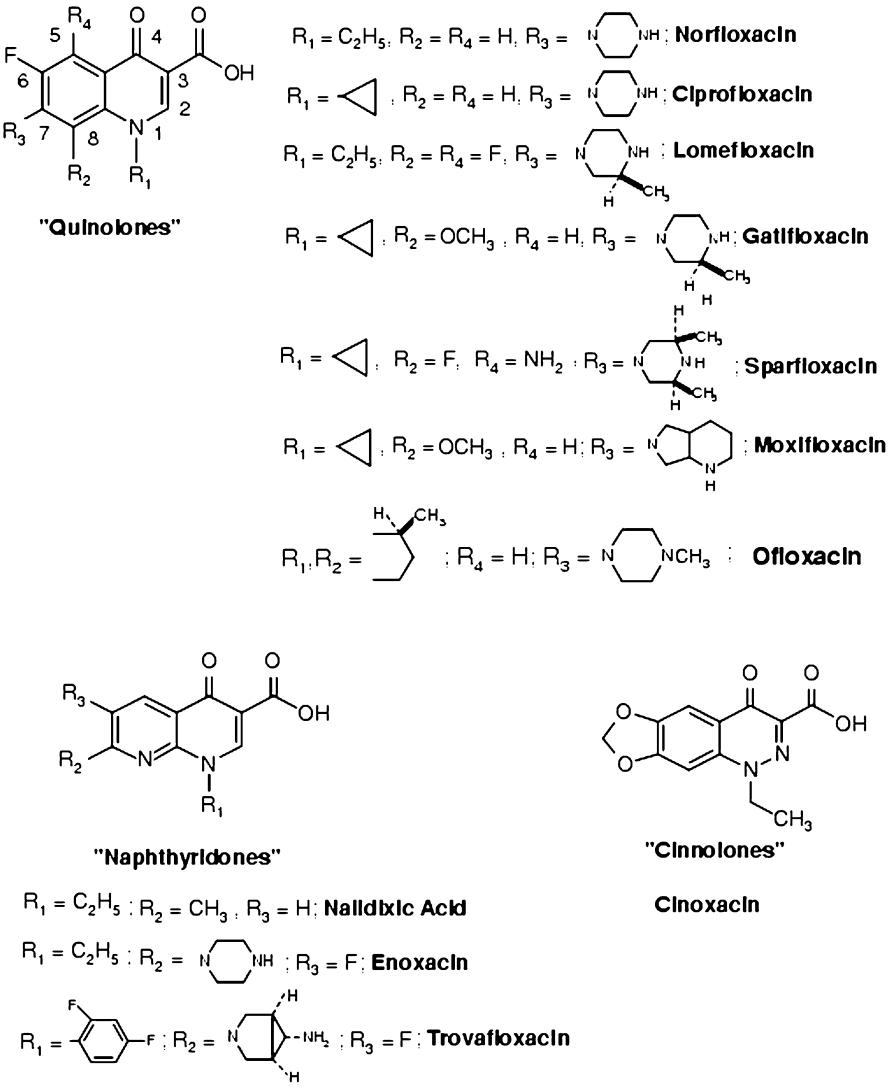


Fig. 1 Structural categorization of quinolones

strong chelating characteristics, and plays a crucial role in the interaction of quinolones with the active sites of DNA gyrase and topoisomerase IV. The pyridine system must be annulated with an aromatic ring [2]. Isosteric replacements of nitrogen for carbon atoms at positions 2 (cinnolones) and 8 (naphthyridones) are consistent with retention of antibacterial activity.

In general, substitution at position 2 results in loss of antibacterial activity, while substitutions on positions 5, 6, 7, and 8 of the annulated ring improve the activity. It is suggested that the presence of fluorine at the C-6

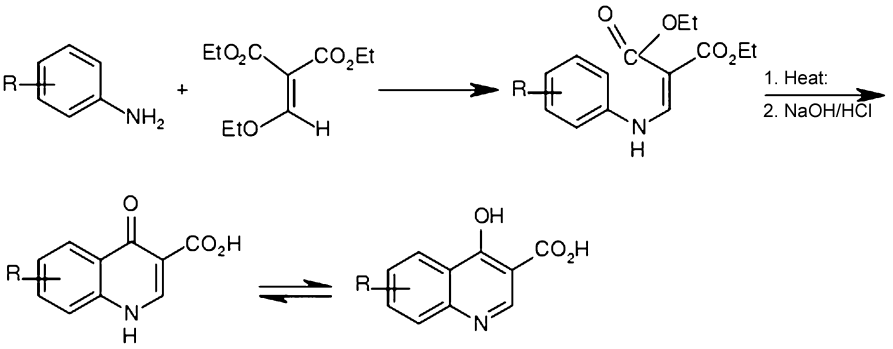
position and a cyclic amine at C-7 of the annulated ring have a major effect on the antibacterial activity of these compounds. An alkyl or aryl substituent at the N-1 position of the pyridine system is essential for antibacterial activity. Ring condensations at the 1,8, 5,6, 6,7, and 7,8 positions also lead to active compounds.

Since the introduction of nalidixic acid in 1963, structural modifications on the quinolones have been performed to improve either the antibacterial efficacy or pharmacokinetic/toxicologic profiles of these compounds. The newest quinolones possess broad-spectrum activity, favorable pharmacokinetic/toxicologic profiles, and potency against bacterial strains that are resistant to older generations of quinolones. This section describes the synthetic procedures for the new generation of quinolones that were studied during the 1995–2005 period.

2.1 General Synthetic Procedure

Although there is versatility in the synthetic methodologies of each individual quinolone antibacterial, two different methods are utilized to synthesize the basic skeleton of 1,4-dihydro-4-oxoquinoline-3-carboxylic acid. The first method is based on the Gould–Jacobs reaction [9] using appropriately substituted aniline derivatives and diethyl ethoxymalonate, which results in the formation of the intermediate anilinomethylenemalonate. Further thermal cyclization of this intermediate followed by hydrolysis gives rise to the targeted 1,4-dihydro-4-oxoquinoline-3-carboxylic acid, according to Scheme 1.

Although the thermal cyclization step in the Gould–Jacobs method proceeds with good yield for simple quinolones, the yields in multisubstituted analogues are low and unsatisfactory. In order to bypass the thermal cyclization step, a second method was introduced in the late 1980s and early 1990s via *o*-halobenzoic acid derivatives. This method, which is now the most pop-

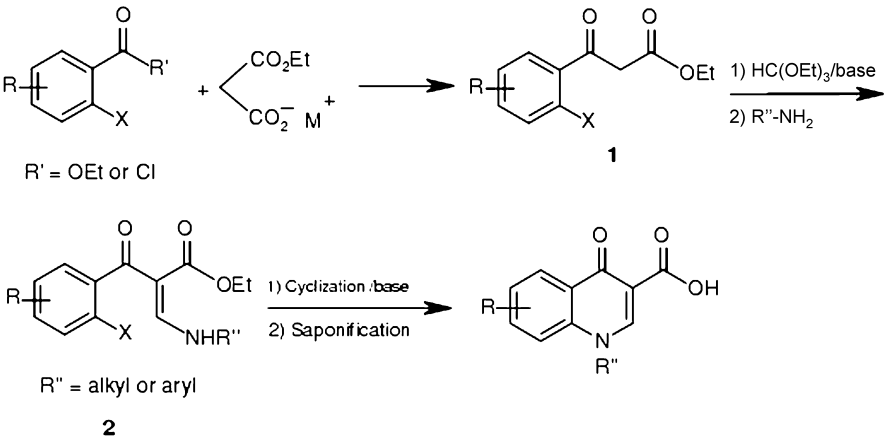


Scheme 1 Gould–Jacobs quinolone synthesis

ular, consists of the formation of an *o*-halobenzoylacetic acid ester (**1**) via the reaction of either a substituted *o*-halobenzoic ester or an *o*-halobenzoyl chloride with an alkali salt of a monomalonate ester followed by decarboxylation. The resulting *o*-halobenzoylacetic acid ester is allowed to react with ethyl orthoformate followed by an aryl- or alkyl-substituted amine to afford the relevant alkyl/aryl aminovinylyl intermediate (**2**). Nucleophilic cyclization of this intermediate under basic conditions followed by saponification affords the targeted quinolone carboxylic acid derivative, as depicted in Scheme 2.

The second method is advantageous over the Gould–Jacobs method with respect to (a) the cyclization step which proceeds smoothly under mild conditions; (b) one-pot synthesis of the 1-substituted derivatives and bypassing the alkylation/arylation of the N_1-position of 1,4-dihydro-4-oxoquinoline-3-carboxylate, which usually gives rise to mixed N_1/O_4-substituted products; and (c) product versatility due to the ability to utilize a wide range of alkyl- and arylamines in the step before cyclization.

Syntheses of naphthyridone derivatives follow the same procedures as those of quinolones, except that substituted 2-aminopyridines (Gould–Jacobs modification) or substituted nicotinic ester/nicotinoyl chloride are used instead of anilines or *o*-halobenzoic acid derivatives. Most of the recently introduced quinolone antibacterials possess bicyclic or chiral amino moieties at the C-7 position, which result in the formation of enantiomeric mixtures. In general, one of the enantiomers is the active isomer, therefore the stereospecific synthesis and enantiomeric purity of these amino moieties before proceeding to the final step of nucleophilic substitution at the C-7 position of quinolone is of prime importance. The enantiomeric purity of other quinolones such as ofloxacin (a racemic mixture) plays a major role in the improvement of the antibacterial efficacy and pharmacokinetics of these enan-



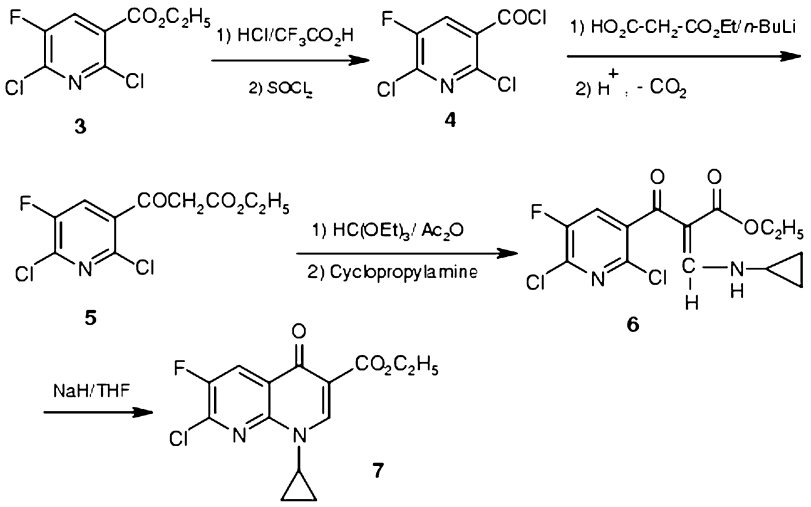
Scheme 2 Alternative quinolone synthesis

tiomers. In fact levofloxacin, the (–) isomer of ofloxacin, exhibits a superior antibacterial efficacy and pharmacokinetic profile compared to those of the racemic parent compound, ofloxacin. In conclusion, the Gould–Jacobs and *o*-halobenzoic acid methods are still the most widely utilized procedures for the syntheses of the 1,4-dihydro-4-oxoquinoline-3-carboxylic acid scaffolds, followed by additional nucleophilic substitution reactions at positions 5, 7, and 8 to provide products with favorable biological activity profiles.

2.1.1 Synthesis of Gemifloxacin (Factive, LB20304a)

The synthesis of gemifloxacin, 7-(4-(aminomethyl)-3-(methoxyimino)pyrrolidin-1-yl)-1-cyclopropyl- 6 -fluoro- 4 -oxo-1, 4-dihydro[1, 8]naphthyridine-3-carboxylic acid, was first reported by a research group from Biotech Research Institute, Korea, in 1997 [10, 11]. This compound was licensed to SmithKline Beecham (SKB). After successful completion of Phase II and III clinical trials, SKB filed a New Drug Application (NDA) with the Food and Drug Administration (FDA) in 1999. This drug entered the global market in 2002. Gemifloxacin and its analogues were initially designed based on the structure of tosufloxacin (a trovafloxacin analogue in which the bicyclic amino group at the C-7 position of the naphthyridine ring was replaced by a 3-aminopyrrolidinyl group) to improve the antibacterial activity and toxicity profile. The initial idea behind this study was to explore the effect of replacing the 3-amino group of the pyrrolidinyl with an oximino group, based on the hypothesis that (a) this functional group can be readily obtained from the corresponding ketone; (b) it is a common and quite stable functional group employed in current drugs; (c) the lone pair on the nitrogen of oxime can participate in hydrogen bonding with the drug target (in this case DNA gyrase); and (d) by changing the R group on the oxygen of the oximino group the lipophilicity of these compounds could be tuned in order to provide the highest potency and most favorable physicochemical properties.

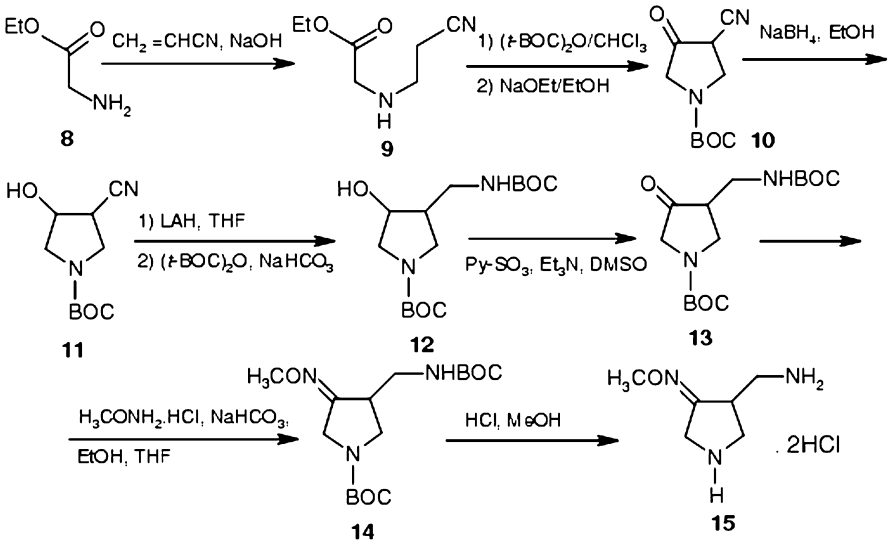
The synthesis of the corresponding naphthyridone scaffold was carried out according to the methods reported by Chu et al. [12] and Sanchez et al. [13]. Namely, the hydrolysis of ethyl 2,6-dichloro-5-fluoronicotinate (**3**) [14] followed by reaction with thionyl chloride results in the formation of 2,6-dichloro-5-fluoronicotinyl chloride (**4**). Treatment of this compound with monoethyl malonate in THF under *n*-butyllithium followed by acidification and decarboxylation gives rise to ethyl 2,6-dichloro-5-fluoronicotinylacetate (**5**). Reaction of compound **5** with ethyl orthoformate in acetic acid followed by cyclopropylamine results in the formation of 3-cyclopropylamino-2-(2,6-dichloro-5-fluoronicotinyl)acrylate (**6**), the cyclization reaction of which under NaH/THF gives rise to the required ethyl 1-cyclopropyl-6-fluoro-7-chloro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylate (**7**), as shown in Scheme 3.



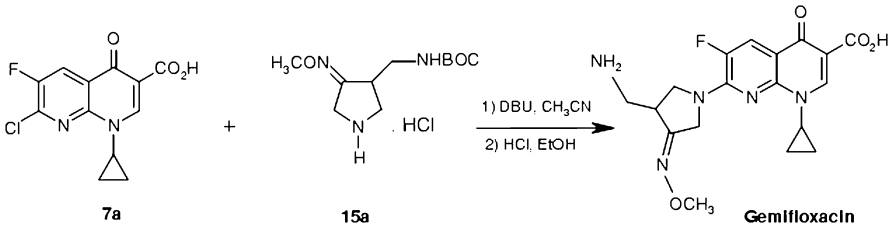
Scheme 3 Synthesis of naphthyridone ring of gemifloxacin

The relevant cyclic amino moiety for coupling with compound **7** is prepared according to the method published by Hong et al. [10]. Namely, ethylglycine hydrochloride (**8**) is allowed to react with acrylonitrile in aqueous NaOH, and the resulting Michael adduct (**9**) is subsequently treated with di-*t*-butyl dicarbonate to yield a BOC-protected cyano ester intermediate. Further cyclization of this intermediate under NaOEt/EtOH gives rise to the BOC-protected cyclic cyanopyrrolidine-3-one (**10**). Reduction of the keto group of **10** with $NaBH_4$ results in the formation of the cyano alcohol (**11**), followed by subsequent reduction of the cyano group to aminomethyl using lithium aluminum hydride (LAH) and protection of the amino group via reaction with *tert*-butyl dicarbonate to afford the di-BOC-protected intermediate (**12**). Parikh–Doering [15] oxidation of **12** produces the ketone (**13**) in good yield. This ketone is then converted to the oxime (**14**) by reaction with *O*-methylhydroxylamine. The geometric configuration of this oxime was assigned as *Z* based on different experimental data. The bis-BOC protective groups of the oxime **14** are easily removed by treatment with hydrochloric acid in MeOH in quantitative yield to afford the required pyrrolidine salt, 4-(aminomethyl)pyrrolidin-3-one-*O*-methyloxime (**15**), as described in Scheme 4.

The final coupling reaction of 1-cyclopropyl-6-fluoro-7-chloro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid (**7a**) and 4-(*tert*-butoxycarbonylaminomethyl)pyrrolidin-3-one-*O*-methyloxime (**15a**) proceeds according to the methods described by Sanchez et al. [13], Domogala et al. [16], and Kimura et al. [17], followed by acid hydrolysis to afford gemifloxacin, 7-(4-(aminomethyl)-3-(methoxyimino)pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro[1,8]naphthyridine-3-carboxylic acid and other corresponding derivatives, according to Scheme 5.



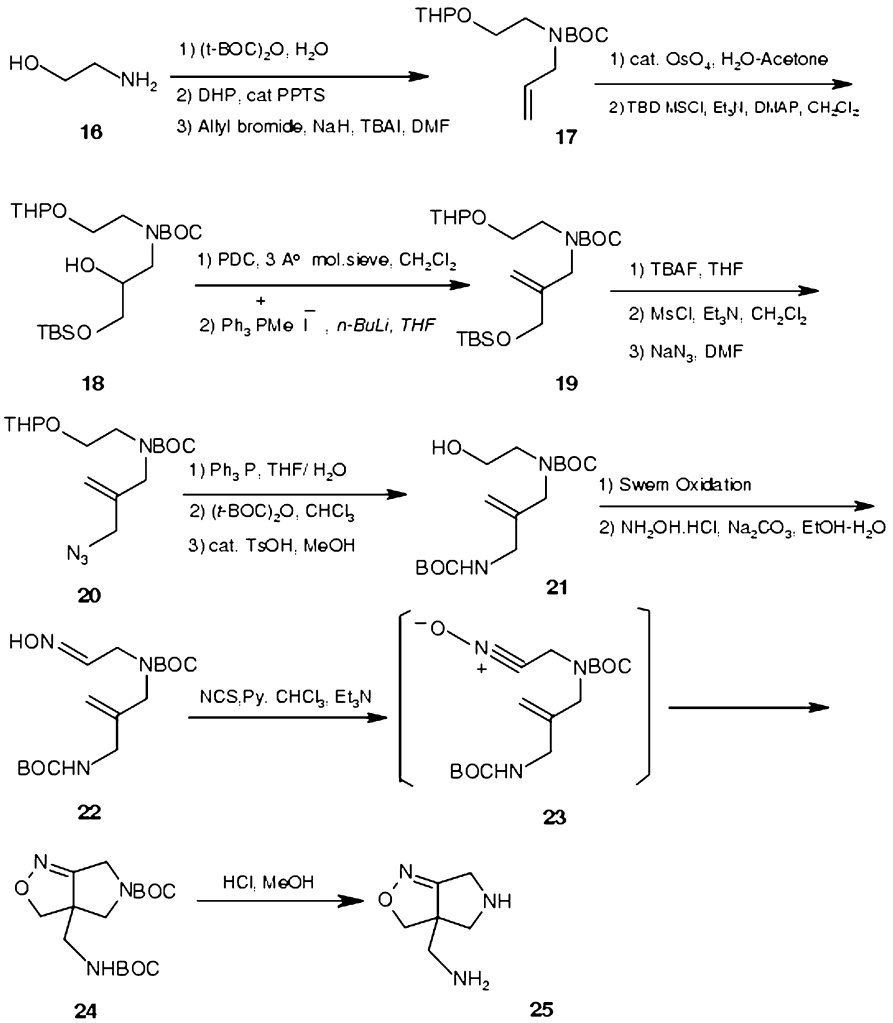
Scheme 4 Synthesis of 4-(aminomethyl)pyrrolidin-3-one-*O*-methyloxime dihydrochloride



Scheme 5 Synthesis of gemifloxacin

The exclusive *Z* configuration of the methyloximino group in the pyrrolidine moiety at the C-7 position of gemifloxacin and its analogues, and its critical contribution to the improvement of biological activity, pharmacokinetics, and toxicological profile of this compound, led the investigators to explore the stereochemical relationship of the alkyloximino group to the biological efficacy of gemifloxacin by the ring-forming modification of 3-(methoxylimino)-4-(aminomethyl)pyrrolidine, which resembles the *E*-alkyloxyimino isomer. On this basis, the 4-aminomethyl-3-oxa-2,7-diazabicyclo[3.3.0]oct-1-ene (**25**) was designed and synthesized as a mimic of the *E*-alkyloximino isomer of the C-7 amine in gemifloxacin [18], and its coupling reactions with various quinolone scaffolds was attempted. The synthetic methodology for the preparation of compound **25** is depicted in Scheme 6.

The emergence of multidrug-resistant Gram-positive bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant enterococci



Scheme 6 Synthesis of 4-aminomethyl-3-oxa-2,7-diazabicyclo[3.3.0]oct-1-ene

(VRE), was the impetus for a global effort to discover new and effective antibacterial agents to fight these infections. In the field of quinolones, compounds such as trovafloxacin [19], moxifloxacin [20], gemifloxacin [10], and gatifloxacin [21] have been introduced as strong antibacterial agents effective against Gram-positive bacteria. However, none has shown acceptable potency against resistant pathogens. In order to overcome the resistance problem, several research groups targeted the substitutions at the C-7 and N-1 positions of the quinolones. For example, Kimura et al. [22] reported that several quinolone derivatives bearing 3-(1-amino-1-substituted methyl)pyrrolidin-1-yl groups, including the 3-(1-aminocyclopropan-1-yl)pyrrolidin-1-yl sub-

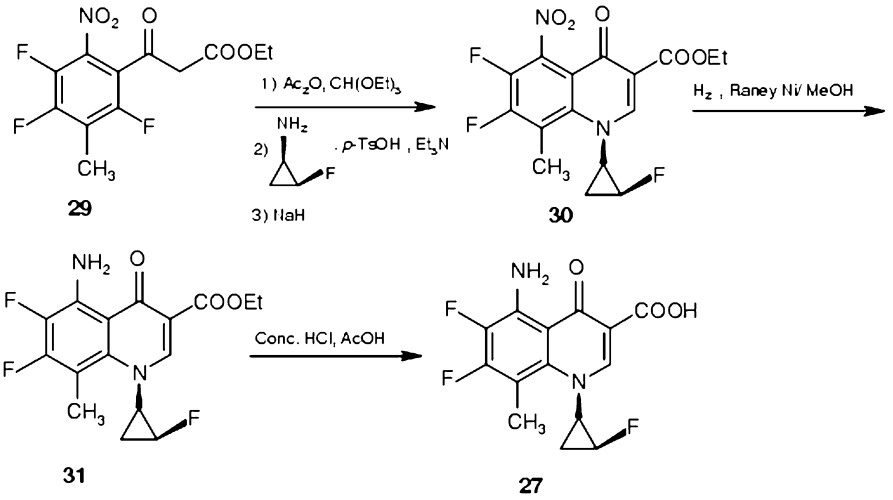
stituent, at the C-7 position demonstrated potent antibacterial activity against Gram-positive bacteria. Although 7-[{(3*R*)-3-(1-aminocyclopropan-1-yl)pyrrolidin-1-yl}]quinolone derivatives exhibited the highest antibacterial potency among them, they demonstrated higher genotoxicity than 7-(piperazin-1-yl)- or 7-(3-aminomethylpyrrolidin-1-yl)quinolone derivatives. Later, the same research team introduced a (1*R*,2*S*)-2-fluorocyclopropan-1-yl substituent at the N-1 position, instead of a cyclopropyl substituent, as an alternative method to reduce the genotoxicity [23, 24]. Additionally, 5-amino-8-methylquinolone derivatives were reported to demonstrate potent antibacterial activity against Gram-positive pathogens and exhibited reduced chromosomal toxicity in comparison with 8-methoxyquinolone derivatives [25, 26]. Based on this information, Inagaki et al. [27] designed and synthesized a 5-amino-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-8-methylquinolone possessing the (3*R*)-3-(1-aminocyclopropan-1-yl)pyrrolidin-1-yl substituent at the C-7 position, which exhibited highly potent activity against Gram-positive bacteria with reduced genotoxicity. Further structural modification of this compound resulted in the discovery of DQ-113, which is currently under preclinical evaluation by Daiichi Pharmaceutical, Japan.

2.1.2 Synthesis of DQ-113

DQ-113, 5-amino-7-[(3*S*,4*R*)-4-(1-aminocycloprop-1-yl)-3-fluoropyrrolidin-1-yl]-6-fluoro-1-[(1*R*,2*S*)-2-fluorocycloprop-1-yl]1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid (**26**), and its analogues were initially synthesized in three stages. In the first stage, the quinolone nucleus, 5-amino-6,7-difluoro-1-[(1*R*,2*S*)-2-fluorocyclopropan-1-yl]-1,4-dihydro-8-methyl-4-oxoquinoline-3-carboxylic acid (**27**), was prepared as a scaffold. The second stage comprised the synthesis of (3*S*,4*R*)-4-(1-aminocycloprop-1-yl)-3-fluoropyrrolidine (**28**), followed by the coupling reaction of **27** and **45** (the *N*-bocylated form of **28**) and deprotection to yield DQ-113, as the third and final stage.

The synthesis of compound **27** was initiated with the treatment of ketoester **29**, reported by Yoshida et al. [25], with ethyl orthoformate in acetic acid, followed by reaction with (1*R*,2*S*)-2-fluoro-1-cyclopropylamine *p*-toluenesulfonic acid salt in the presence of triethylamine to yield an enaminoketoester intermediate, cyclization of which under NaH in dioxane yields the 5-nitroquinolone derivative (**30**). Reduction of the nitro group of compound **30** followed by acid hydrolysis provides compound **27** via the aminoquinolone derivative (**31**), according to Scheme 7.

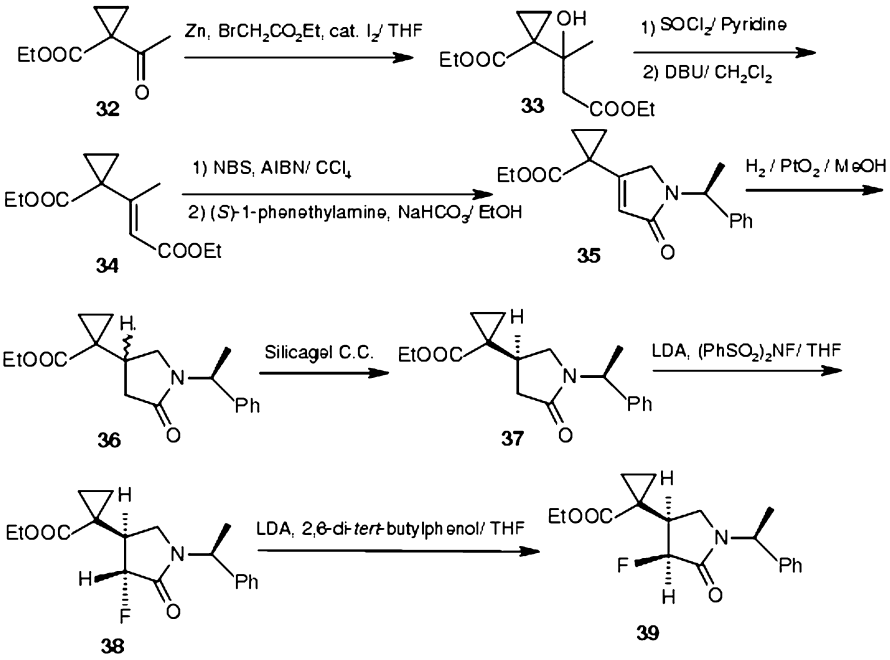
The synthesis of (3*S*,4*R*)-4-(1-aminocycloprop-1-yl)-3-fluoropyrrolidine (**28**) is illustrated in Schemes 9 and 10. Namely, Reformatsky reaction of 1-acetylcyclopropanecarboxylate (**32**) [28] with ethyl bromoacetate yields the hydroxyester intermediate (**33**). Chlorination of this intermediate with



Scheme 7 Synthesis of the quinolone scaffold of DQ-113

thionyl chloride/pyridine, and the subsequent elimination reaction of the chlorinated product with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), results in the formation of the α,β-unsaturated ester (**34**) as the *E*-isomer. Bromination of **34** with *N*-bromosuccinimide (NBS) and treatment of the resultant bromoester with (*S*)-1-phenylethylamine results in the cyclization and formation of pyrroline (**35**). Reduction of **35** catalyzed by platinum oxide gives a mixture of isomers (**36**; 3*S* : 3*R* = 3.5 : 1) which were separated by silica gel column chromatography. Incorporation of a fluorine atom into the pyrrolidinone ring of the major isomer (**37**, 3*S*) is achieved by the treatment of this compound with lithium diisopropylamide (LDA) followed by *N*-fluorobenzenesulfonimide to obtain the *trans*-fluorinated oxopyrrolidinyl ester (**38**). The *cis*-fluorinated compound (**39**) is obtained by treatment of **38** with LDA and subsequent quenching with 2,6-di-*tert*-butylphenol, as shown in Scheme 8.

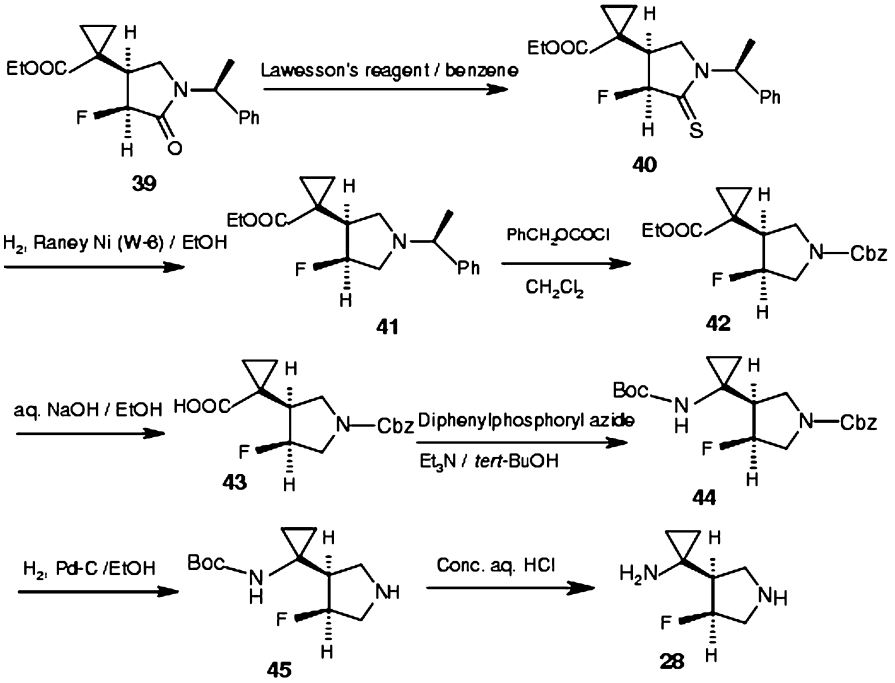
The oxopyrrolidinyl compound (**39**) is treated with Lawesson's reagent to afford the thioxopyrrolidinyl ester (**40**), the reduction of which by Raney nickel gives rise to the pyrrolidinyl ester (**41**). The 1-phenylethyl group of **41** is transformed into the benzyloxycarbonyl group using benzyl chloroformate according to the von Braun conditions to yield the benzyloxycarbonyl compound (**42**). Basic hydrolysis of **42** affords the carboxylic acid derivative (**43**), which upon Curtius rearrangement using diphenylphosphoryl azide (DPPA) and *tert*-butyl alcohol provides compound **44**. Reduction of compound **44** under palladium/carbon gives rise to the pyrrolidine intermediate (**45**), the deprotection reaction of which with concentrated aqueous HCl affords (3*S*,4*R*)-4-(1-aminocycloprop-1-yl)-3-fluoropyrrolidine (**28**), as shown in Scheme 9.



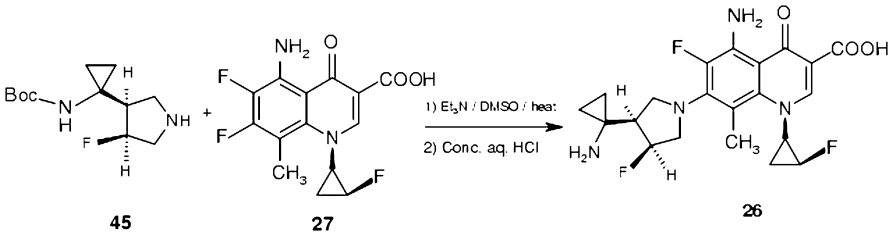
Scheme 8 Synthesis of pyrrolidinone intermediate **39**

In the final stage, as depicted in Scheme 10, the BOC-protected compound **45** and the quinolone carboxylic acid **27** are heated in DMSO under triethylamine, followed by deprotection of the *tert*-butoxycarbonyl group under acidic condition to afford the final product DQ-113 (**26**).

Considering the chronology of the structural modifications on the fluoroquinolones, the critical role of the substitutions at N-1, C-6, and C-7 in the antibacterial potency and spectrum of these compounds becomes evident. In general, the substitution of a cyclopropyl group at the N-1 position of conventional quinolones has proved to be of prime importance. In this regard, compounds such as ciprofloxacin [29] and gatifloxacin [30] are the best representatives of the relationship between N-1 substitution and antibacterial strength in the quinolone series. On the other hand, the critical contribution of the C-7 amino substitution to the antibacterial efficacy of quinolones has been demonstrated in several clinically useful compounds possessing five- or six-membered chiral or achiral cyclic amines at this position, as described in the previous examples. Additionally, compounds such as WIN-57273 [31], the 7-(2,6-dimethyl-4-pyridyl) analogue of ciprofloxacin, which displays about 30-fold more activity than ciprofloxacin against Gram-positive bacteria, and its bicyclic 8-fluoro-7-(isoindoly-1-yl) analogue (Wakanagu/Banyu) [32], with promising efficacy against Gram-positive bacteria, represent examples of quinolones with C-7 cyclic amino



Scheme 9 Synthesis of (3*S*,4*R*)-4-(1-aminocycloprop-1-yl)-3-fluoropyrrolidine



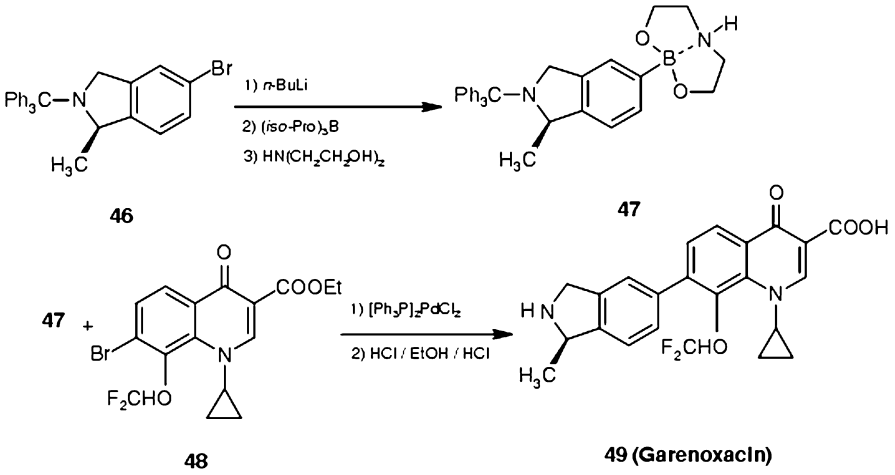
Scheme 10 Synthesis of DQ-113

substituents which are different from those substituents found in traditionally utilized quinolones. Although for decades the existence of fluorine at the C-6 position of quinolones was considered critical for strong antibacterial activity, it was also speculated to be the source of unwanted adverse reactions. Based on these assumptions, the scientists at Toyama Chemical Co. Ltd. designed a new quinolone derivative with no fluorine at the C-6 position, which possessed a (2-methylisoindolin-5-yl) moiety at the C-7 position and a difluoromethoxy group at the C-8 position. This compound, 1-cyclopropyl-8-(difluoromethoxy)-7-[(1*R*)-1-methyl-2,3-dihydro-1*H*-isoindol-5-yl]-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid [33], which possesses strong and

wide-spectrum antibacterial activity, is in the late stages of clinic trials under the name of Garenoxacin.

2.1.3 Synthesis of Garenoxacin

Garenoxacin (**49**) was discovered as a result of extensive and elaborate synthetic and biological assessment studies to obtain new quinolones with strong antibacterial activity and low toxicity [33]. The bicyclic amino derivative used as one of the building blocks in the synthesis of Garenoxacin is (*R*)-7-[1-methyl-2-(triphenylmethyl)isoindolin-5-yl]boronic acid (**47**), which was prepared from (+)-(*R*)-5-bromo-1-methyl-2-(triphenylmethyl)isoindole (**46**) [34, 35] via treatment of this compound with *n*-butyllithium followed by triisopropyl borate and diethanolamine. The Suzuki cross-coupling reaction [36, 37] between **47** and ethyl 7-bromo-1-cyclopropyl-8-(difluoromethyl)-1,4-dihydro-4-oxoquinoline-3-carboxylate (**48**) [34, 35] using dichlorobis(triphenylphosphine)palladium(II) followed by acid hydrolysis results in the formation of Garenoxacin (**49**), as depicted in Scheme 11.

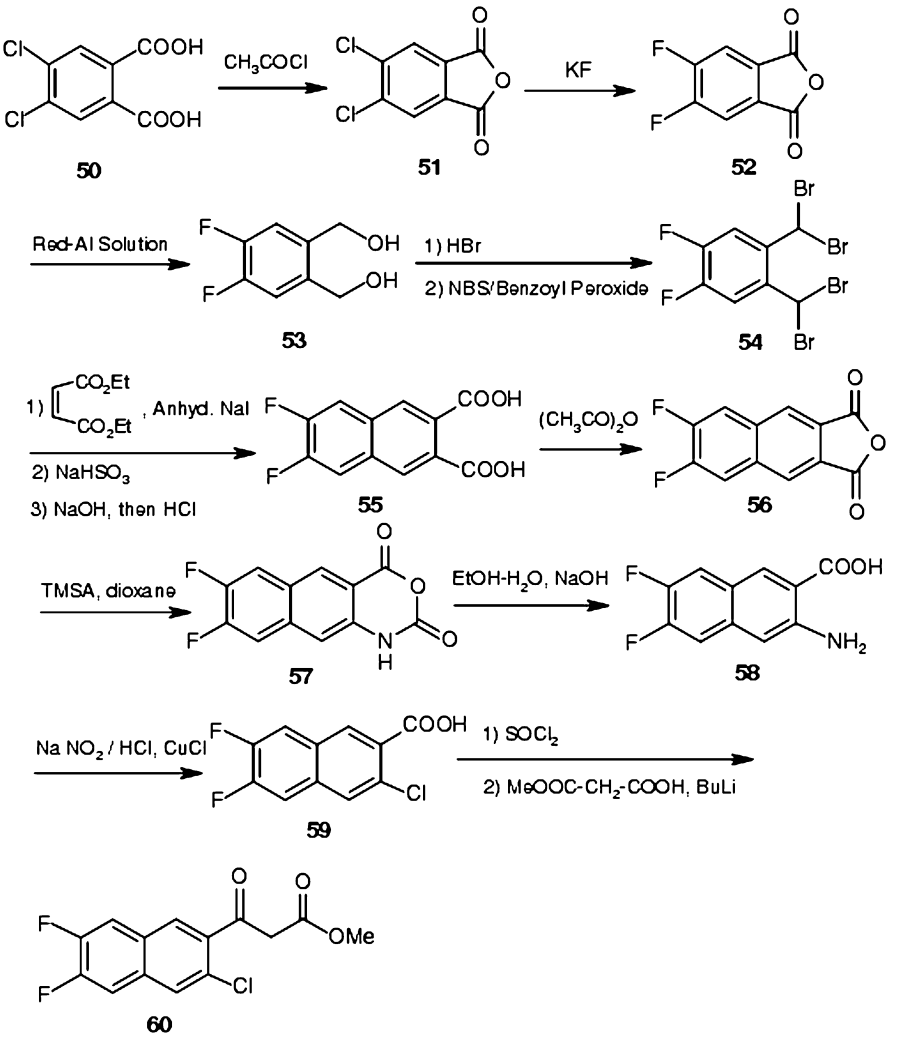


Scheme 11 Synthesis of garenoxacin

2.1.4 Fused Quinolones

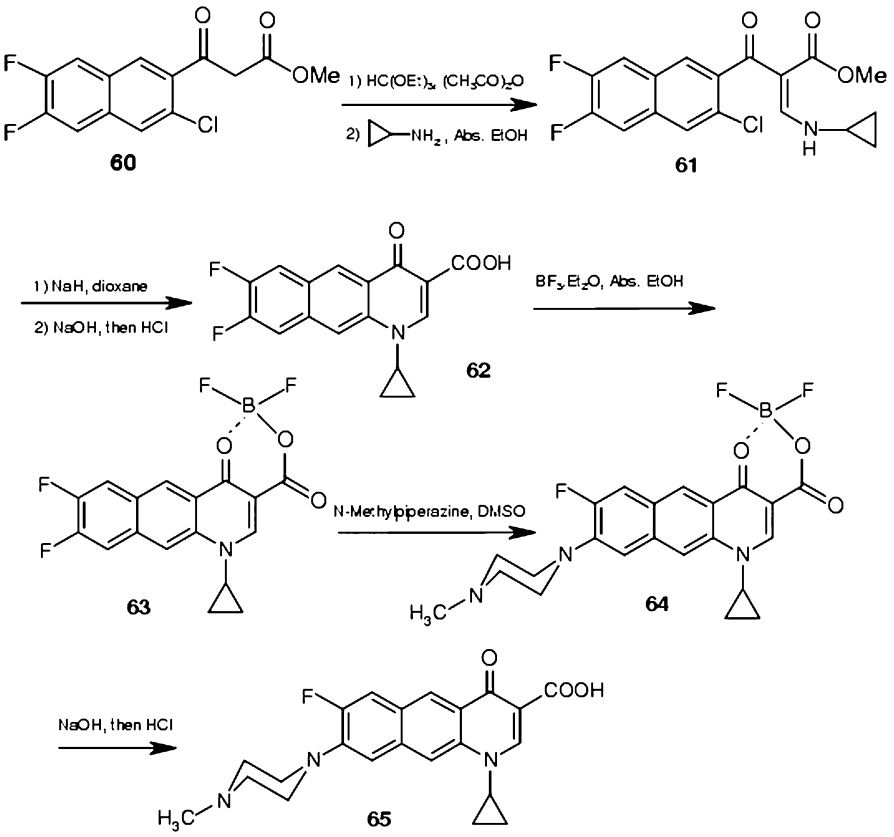
Extension of the ring numbers, bioisosteric replacement of the benzene ring, and conversion of the quinolone system to the corresponding quinalizinone system has been a subject of research interest during the past decade. For example, Jordis et al. [38] recently reported the syntheses of a series of linear benzo- or pyrido-ciprofloxacin (lin-benzo- and lin-pyrido-ciprofloxacin)

derivatives with potential topoisomerase/gyrase inhibitory properties and strong antibacterial activities. The synthetic methodology for the preparation of a representative compound (**65**) is illustrated in Schemes 13 and 14. Namely, 4,5-dichlorophthalic acid (**50**) is used as the starting material, which upon treatment with acetyl chloride gives rise to the corresponding anhydride derivative (**51**). Replacement of the chlorine at the C-4 and C-5 positions of the anhydride with fluorine via potassium fluoride fusion results in the formation of the corresponding fluorinated analogue (**52**). Reduction of **52** using Red-Al solution (sodium bis(2-methoxyethoxy)aluminum



Scheme 12 Synthesis of naphthylacetoacetate **60**

hydride in toluene) gives rise to 1,2-dihydroxymethyl-4,5-difluorobenzene (**53**). Reaction of **53** with hydrogen bromide followed by treatment with NBS in the presence of benzoyl peroxide results in the formation of the 1,2-dibromomethyl derivative (**54**). Reaction of this compound with diethyl maleate in the presence of anhydrous sodium iodide, followed by treatment with sodium hydrosulfite, gives rise to the diethyl 6,7-difluoronaphthalene-2,3-dicarboxylic acid which upon saponification yields the corresponding free carboxylic acid derivative (**55**). Dehydration of this dicarboxylic acid via treatment with acetic anhydride results in the formation of the corresponding cyclic anhydride (**56**). Treatment of this cyclic anhydride with trimethylsilyl azide (TMSA) in dioxane yields the ring-converted naphthoxazine dione derivative (**57**) from which, upon alkaline hydrolysis, the benzoanthranilic acid derivative (**58**) is obtained. The Sandmeyer reaction of **58** results in the formation of the corresponding chloronaphthoic acid derivative (**59**), which upon reaction with thionyl chloride followed by monomethyl malonate ester



Scheme 13 Synthesis of lin-benzo-ciprofloxacin analogue

under the catalytic action of *n*-butyllithium gives rise to methyl 1-(3-chloro-6,7-difluoronaphth-2-yl)acetoacetic acid (**60**), as depicted in Scheme 12.

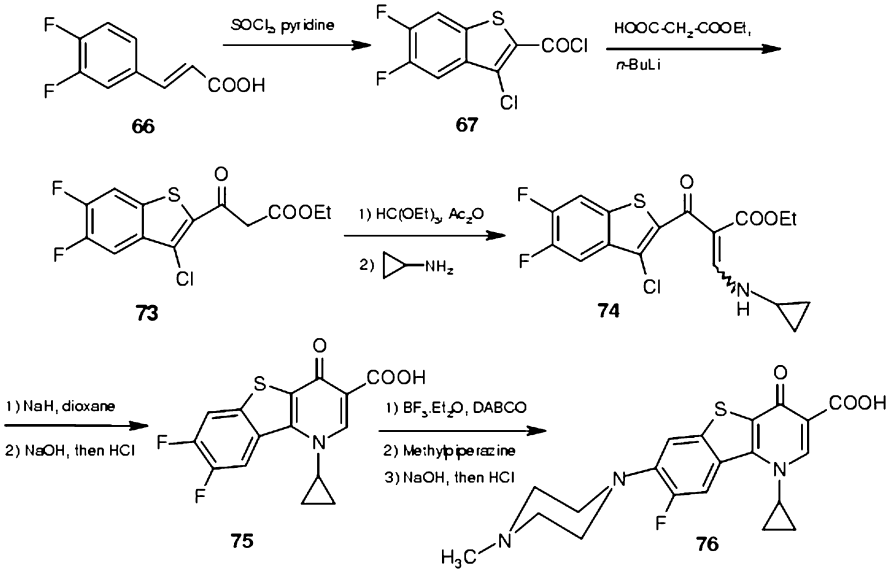
Methoxyvinylation of compound **60** using triethyl orthoformate in acetic anhydride, followed by the reaction of the methoxyvinylated intermediate with cyclopropylamine in absolute ethanol, gives rise to the cyclopropylaminovinyl derivative (**61**). Cyclization of this compound using NaH in dioxane followed by saponification of the cyclized product results in the formation of the linear benzoquinolone derivative (**62**). The final step in the synthesis of lin-benzo-ciprofloxacin (**65**) consists of the formation of the boron complex of **62** via reaction with boron trifluoride/ether to yield compound **63**, which upon reaction with *N*-methylpiperazine in dimethyl sulfoxide gives rise to the boron complex form of lin-benzo-ciprofloxacin (**64**). Treatment of this compound with sodium hydroxide followed by acidification yields the final product (**65**) in good yield, as depicted in Scheme 13.

In the same context, Sauter et al. reported the synthesis of the isosteric benzothieno[3,2,*b*]pyridone-3-carboxylic acid [39] and Jordis et al. reported the synthesis of thieno[2′,3′:4,5]thieno[3,2-*b*]pyridone-3-carboxylic acid derivatives as potential antibacterial agents [40].

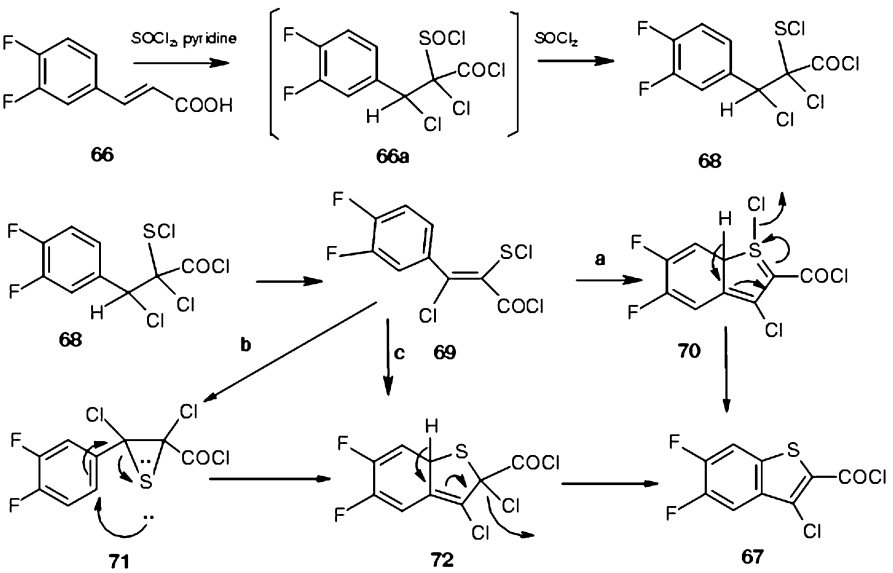
2.1.5
Synthesis of Benzothieno[3,2-*b*]pyridone-3-carboxylic Acids

The target compound, 1-cyclopropyl-8-fluoro-1,4-dihydro-7-(4-methylpiperazin-1-yl)-4-oxo-benzothieno[3,2-*b*]pyridine-3-carboxylic acid (**76**), is prepared sequentially using 3,4-difluorocinnamic acid (**66**) as the starting material. The reaction of the aromatic acid **66** with thionyl chloride and catalytic amounts of pyridine yields 3-chloro-5,6-difluorobenzothiophene-2-carboxylic acid chloride (**67**), according to the method developed by Higa and Krubsack [41]. Reaction of compound **67** with monoethyl malonate in the presence of *n*-butyllithium results in the formation of the corresponding acetoacetic ester (**73**), which upon treatment with ethyl orthoformate in acetic anhydride followed by cyclopropylamine yields the enamine derivative (**74**). Ring closure of **74** using sodium hydride in dioxane followed by saponification gives rise to the benzothieno[3,2-*b*]pyridone-3-carboxylic acid derivative (**75**) as the quinolone isosteric building block. Complexation of the β-ketocarboxylic function of compound **75** with boron trifluoride etherate, followed by reaction with *N*-methylpiperazine and decomplexation with NaOH, gives rise to compound **76**, as depicted in Scheme 14.

The mechanism of the formation of compound **67** has been studied by Higa and Krubsack [41] in detail, as shown in Scheme 15. Namely, the initial step of the reaction of the cinnamic acid derivative **66** with thionyl chloride is an electrophilic addition of thionyl chloride across the double bond of cinnamoyl chloride to form the sulfinyl chloride intermediate (**66a**), which is then converted to **68** by the Pummerer reaction. Dehydrochlorination of **68**



Scheme 14 Synthesis of benzothieno[2,3-*b*]pyridone-3-carboxylic acid derivative



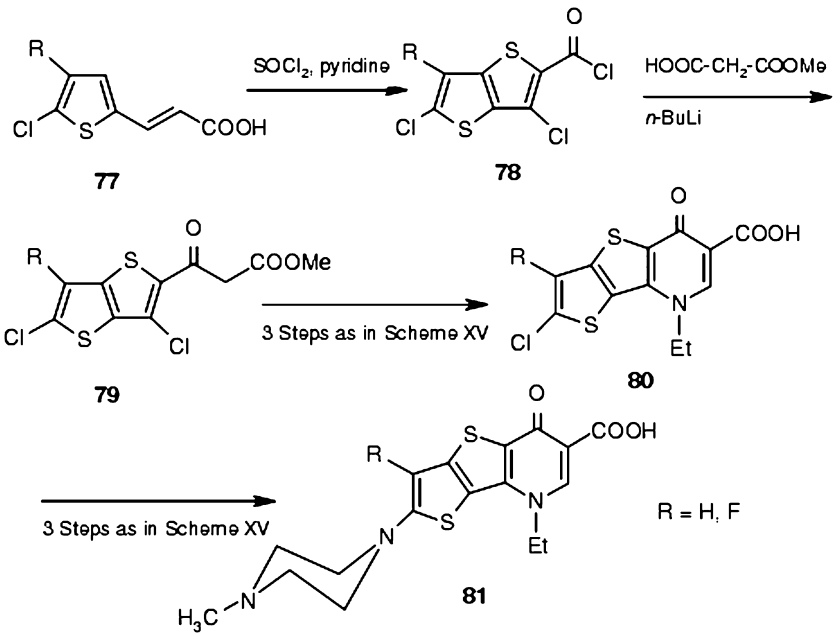
Scheme 15 Mechanism of formation of compound **67**

results in the formation of the cinnamoyl chloride derivative (**69**). Cyclization of **69** to the benzothiophene derivative (**67**) might proceed through various pathways, the most likely of which are either a concerted transformation of **69** via **70** (pathway **a**), which can be regarded as a six-π-electron system, or

rearrangement of **69** to the episulfide **71** (pathway **b**), which could then be transformed to compound **67** either by a nucleophilic attack by sulfur or by a concerted process (pathway **c**) via intermediate **72**.

2.1.6
Synthesis of Thieno[2′,3′:4,5]thieno[3,2-*b*]pyridone-3-carboxylic Acids

The synthesis of the representative compound of this series, 1,4-dihydro-1-ethyl-6-fluoro (or 6-H)-4-oxo-7-(piperazin-1-yl)thieno[2′,3′:4,5]thieno[3,2-*b*]pyridine-3-carboxylic acid (**81**), follows the same procedure as that utilized for compound **76**. Namely, the β-thienylacrylic acid (**77**) reacts with thionyl chloride to form the thieno[2′,3′:4,5]thiophene-2-carboxyl chloride (**78**). Reaction of this compound with monomethyl malonate and *n*-butyllithium gives rise to the acetoacetate derivative (**79**). Transformation of compound **79** to the thieno[2′,3′:4,5]thieno[3,2-*b*]pyridone-3-carboxylic acid derivative (**80**) proceeds in three steps in the same manner as that shown for compound **75** in Scheme 15. Complexation of compound **75** with boron trifluoride etherate, followed by reaction with piperazine and decomplexation, results in the formation of the target compound (**81**), as shown in Scheme 16. The 6-desfluoro derivative of **81** does not show antibacterial activity in vitro.



Scheme 16 Synthesis of thieno[2′,3′:4,5]thieno[3,2-*b*]pyridone-3-carboxylic acid derivatives

According to molecular modeling studies, at the ground state the 6-fluoro analogue of **81** overlaps perfectly with the structure of norfloxacin (a well-established quinolone antibacterial agent) and is predicted to exhibit promising antibacterial activity.

In addition to the above quinolone derivatives with interesting synthetic profiles, a large number of other quinolone derivatives have been synthesized in the past decade and are in different stages of clinical evaluation. These compounds are mostly synthetic modifications of the above-mentioned quinolones, and the goal is to improve their pharmacological/toxicological or pharmacokinetic profiles. Among these, a minor C_7-modification of gemifloxacin (DW286) [42], N_1-(2-fluorovinyl) derivatives of conventional quinolones [43], C_7-azetidinyl-C_8-chloro derivatives of ciprofloxacin (E-4767 and E-5065) [44], enantiomeric C_7-azetidinyl-substituted quinolones [45], and N_1-trifluoromethyl-substituted quinolones [46] are of biological interest. The synthetic methodologies for these compounds are similar to those of their parent molecules and are not covered in this chapter.

3 Novel Oxazolidinone Antibacterials

The discovery of oxazolidinones as potential antibacterial agents dates back to 1987 when a group of scientists from the DuPont Company presented the antibacterial profile of two new compounds, Dup-105 and Dup-721, at the 1987 Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) in New York [47]. These two compounds were the first clinical candidates representing a totally novel class of antimicrobial agents, the oxazolidinones. Subsequent structural modifications of these two compounds by the scientists at Pharmacia resulted in the drawing of structure–activity relationships (SAR) and the identification of the pharmacophoric groups in the oxazolidinone molecule [48]. Based on the preliminary information provided by SAR, it was suggested that the presence of an acetamidomethyl group at C-5 (*S* configuration at the C-5 position) and a 4-substituted phenyl group at the N-3 position of the oxazolidin-2-one nucleus were essential for antibacterial activity. Further modification of Dup-721 by replacing the 4-substituted phenyl ring with a fused bicyclic ring system resulted in the discovery of a potent antibacterial agent, PNU-82965 [49], with an indanone moiety in place of the 4-substituted phenyl ring. The impressive in vitro and in vivo activity profiles of PNU-82965 persuaded the scientists at Pharmacia to view this compound as a lead structure, and to synthesize its bioisosteric analogues in order to improve the pharmacokinetic and toxicological profiles of this class of compounds. As a result, PNU-85112 [50], an indoline analogue of PNU-82965, was discovered as a racemic mixture which possessed an improved biological activity and pharmacokinetic profile compared to

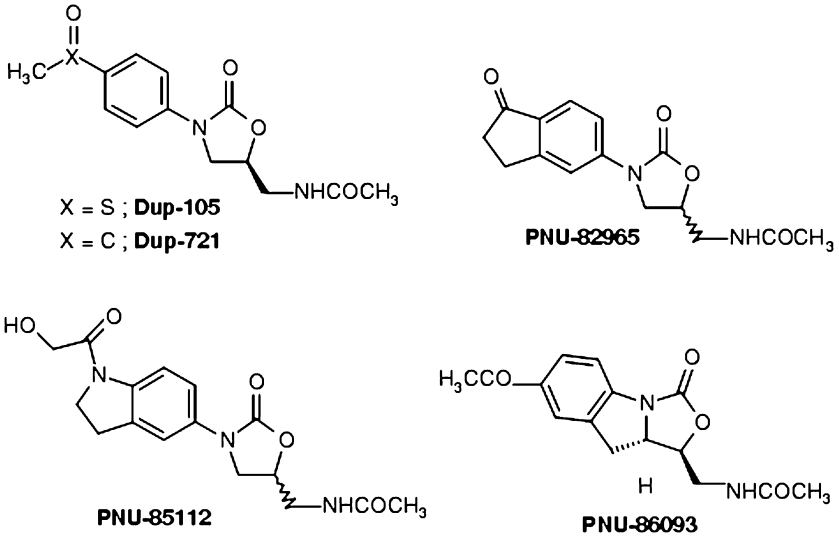


Fig. 2 Structures of the lead compounds in oxazolidinone discovery

its parent molecule. Further modifications include a tricyclic fused system, PNU-86093 [51]. The structures of these compounds are depicted in Fig. 2.

Further development in the chemistry of oxazolidinone antibacterials was based mainly on the assumption that the 4-pyridyl moiety of one of Dupont's lead compounds, E-3709, might be amenable to replacement by suitably saturated heterocyclic bioisosteres [48]. This assumption was based on an example in which successful replacement of the piperazine ring system in the quinolone antibacterials, such as ciprofloxacin, with a pyridine fragment, such as seen in Win-57273, results in improvement of both the antibacterial and the pharmacokinetic profiles of the compounds. Similarly, as in the case of ciprofloxacin and Win-57273, it was predicted that the presence of a small but highly electron-withdrawing fluorine atom would be tolerated at the *meta* position(s) of the central phenyl ring, and would confer enhanced antibacterial activity and/or other desirable properties to the targeted oxazolidinones, as shown in Fig. 3.

On this basis, a large number of piperazinylphenyl derivatives, such as PNU-97665 [52], PNU-100592 (eperezolid) [53], and their morpholino and thiomorpholino analogues PNU-100766 (linezolid) [53] and PNU-10048 [54], were synthesized. These compounds are either on the market or in the later stages of clinical trials for the treatment of infections caused by strains resistant to conventional antibacterials. See Fig. 4 for the corresponding structures.

The oxazolidinones have a novel mechanism of action that involves the inhibition of bacterial protein synthesis at the very early stage, prior to chain initiation [55–58]. They are effective against a broad range of Gram-

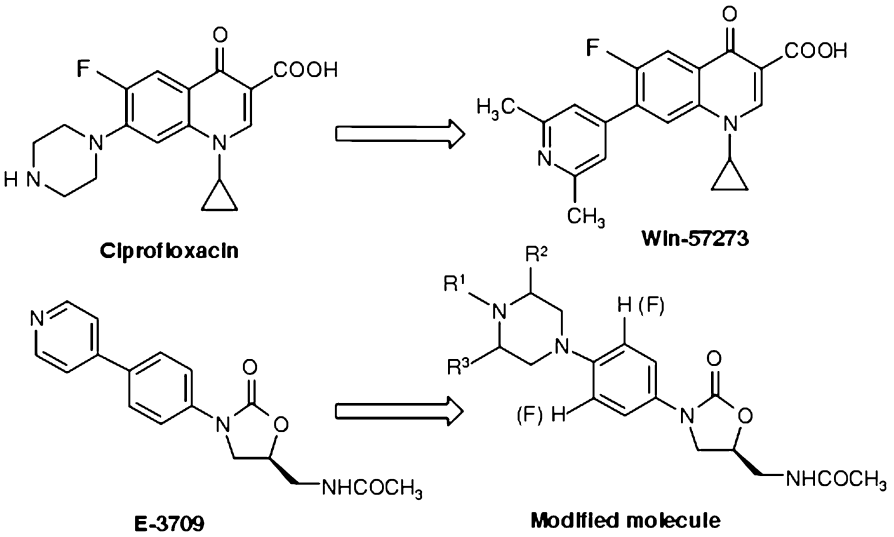


Fig. 3 Development of the piperazinylphenyl oxazolidinones

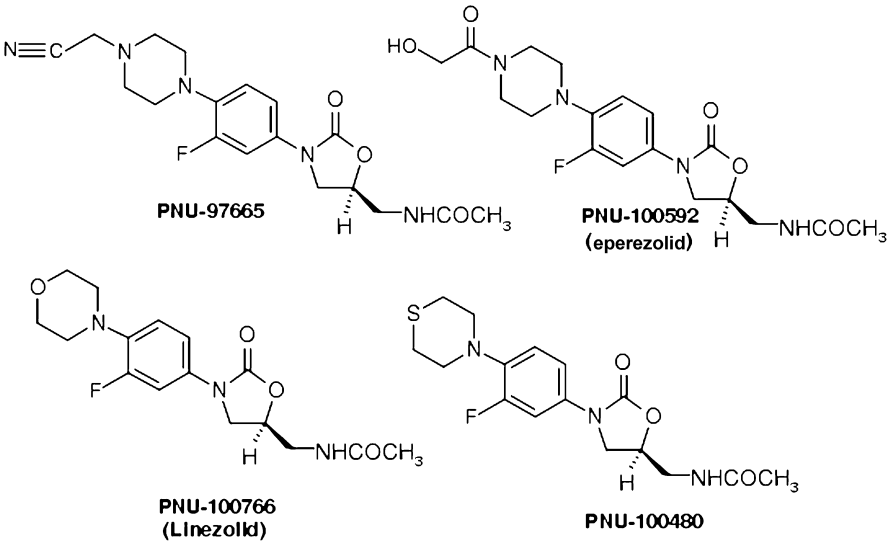
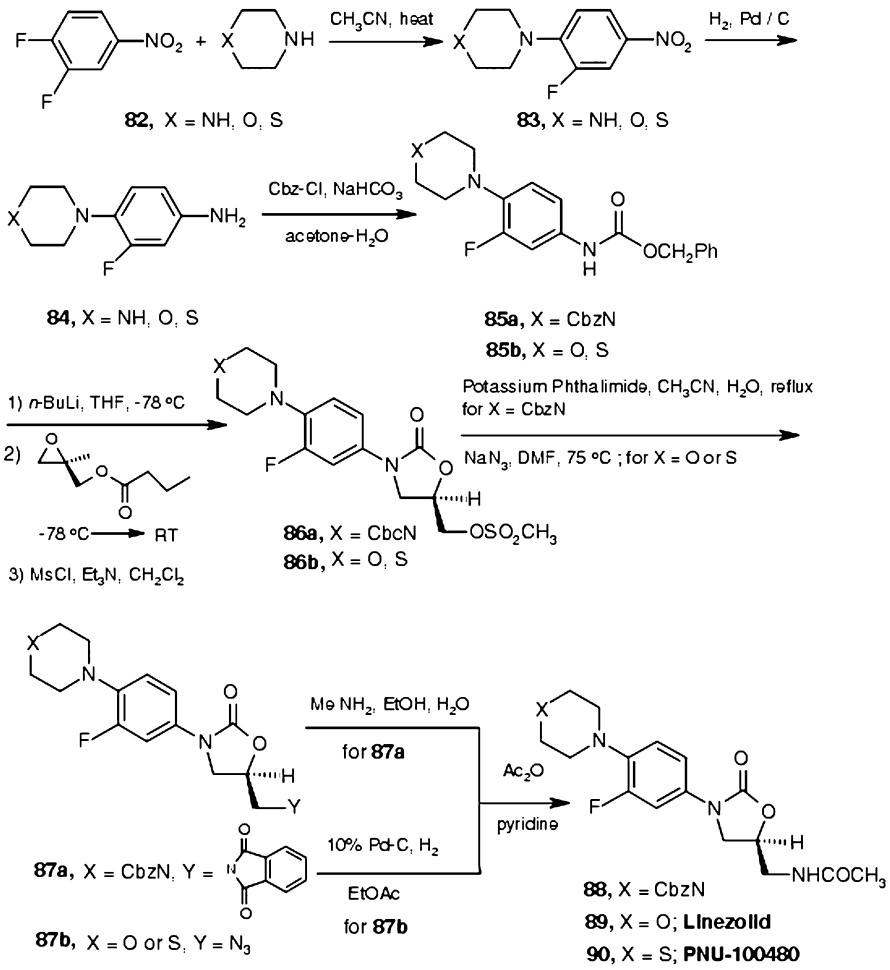


Fig. 4 Structure of some clinically useful oxazolidinones

positive, Gram-negative, and anaerobic pathogens including antibiotic-resistant strains, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VER), and *Clostridium* species (anaerobic pathogen responsible for pseudomembranous colitis) [48]. In this section, the synthetic procedures for the oxazolidinones that have been published since 1995 are discussed.

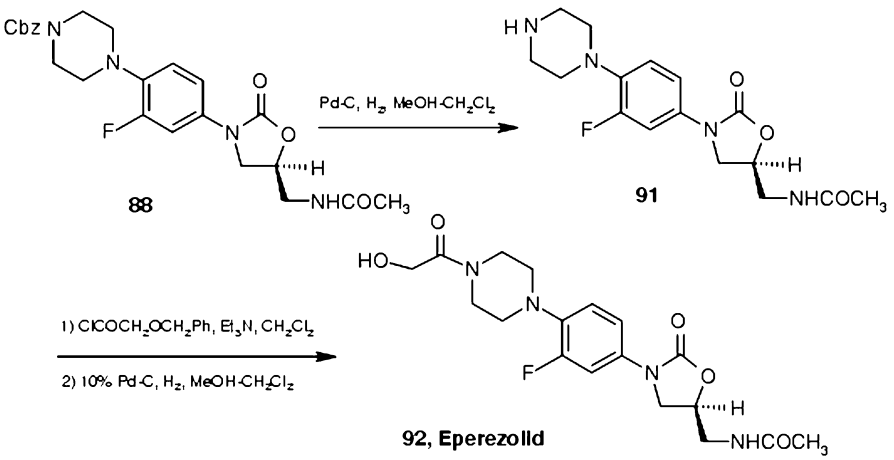
3.1
Synthesis of Eperezolid (PNU-100592), Linezolid (PNU-100766), and PNU-10048

The syntheses of these three compounds share a common route as described by Brickner et al. [53] and Barbachyn et al. [54]. Namely, the coupling reaction of 3,4-difluoronitrobenzene (**82**) with piperazine, morpholine, or thiomorpholine to yield the corresponding 4-substituted 3-fluoronitrobenzene (**83**), which upon reduction gives rise to the aniline derivative (**84**). Carbobenzoxy protection of the active nitrogen of **84** using benzyloxycarbonyl chloride (CbzCl) results in the formation of carbamates **85a** and **85b**. Treatment of **85a,b** with *n*-BuLi and (*R*)-glycidyl butyrate yields a 5-(*R*)-

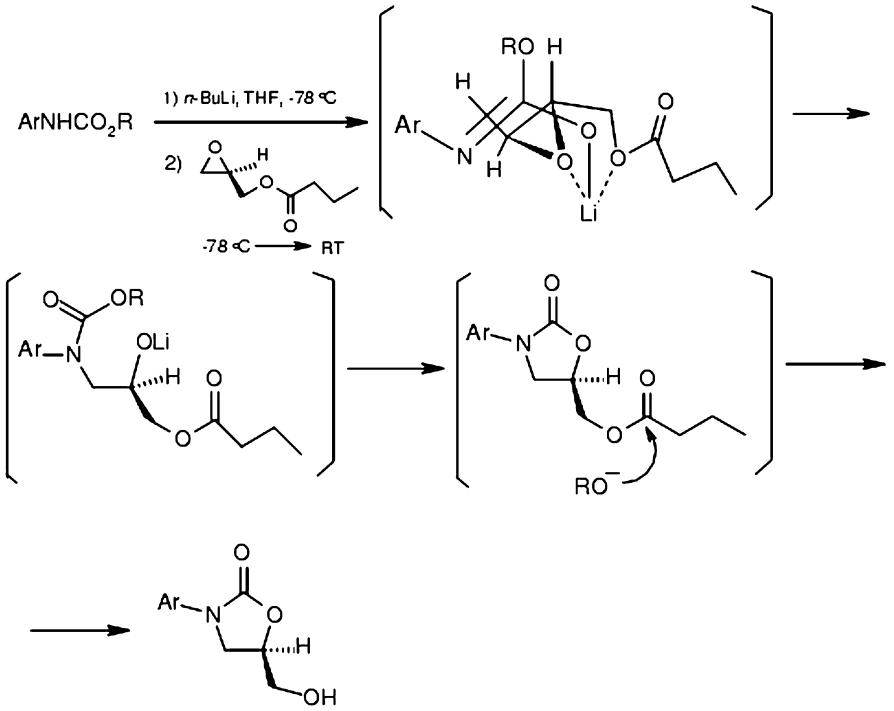


Scheme 17 Synthesis of linezolid and PNU-100480

hydroxymethyloxazolidinone intermediate, which is converted to the corresponding 5-(*S*)-(acetamidomethyl)-2-oxazolidonones linezolid, PNU-100480, and eperezolid according to the method published by Wang et al. [59]. In



Scheme 18 Synthesis of eperezolid



Scheme 19 Mechanism of enantiomeric synthesis of aryl oxazolidinones

general, mesylation of the primary alcohol group of the newly formed oxazolidinone results in the formation of compounds **86a** and **86b**. Displacement of the methanesulfonyl group of **86a,b** with potassium phthalimide gives compound **87a**, while displacement of the same group in **86b** with sodium azide yields the azide derivative **87b**. Deblocking of the phthalimide **87a** with aqueous methylamine or reduction of the azide **87b** yields the intermediate 5-(aminomethyl)-2-oxazolidinone, which upon treatment with acetic anhydride and pyridine provides the *N*-carbobenzoxy derivative (**88**), linezolid (**89**), or PNU-100480 (**90**), as depicted in Scheme 17.

Formation of eperezolid (**92**) proceeds via the catalytic hydrogenation of **88** to the deprotected intermediate (**91**), which is then acylated with benzyloxyacetyl chloride. Finally, the benzylic hydrogenolytic cleavage of this intermediate gives rise to the targeted compound eperezolid (**92**), as depicted in Scheme 18.

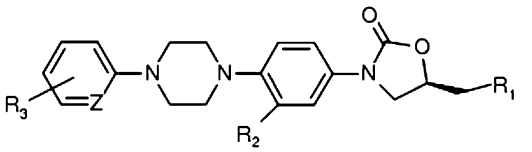
The mechanism of the stereoselective syntheses of (*R*)-3-aryl-5-(hydroxymethyl)oxazolidinones via the Mannenin reaction of aryl carbamic acid esters with (*R*)-glycidyl butyrate has been explored in detail by Brickner et al. [60]. Namely, *N*-lithiated carbamate derivatives of anilines are allowed to react with the commercially available (*R*)-glycidyl butyrate (96–98% enantiomeric excess; *ee*) under appropriate conditions to obtain enantiomerically pure (*R*)-3-aryl-5-(hydroxymethyl)oxazolidinones in 85–99% yields, according the pathways depicted in Scheme 19.

3.1.1
Other Structurally Related Nonfused Oxazolidinones

Further exploration of the structure–activity relationships of oxazolidinone has resulted in the discovery of different series of compounds with potent antibacterial activity and promising pharmacokinetic/toxicological profiles. The synthetic methodologies for the preparation of these compounds (excluding the side-chain substituents) follow the same procedures as those described for the parent oxazolidinones. These include the arylpiperazinyl oxazolidinones reported by Jang et al. [61], AZD2563 reported by Anderegg et al. [62], DA-7867 reported by Yong et al. [63], and the 5-triazolylmethyl analogue of linezolid, PH-027, reported by Phillips et al. [64]. The structures of these compounds are depicted in Fig. 5.

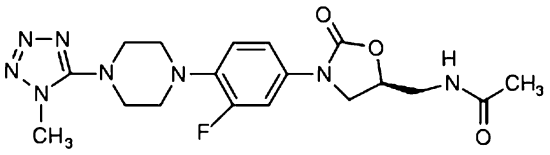
3.2
Synthesis of [6,5,5] and [6,6,5] Tricyclic Fused Oxazolidinones

This class of rigid tricyclic fused oxazolidinones was synthesized in order to gain an understanding of the importance of the spatial relationship and torsional angle between the aryl and oxazolidinone rings with regard to antibacterial activity [51, 65]. Considering the structure of an early lead compound,

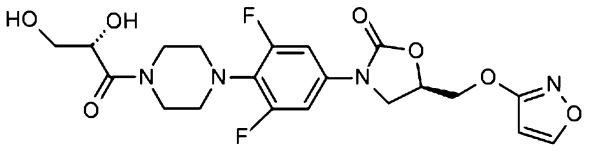


R_1 = *O*-2-pyridyl, *O*-2-pyrazinyl, *O*-2-isoxazolyl, $N(C{=}S)CH_3$, $N(C{=}S)SCH_3$, $N(C{=}S)NH_2$, or $N(C{=}S)OCH_3$
R_2 = F or H
R_3 = Various substituents
Z = C or N

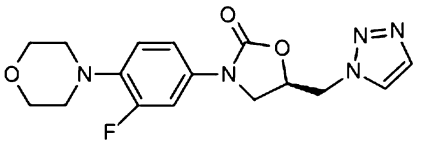
arylpiperazinyl oxazolidinones



DA-7867



AZD2563



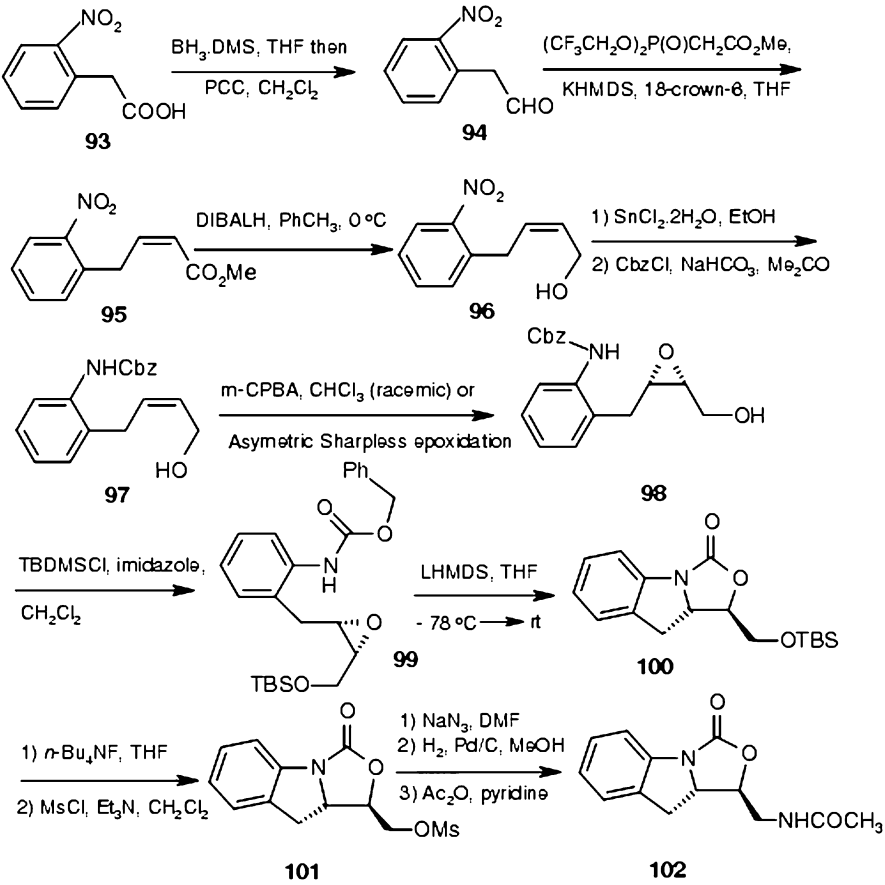
PH-027

Fig. 5 Other structurally related nonfused oxazolidinones

Dup-721 (please see Fig. 2 for the structure), these tricyclic fused oxazolidinones have their aryl and oxazolidinone rings joined together by either one or two carbon linkers, resulting in the formation of the [6,5,5] and the [6,6,5] series, respectively. The preliminary in vitro antibacterial evaluation of these compounds revealed that the [6,6,5] series possessed no antibacterial activity, while several potent compounds were identified among the [6,5,5] series. Due to the similarity of the synthetic methodologies used for both classes of compounds, the synthetic procedure for the [6,5,5] series, including PNU-86093 and its aryl and sulfonamide analogues, is described in detail.

3.2.1
Synthesis of PNU-86093 and its Analogues

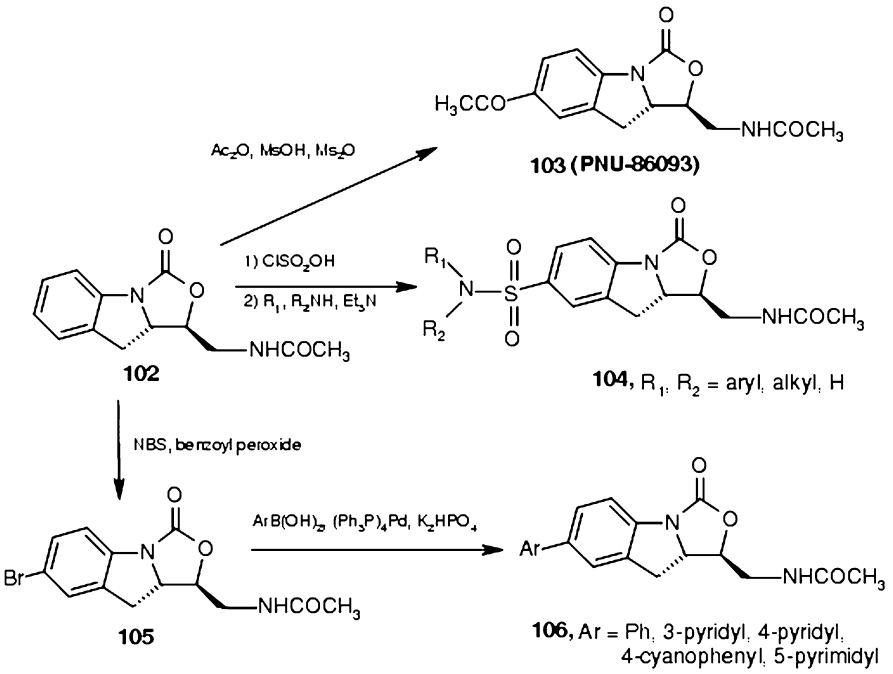
The starting material, 2-nitrophenylacetic acid (**93**), was converted to its corresponding aldehyde, 2-nitrophenyl acetaldehyde (**94**), using BH_3.DMS followed by purification via column chromatography and hydrolysis. This compound then undergoes the Horner–Wadsworth–Emmons reaction using Still's conditions [66] with methylbis(trifluoroethyl)phosphonoacetate and 5 equivalents of 18-crown-6 to obtain compound **95** with a $Z:E$ ratio of 15 : 1. These two isomers can be easily separated by column chromatography. The desired isomer (*Z*)-**95** is then reduced with diisobutylaluminum hydride (DIBALH), according to the method published by Yoon et al. [67], to the allylic alcohol (**96**). Reduction of the nitro group of this compound with



Scheme 20 Synthesis of [6,5,5] fused tricyclic oxazolidinones

stannous chloride, according to the method of Bellamy et al. [68], followed by protection of the amino group by benzyl chloroformate gives rise to the benzyl carbamylated intermediate (**97**) as the *Z*-isomer. The epoxidation reaction of **97** can proceed either through treatment with *m*-chloroperbenzoic acid to give a mixture of isomers with a 96% yield, or via a controlled asymmetric Sharpless epoxidation [65] using $Ti(OiPr)_4$ and L-(+)-diethyl tartrate reagents in the presence of 4-Å molecular sieves to give compound **98** in $\geq$ 95% enantiomeric excess. Reaction of **98** with (*tert*-butyldimethyl)silyl chloride (TBDMSCl) provides compound **99**, which upon treatment with lithium hexamethyldisilazide (LHMDS) yields the desired silyl-protected tricyclic oxazolidinone (**100**). Deprotection of the silylated oxazolidinone with Bu_4NF furnishes the free alcohol, which is then converted to the corresponding mesylate ester (**101**) in $\geq$ 95% enantiomeric excess. Replacement of the mesylate ester with an azide group followed by the reduction of the azide intermediate to the amino intermediate and further acetylation results in the formation of the acetamino derivative (**102**), as depicted in Scheme 20.

Compound **102** is used as the building block for the syntheses of PNU-86093 and its analogues, as described in Scheme 21. Namely, the Friedel–Crafts acetylation reaction of **102** under acetic anhydride, methanesulfonic acid, and methanesulfonyl anhydride [51, 65] affords compound **103** (PNU-



Scheme 21 Synthesis of PNU-86093 and its analogues

86093) with a 67% yield. This compound has shown potent antibacterial activity and a favorable toxicological profile. Treatment of compound **102** with chlorosulfonic acid followed by a primary or secondary amine in the presence of triethylamine gives rise to compound **104**, while treatment of the same compound with NBS in the presence of benzoyl peroxide affords the bromo intermediate (**105**). Treatment of (±)-**105** with various aryl boronic acids under Suzuki palladium-catalyzed cross-coupling conditions yields a variety of racemic aryl- and heteroaryl-substituted [6,5,5] tricyclic oxazolidinones (**106**) [51].

The in vitro and in vivo antibacterial evaluations of these compounds revealed that the active compounds are those that correspond to structure **106**, with the 3-pyridyl analogue being the most active. Unfortunately, the 30-day toxicological evaluation of this compound in mice demonstrated severe toxicity, and therefore further development of this compound was halted. Compound **103** (PNU-86093), although weaker than Dup-721, has a promising toxicological profile and is considered as a lead compound for future studies [51].

4
Peptide Deformylase Inhibitors as Novel Antibacterial Agents

The prevalence of bacterial resistance to the conventional antibacterial agents has prompted scientists to search for new antibacterial agents that act on different bacterial targets from those already exploited. Among the bacterial targets, many bacterial enzymes have been well characterized and hold promise for the discovery of novel antibacterial agents. One such target that has recently attracted a great deal of attention is peptide deformylase (PDF). Protein synthesis has proven to be a reliable source of targets for antibacterial drugs. In spite of the similarity between the protein synthesizing machineries of bacterial and mammalian cells, there are sufficient differences to allow for a selective blocking of the process in bacteria. One significant difference is the transformylation followed by deformylation of the initiating methionine in bacterial translation. In bacteria, the *N*-formylmethionine of the nascent protein is removed by the sequential action of PDF and a methionine aminopeptidase to afford the mature protein. This characteristic role of PDF in protein synthesis provides a rational basis for selectivity and makes it an attractive target for drug discovery. Recently, the possible use of PDF for an antibacterial target has been reported by Giglione et al. [69] and Yuan et al. [70]. Bacterial PDF belongs to a new class of metalloproteases that utilize the Fe^{2+} ion as the catalytic metal ion. The 3-D structures of various PDF molecules, including structures of the enzyme-inhibitor complex, have been solved and published [71–73]. It was noted that, in spite of the difference between the primary sequence of PDF and other metalloproteases, the environment sur-

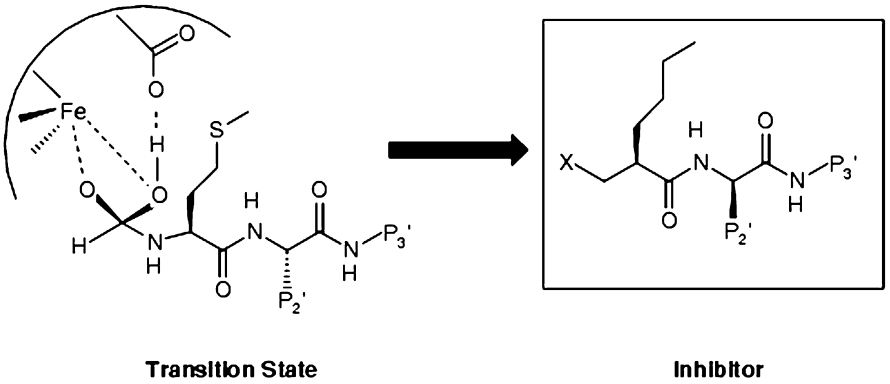


Fig. 6 Generic PDF inhibitor structure derived from the transition state of PDF reaction

rounding the catalytic metal ion of PDF appears to be very similar to the active sites of thermolysin and the matrix metalloproteases (MMPs). Based on the mechanistic and structural information, together with an understanding of the general principles of inhibiting metalloproteases, Yuan et al. [71] proposed a generic PDF inhibitor structure as depicted in Fig. 6.

In the structure of the proposed inhibitor, X represents a chelating pharmacophore that provides binding energy, the *n*-butyl group mimics the methionine side chain, and P_2' and P_3' are regions of the inhibitor that can provide additional binding energy, selectivity, and favorable pharmacokinetic properties. Based on the proposed generic structure of PDF inhibitors in Fig. 6, several research groups attempted the design and syntheses of new molecules with potential PDF inhibitory and antibacterial activity. The syntheses of the representative molecules with promising antibacterial activity are described in this section.

4.1 *N*-Alkyl Urea Hydroxamic Acids as PDF Inhibitors with Antibacterial Activity

The hydroxamic acid moiety plays an important role as a pharmacophore in a variety of biologically active compounds such as enzyme inhibitors, antimicrobial agents, cardiovascular agents, and anticancer agents [74]. Synthesis of the first series of β-sulfonyl- and β-sulfinylhydroxamic acids with potent PDF inhibitory activity was reported by Apfel et al. [75]. The driving force behind this research was the identification of a naturally occurring compound, actinonin, as a potential PDF inhibitor and antibacterial agent [76]. The structures of actinonin and the sulfonyl-/sulfinylhydroxamic acid derivatives are depicted in Fig. 7.

Based on the proposed generic PDF inhibitor structure and by incorporating the hydroxamic acid moiety, Hackbarth et al. [77] and Lewis et al. [78]

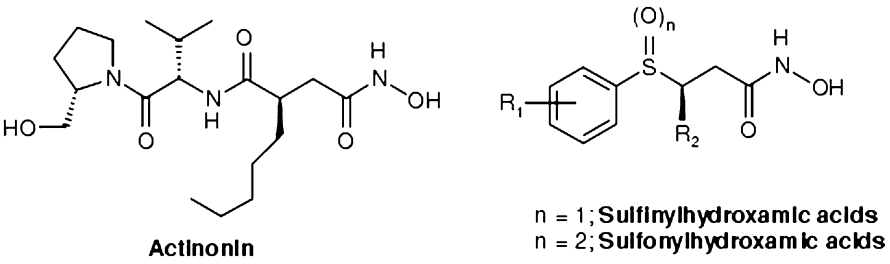
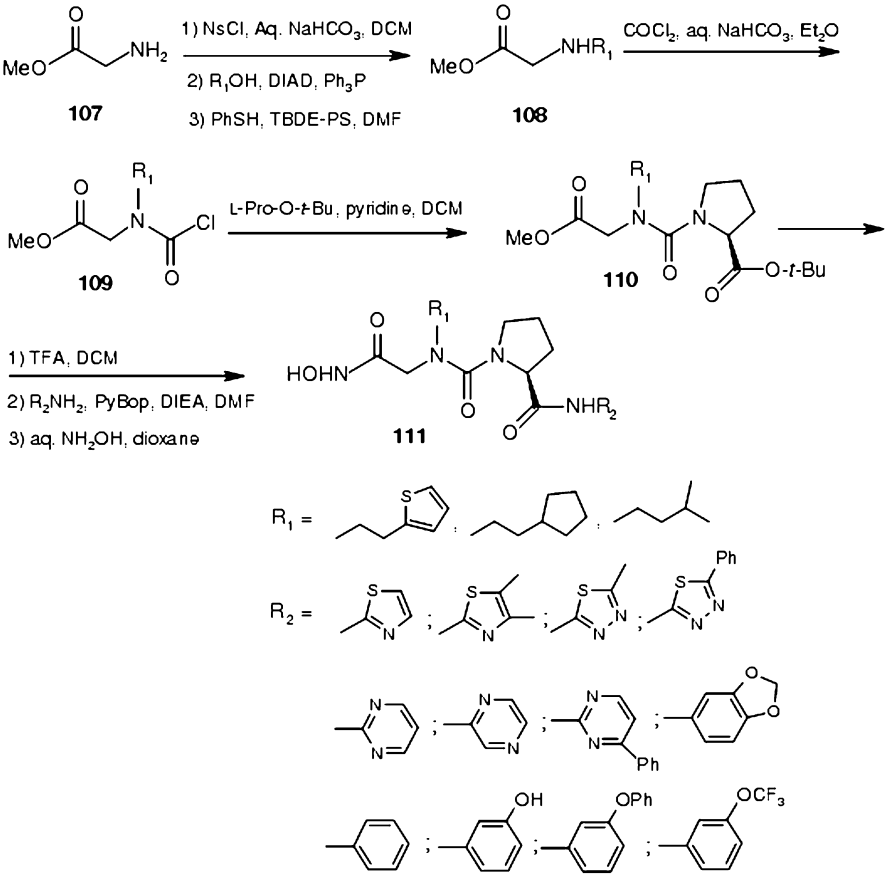


Fig. 7 Structures of actinonin and sulfinyl-/sulfonylhydroxamic acids



Scheme 22 Synthesis of *N*-alkyl urea hydroxamic acids as PDF inhibitors

designed and synthesized a series of *N*-alkyl urea hydroxamic acid derivatives (**111**). The synthetic methodology is depicted in Scheme 22. Namely, glycine monomethyl ester (**107**) under Fukuyama–Mitsunobu conditions yields the *N*-alkylated intermediate (**108**) in three steps. Chloroacylation of compound

108 with excess phosgene gives rise to *N*-alkyl-*N*-chlorocarbamoyl-glycine methyl ester (**109**). The reaction of intermediate **109** with L-proline-*tert*-butyl ester in pyridine provides the tetrasubstituted urea derivative (**110**), which upon trifluoroacetic acid deprotection followed by coupling of the R_2NH_2 with PyBop (benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate) peptide coupling reagent and treatment with hydroxylamine provides the targeted *N*-alkyl urea hydroxamic acids (**111**) in good yield.

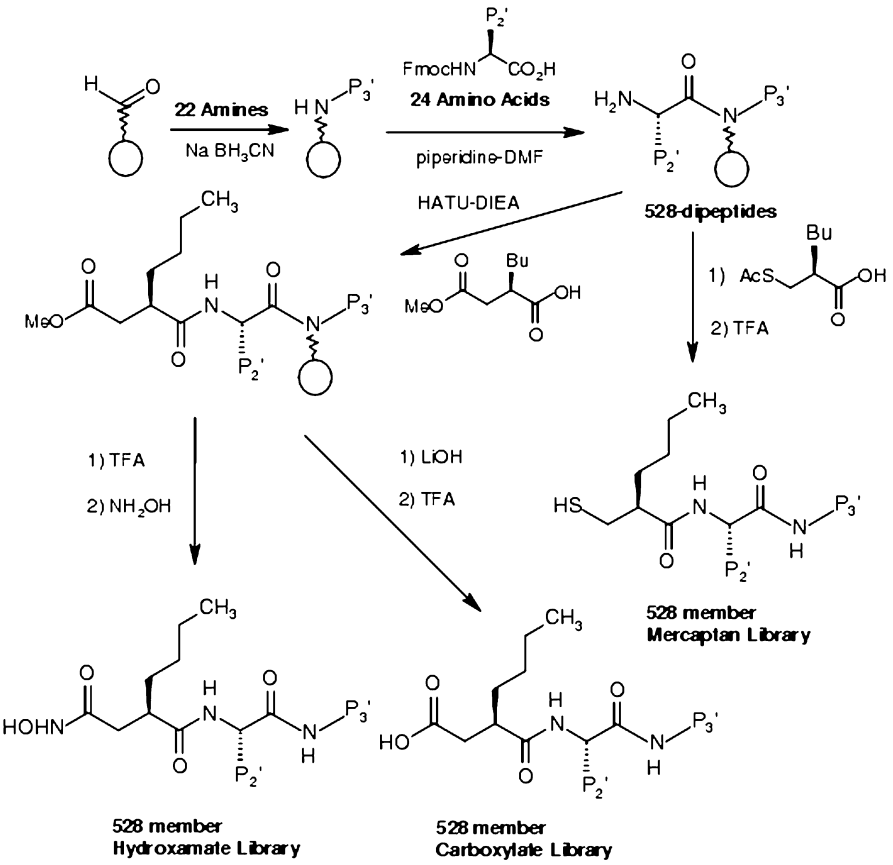
Most of the compounds in this series exhibited strong PDF inhibitory activity, low cytotoxicity, and potent antibacterial activity. Two compounds, VRC-4307 (**111**; R_1 = cyclopentylethyl, R_2 = 4,5-dimethylthiazol-2-yl) and VRC-4232 (**111**; R_1 = isopentyl, R_2 = 4,5-dimethylthiazol-2-yl), have been selected for further preclinical studies due to their promising therapeutic profiles.

4.2 Synthesis of VRC3375, a Proline-3-alkylsuccinyl Hydroxamate Derivative

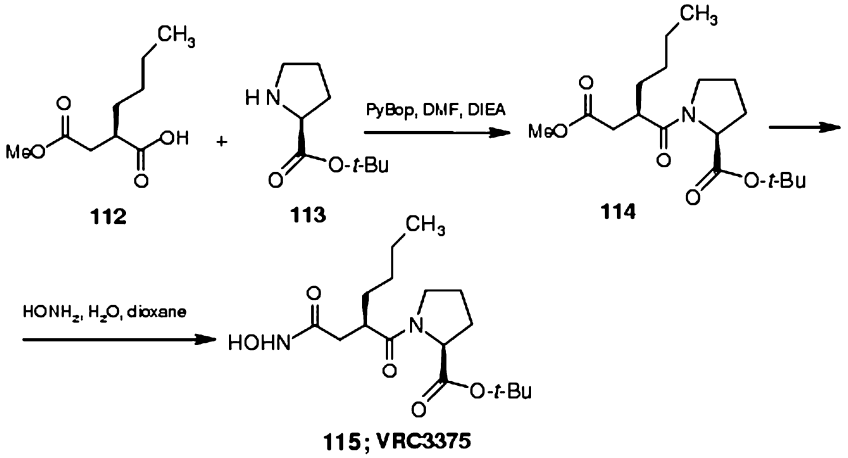
The discovery of this lead compound as a potent PDF inhibitor was a result of an integrated combinatorial and medicinal chemistry approach based on the proposed generic PDF inhibitor structure. This focused chemical library was designed by Chen et al. [79], and was prepared using solid-phase parallel synthesis in which 22 amines and 24 amino acids were used as building blocks, as outlined in Scheme 23.

Namely, 528 compounds are prepared in each set of mercaptan, carboxylate, and hydroxamate libraries. Twenty-two amines for P_3' substitution are mobilized via a 5-(4-formyl-3,5-dimethoxyphenoxy)valeric (BAL) aldehyde linker on a PEG resin through reductive amination using trimethyl orthoformate-$NaBH_3CN$. Each amine resin is coupled with 24 different 9-fluorenylmethoxycarbonyl-protected natural and unnatural amino acids (P_2') 2-(1*H*-9-azobenzyltriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate–diisopropylethylamine (HATU-DIEA) followed by removal of the 9-fluorenylmethoxycarbonyl protecting group by using 20% piperidine in DMF to give 528 dipeptides immobilized on a solid phase. The thiol library is prepared by reacting the amine of the 528 dipeptides on the resin with 2-acetylsulfanilmethylhexanoic acid followed by cleavage from the resin using trifluoroacetic acid (TFA). Alternatively, the dipeptides are coupled with the 4-monomethyl 2-(*R*)-butylsuccinic ester by using the HATU-DIEA method. Cleavage of the methyl ester from the resin by using TFA followed by reaction with hydroxylamine in dioxane/water yields the 528-member hydroxamate library. The carboxylate library is prepared by the alkaline hydrolysis of the corresponding methyl ester on resin using $LiOH/THF/H_2O$, followed by TFA cleavage of the corresponding carboxylate dipeptide from the resin.

VRC3375 was selected from among the 1548 compounds prepared based on its strong PDF inhibitory properties, potent antibacterial activity, and



Scheme 23 Synthesis of chemical libraries of focused PDF inhibitors

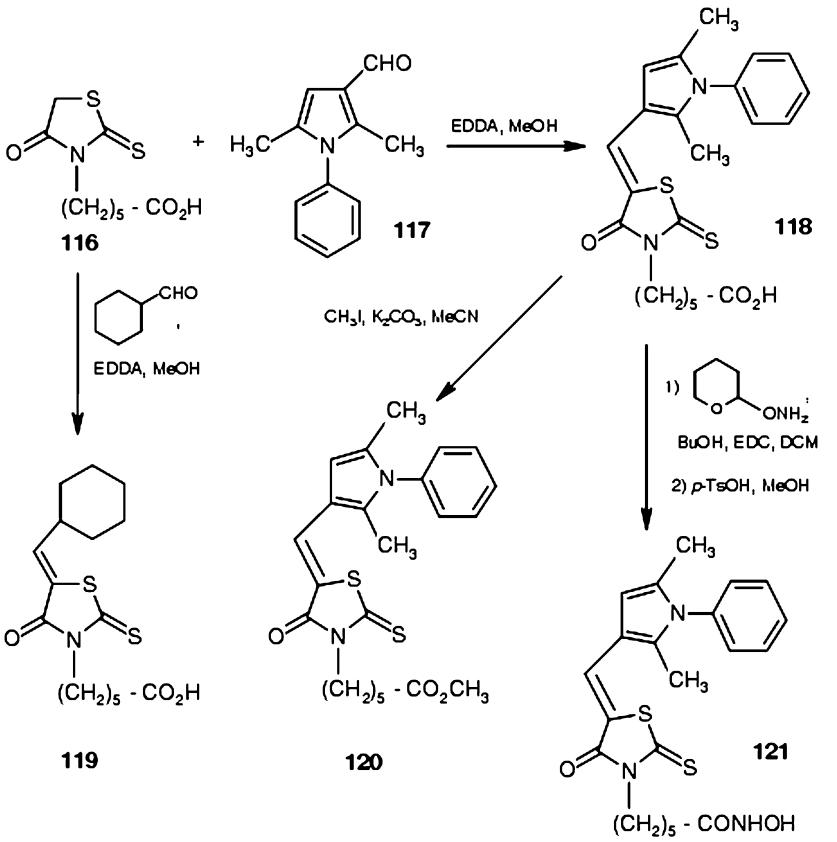


Scheme 24 Synthesis of VRC3375

promising pharmacokinetic profile. This compound was then synthesized on a large scale, according to Scheme 24, for further biological assessment. Namely, 4-monomethyl 2-(*R*)-*n*-butylsuccinic acid (**112**) is allowed to couple with *t*-butyl L-proline hydrochloride (**113**) in DMF in the presence of the Py-Bop peptide coupling reagent and DIEA to afford the ester **114**, which upon reaction with aqueous hydroxylamine in dioxane yields the desired proline-3-*n*-butylsuccinyl hydroxamate (**115; VRC3375**) with a good yield.

4.3
Synthesis of 5-Arylidene-2-thioxothiazolidin-4-one-3-hexanoic Acid Derivatives

This novel class of PDF inhibitors was discovered through the high-throughput screening (HTS) and the virtual ligand screening (VLS) of over 10 000 members of a library composed of molecules selected from DuPont's corporate collection, and designed to represent the broad diversity of bio-



Scheme 25 Synthesis of 5-arylidene-2-thioxothiazolidin-4-one-3-hexanoic acid derivatives

logically relevant chemical space encompassed by the larger collection [80]. Among several hits, 2-thioxo-4-thiazolidinone *N*-hexanoic acids exhibited the most PDF inhibitory activity and were selected for further evaluation. Structural optimization resulted in the identification of several 5-arylalkylidene analogues, of which 5-[(2,5-dimethyl-1-phenylpyrrol-3-yl)methylidene]-2-thioxothiazolidin-4-one *N*-hexanoic acid (**118**) proved to be the most active compound. On this basis, the corresponding hydroxamate derivative (**121**), as well as the methyl ester (**120**), were also synthesized along with the 5-(cyclohexyl)methylidene analogue (**119**). The PDF inhibitory and antibacterial assessment of these compounds demonstrated that, with the exception of the methyl ester **120**, three compounds possess strong PDF inhibitory properties as well as medium to strong antibacterial activity against both Gram-positive and Gram-negative pathogens. The synthetic methodology for the preparation of these compounds is depicted in Scheme 25.

Namely, the reaction of 2-thioxothiazolidin-4-one *N*-hexanoic acid (**116**) with 2,5-dimethyl-1-phenylpyrrol-3-carboxaldehyde (**117**) in methanol under the catalytic action of ethylenediamine diacetate (EDDA) yields 5-[(2,5-dimethyl-1-phenylpyrrol-3-yl)methylidene]-2-thioxothiazolidin-4-one *N*-hexanoic acid (**118**) in 79% yield. The hydroxamate derivative of **118** is prepared by reacting this compound with *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine followed by treatment with *p*-toluenesulfonic acid in methanol to afford compound **121** in 60% yield. Esterification of compound **118** is carried out by using methyl iodide in acetonitrile in the presence of sodium carbonate to give compound **120**. The 5-(cyclohexyl)methylidene analogue (**119**) is obtained in 42% yield by direct reaction of compound **116** with cyclohexanecarboxaldehyde in methanol under the catalytic action of EDDA.

4.4 Synthesis of Macrocyclic Peptidomimetic Inhibitors of PDF

The idea for macrocyclic peptidomimetic inhibitors of PDF originated from the structure of the reverse hydroxamate BB-3497 that was reported by Clements et al. [73]. On this basis, Hu et al. [81] designed the cyclic compound **135**, in which a nonyl group serves as the cross-linked P1′ and P3′ side chain, as depicted in Fig. 8.

Synthesis of acid **129** starts from the commercially available 6-heptenoic acid (**122**), which upon reaction with (4*S*)-benzyloxazolidin-2-one (**123**) as the chiral auxiliary group yields the intermediate **124**, hydroxymethylation of which affords compound **125**. Hydrolysis of compound **125** followed by condensation with *O*-benzylhydroxylamine gives rise to the hydroxamate (**126**), which is then converted into β-lactam **127** via an intramolecular Mitsunobu reaction. Hydrolysis of the β-lactam **127** affords acid **128**, which is subsequently formylated at the benzyloxyamine moiety to give the required intermediate acid (**129**) in quantitative yield, as depicted in Scheme 26.

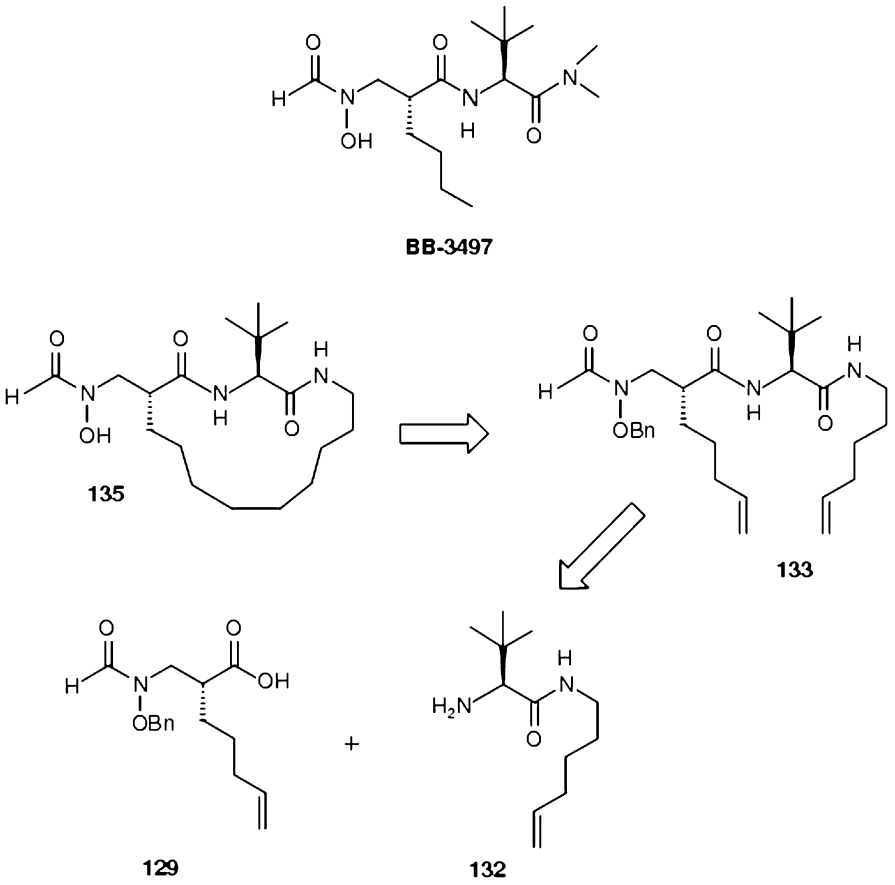
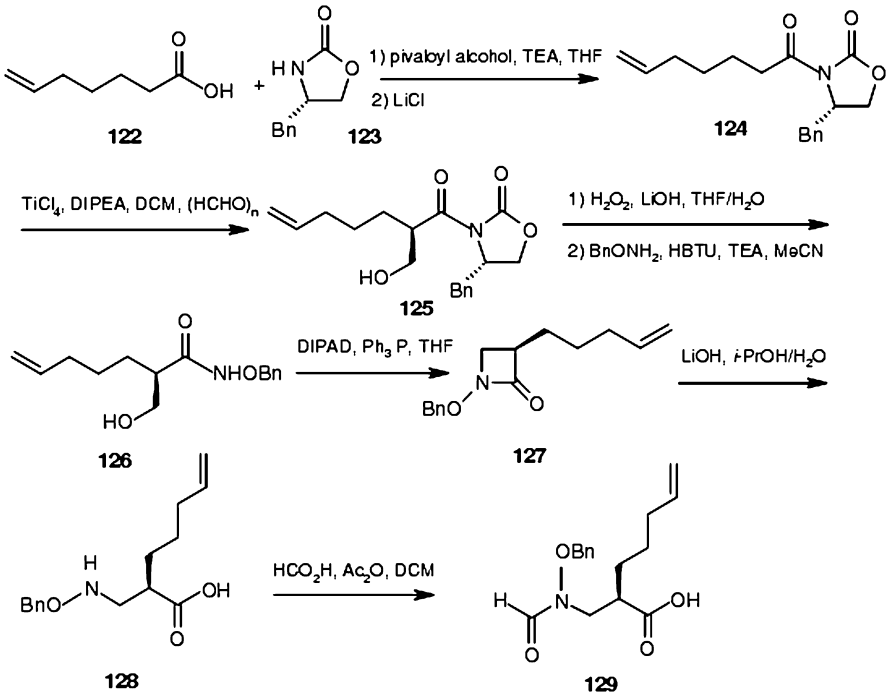


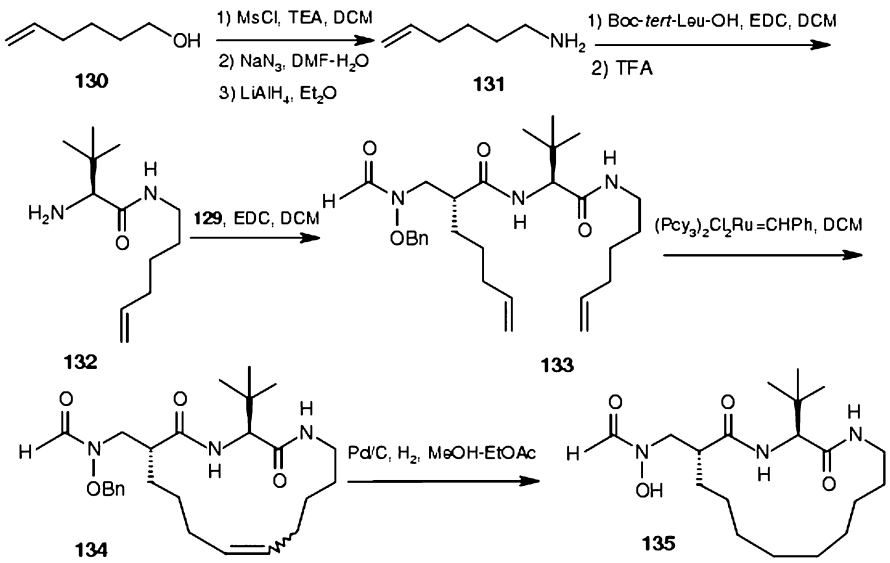
Fig. 8 Retrosynthetic design of the macrocyclic PDF inhibitor **135**

Synthesis of the macrocyclic compound **135** is depicted in Scheme 27. Namely, the amine **131**, which is obtained from the corresponding alcohol **130** in three steps, is condensed with *N*-BOC-*tert*-leucine. Treatment of the resulting amide intermediate with trifluoroacetic acid gives rise to amine **132**, which is then coupled with the acid **129** to give the diene **133**. The terminal alkenes are cross-linked using Grubb's ruthenium catalyst [82] to produce the 15-membered macrocyclic compound **134**. Catalytic hydrogenation of **134** results in the simultaneous reduction of the double bond and removal of the benzyl group from the *N*-hydroxy moiety to give the *N*-formylhydroxylamine (**135**) as the final product.

This macrocyclic peptidomimetic compound exhibits potent inhibitory activity against *Escherichia coli* deformylase as well as strong antibacterial activity against both Gram-positive and Gram-negative bacteria.



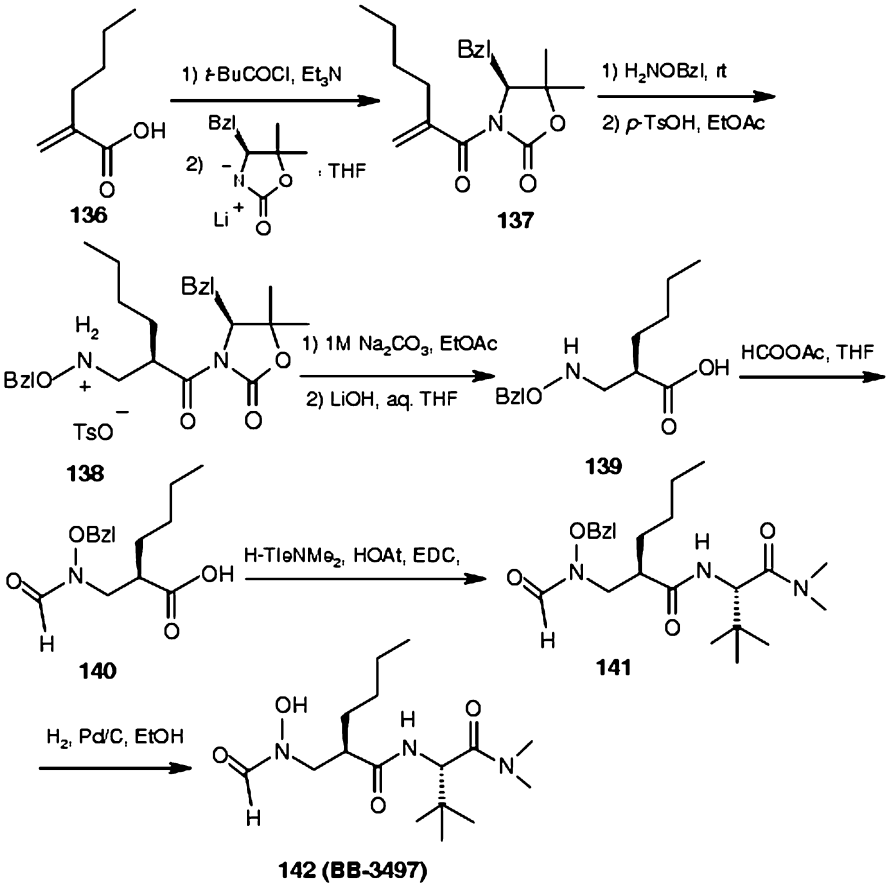
Scheme 26 Synthesis of the intermediate acid **129**



Scheme 27 Synthesis of macrocyclic peptidomimetic PDF inhibitors

4.5 Asymmetric Synthesis of BB-3497

The discovery of BB-3497 was the result of screening a proprietary library for potential metalloenzyme inhibitors at the British Biotech Pharmaceutical Co. Ltd. [73]. This compound was originally prepared in a nonstereoselective manner and its stereochemistry was assigned on the basis of matrix metalloprotease (MMP) inhibitory activity. The asymmetric synthesis of BB-3497 and the establishment of its stereochemistry by small-molecule X-ray crystallography was later reported by Pratt et al. [83]. Further structure–activity relationship studies of BB-3497 with respect to the modification of the P2′ and P3′ side chains [84] and metal binding group [85] were later reported by the scientists at British Biotech. These studies revealed that none of the



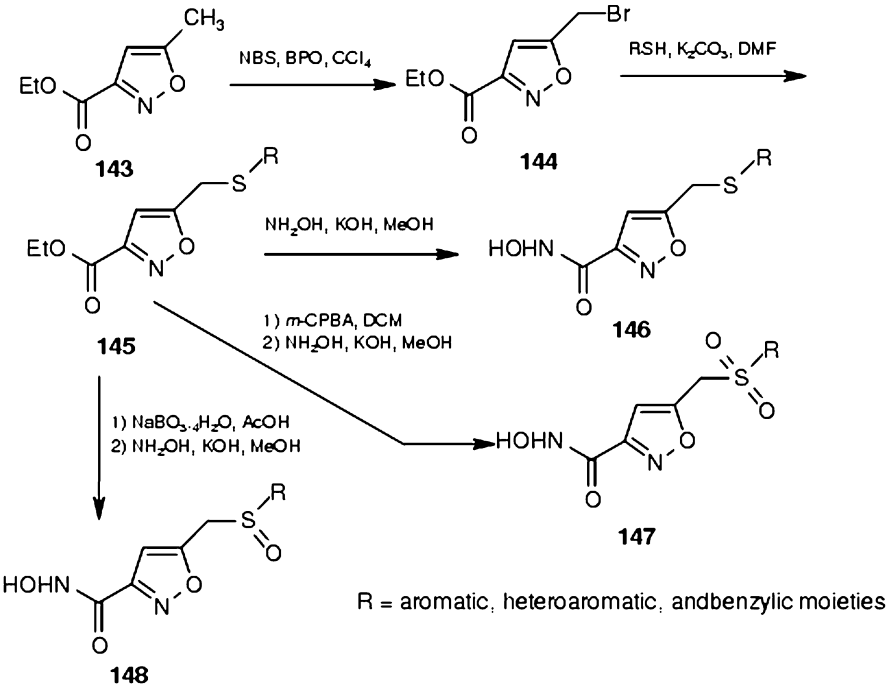
Scheme 28 Synthesis of BB-3897

modifications of BB-3497 was as active as the parent compound, and therefore BB-3497 was selected for further preclinical and clinical studies. The asymmetric synthesis of BB-3497 is outlined in Scheme 28. Namely, the homochiral acrylate (**137**) is prepared via the reaction of 2-butylacrylic acid (**136**) with 3-lithio-4-benzyl-5,5-dimethyl-oxazolidin-2-one under mixed anhydride conditions. Reaction of *O*-benzylhydroxylamine with **137** at ambient temperature under *p*-TsOH affords the Michael addition product (**138**) as a tosyl salt. Removal of the chiral auxiliary (4*S*)-benzyloxazolidin-2-one via the hydrolysis of **138** results in the formation of the benzylhydroxylamine derivative (**139**). Formylation of compound **139** is carried out by reaction with the mixed anhydride $HCOOCOCH_3$ in THF to afford compound **140**. Coupling of compound **140** with *N*,*N*-dimethyl-*tert*-leucine proceeds in the presence of 1-hydroxybenzotriazole to give compound **141**, which upon deprotection of the benzyl group via catalytic hydrogenation affords compound **142** (BB-3497). The original assignment of the stereochemistry of BB-3497 was supported by the small-molecule crystal structure of the benzyl precursor **141**, and later by the crystal structure of the *E. coli* PDF/BB-3497 complex [73].

4.6
Isoxazole-3-hydroxamic Acid Derivatives as Potential PDF Inhibitors

Due to the significant peptide characteristics of most of the PDF inhibitors, there are concerns about their selectivity and in vivo metabolic stability. In this context, Cali et al. [86] attempted the syntheses and evaluation of a new series of non-peptidic PDF inhibitors possessing an isoxazole-3-hydroxamic acid as the central core. The general route for the synthesis of these compounds is outlined in Scheme 29. Namely, the radical bromination of 5-methyl-isoxazole-3-carboxylate (**143**) using *N*-bromosuccinimide and catalytic amounts of benzoyl peroxide (BPO) yields the corresponding 5-bromomethyl analogue (**144**), which upon alkylation with an array of aromatic, heteroaromatic, and benzylic thiols in the presence of potassium carbonate affords the desired thioethers (**145**) in good yield. Reaction of the ethyl ester **145** with a methanolic solution of hydroxylamine and KOH gives rise to the hydroxamic acid derivatives (**146**). On the other hand, oxidation of **145** with *m*-chloroperbenzoic acid followed by reaction with a methanolic solution of hydroxylamine and KOH affords the sulfonyl analogues (**147**). Partial oxidation of **145** with sodium borate in acetic acid results in the formation of a sulfinyl intermediate, which upon treatment with a methanolic solution of hydroxylamine and KOH gives rise to the sulfinyl derivatives (**148**).

In spite of the reasonable PDF inhibitory activity and antibacterial potency, development of this class of compounds was halted due to their weak potency compared to those of the actinonin, BB-3497, and its derivative BB-38698 [87, 88], which is currently in Phase I clinical trials.



Scheme 29 Synthesis of isoxazole-3-hydroxamic acid derivatives

5
Inhibitors of Bacterial Fatty Acid Synthesis as Potential Antibacterial Agents

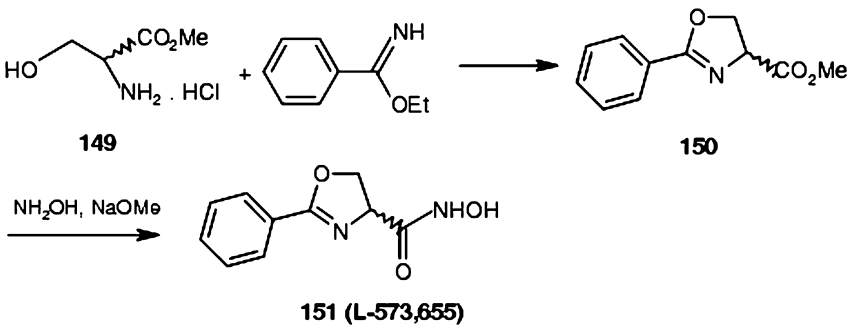
Septic shock, which is caused by systemic Gram-negative bacterial infection, is the most frequent cause of death in hospital intensive care units. Gram-negative bacterial sepsis arises from the systemic response to infection, mainly the overexpression of cytokines and inflammatory mediators in response to macrophage activation by endotoxins (also known as lipopolysaccharides or LPS) [89]. Approximately 2×10^6 LPS molecules assemble to form the outer monolayer of the outer membrane of the Gram-negative bacterium, and serve as a permeability barrier that protects the bacterium from many antibiotics. The hydrophobic anchor of LPS is lipid A, a phosphorylated, $\beta(1 \rightarrow 6)$-linked glucosamine disaccharide hexa-acylated with *N*-linked and *O*-linked fatty acids. Lipid A is essential for LPS assembly in the outer membrane of the Gram-negative bacteria; as a result lipid A-defective bacterial strains are remarkably hypersensitive to antibiotics [90, 91]. Considering that lipid A is the toxic component of LPS and is essential for bacterial survival, inhibitors of the enzymes in the lipid A biosynthetic pathway may comprise antibacterial agents that target Gram-negative bacteria.

UDP- 3 - *O* - [(*R*) - 3 - hydroxymyristoyl] - *N*-acetylglucosamine deacetylase (LpxC) catalyzes the deacetylation of UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-*N*-acetylglucosamine, the first committed step in the biosynthesis of lipid A, and is essential for bacterial growth and virulence [89, 92]. LpxC is a metalloenzyme from a class of zinc-dependent deacetylases [93] and functions via a general acid–base catalyst pair mechanism [94]. Inhibitors of bacterial enzyme LpxC have been demonstrated to have strong antibacterial activity, validating LpxC as a drug target for the development of antibacterial agents selective against Gram-negative bacteria. These inhibitors mainly contain hydroxamate or phosphonate zinc-binding motifs. The synthesis of some LpxC inhibitors showing significant antibacterial activity is described in this section.

5.1
Oxazoline Hydroxamates as Potential LpxC Inhibitors and Antibacterial Agents

The discovery of oxazoline hydroxamates as potential inhibitors of LpxC was the result of high-throughput screening of large libraries of compounds at the Merck Research Laboratories in collaboration with the Department of Biochemistry, Duke University Medical Center [95]. The lead compound, L-573,655, was a racemic mixture of 4-carbohydroxamido-2-phenyl-2-oxazoline, which had been previously made by Stammer et al. [96] as a precursor in the chemical synthesis of cyclosporine. Namely, (*R*,*S*)-serine methyl ester hydrochloride (**149**) is converted into (*R*,*S*)-4-carbomethoxy-2-phenyl-2-oxazoline (**150**) via treatment with ethyl benzimidate using the Elliot procedure [97]. Treatment of this ester with one equivalent each of hydroxylamine and sodium methoxide in methanol at room temperature affords the desired (*R*,*S*)-4-carbohydroxamido-2-phenyl-2-oxazoline (**151**), as depicted in Scheme 30.

The LpxC inhibitory and antibacterial activity of L-573,655 was not satisfactory. In an attempt to measure the enantiomeric selective activity of



Scheme 30 Synthesis of L-573,655

this compound, the authors synthesized the pure *R* and *S* enantiomers of L-573,655 and identified the *R* isomer (L-159,463) as the active enantiomer [95]. Following the Stammer method for the synthesis of 2-oxazoline hydroxamates [96] by using (*R*)-serine methyl ester and appropriately substituted ethyl arylimidates, over 200 analogues of L-573,655 were synthesized by the same group in order to optimize the LpxC inhibitory and antibacterial activities of this class of compounds [98]. Among the synthesized compounds, (*R*)-4-carbohydroxamido-2-(*p*-methoxyphenyl)-2-oxazoline (L-159, 692) and (*R*)-4-carbohydroxamido-2-(3-propyl-4,5-dimethoxyphenyl)-2-oxazoline (L-161,240) exhibited strong LpxC inhibitory and antibacterial activities, with L-161,240 being the most active compound [95] (Fig. 9).

The first attempt in making a large library of oxazoline LpxC inhibitors was reported by Pirrung et al. [99] via a "catch-and-release" ring-forming reaction that utilizes β-hydroxyamines and acid chlorides as the diversity inputs. In this procedure, the initial *N*-acylated product can be captured onto polymer-bound tosyl chloride, eliminating the need for an extractive purification in the first step. The resin-bound intermediate is washed extensively to remove nonnucleophilic impurities then treated with a volatile, nonnucleophilic base to initiate ring-forming cleavage from the resin. This sequence of steps has proven to be highly amenable to parallel and semiautomated methods of synthesis.

Using this procedure, Pirrung et al. [100] synthesized L-159,692 and its analogues according to Scheme 31. Namely, the key intermediate *O*-protected serine hydroxamate (**156**) is prepared in a reaction sequence starting from 2,4-dimethoxybenzyl alcohol (**152**), which then undergoes a modified Barlaam's procedure [101] to yield the *O*-protected hydroxylamine (**153**). Reaction of this intermediate with BOC-D-serine (**154**) results in the formation of the diprotected serine derivative (**155**). The BOC protecting group is removed using a significantly modified Sakaitani procedure [102], in which intermediate **155** is treated with 4 equivalents of TMS-triflate in the presence of an excess of 2,6-lutidine to give the corresponding TMS-carbamate. The TMS-carbamate is then easily removed by treatment with triethylamine in methanol to give the desired compound **156**. Compound **156** is acylated with *p*-anisoyl chloride to give the acylated intermediate (**157**), which is then loaded onto an excess (3 equivalents) of tosyl chloride resin at $-15\,^{\circ}C$ to give the resin-bound species (**158**). The resin is washed extensively and then

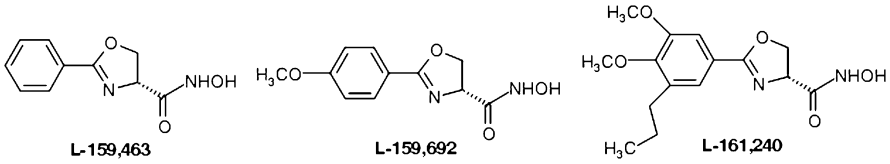
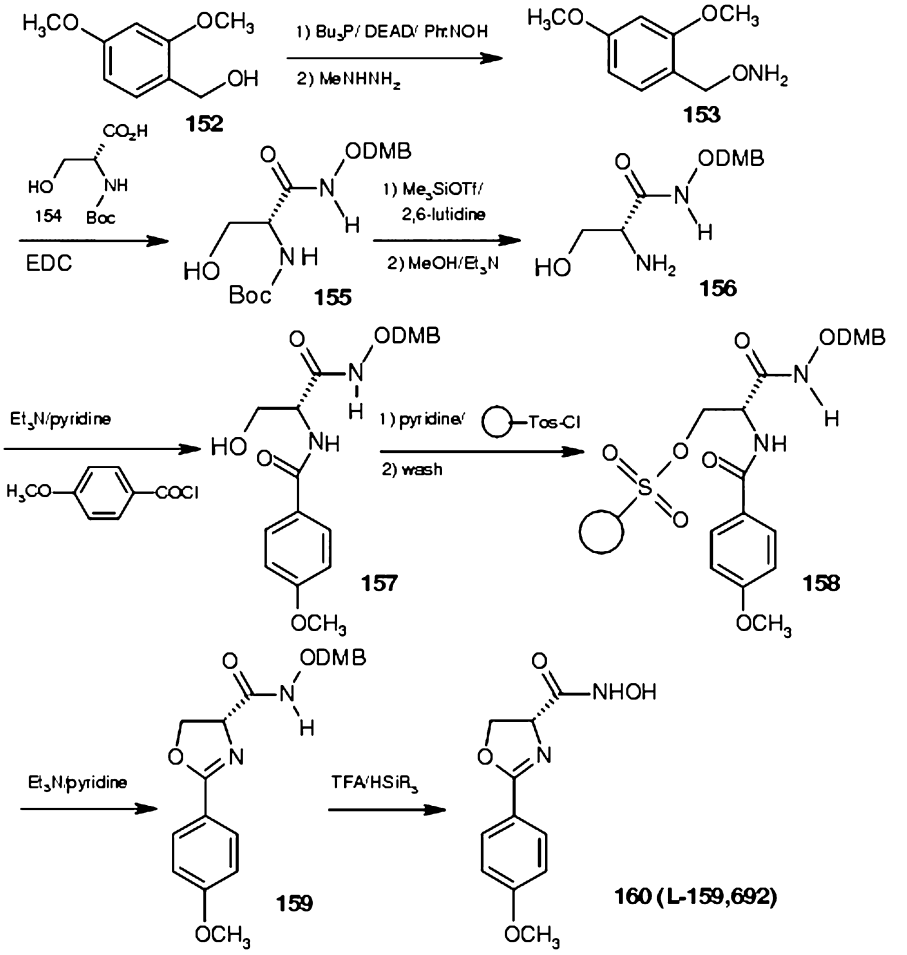


Fig. 9 Structures of L-159,463, L-159,692, and L-161,240



Scheme 31 Catch-and-release synthesis of L-159,692 and its analogues

treated with a mixture of pyridine and triethylamine in THF to give compound **159**. Removal of the 2,4-dimethoxybenzyl protecting group from **159** can be easily carried out by treatment with dilute TFA in the presence of trialkyl silane to give the hydroxamate derivative (**160**; L-159,692), as shown in Scheme 31.

This procedure was utilized by the same authors to synthesize a large library of 4-carbohydroxamido-2-(aryl or alkyl)-2-imidazolines, some of which exhibited excellent LpxC inhibitory and antibacterial activities. The technical maneuvers in the syntheses of these compounds and their structure–activity relationships are comprehensively covered by the authors in their paper.

5.2 Synthesis of Isoxazolone Analogues of L-159,692

One problem in obtaining analogues of the original lead oxazoline hydroxamate bearing various metal binding groups is the difficulty in finding the appropriate serine analogues to serve as precursors. Furthermore, oxazolines are essentially sensitive to nucleophiles such as the thiols that would be of great interest to incorporate into the inhibitors [103]. A potential solution to these problems is to replace the oxazoline scaffold with isomeric heterocycles, to increase its stability toward nucleophiles and to allow the facile incorporation of a wide variety of groups in place of hydroxamic acid. One class of heterocycles that fits these criteria is the 4,5-dihydro-isoxazoles (Fig. 10). These isoxazolines are reasonably stable toward nucleophiles and are easily derived from 1,3-dipolar cycloaddition with an acrylate or substituted ethylene [104]. Ethylenes substituted with an electron-withdrawing group generally react to give a single regioisomer, as depicted in Fig. 10 [105].

Using the above procedures, the racemic isoxazoline analogue of L-159,692 was synthesized according to Scheme 32. Namely, the diprotected hydroxylamine derivative TMB-NHO-DMB (**161**), prepared according to Barlaam's method [101] from the reaction of *O*-(2,4-dimethoxybenzyl)hydroxylamine (**153**) and 2,4,6-trimethoxybenzaldehyde followed by reduction with sodium cyanoborohydride, is allowed to react with acryloyl chloride to yield the diprotected hydroxamoyl intermediate (**162**). Further, the 1,3-dipolar cycloaddition reaction of **162** with 4-methoxybenzohydroximinoyl chloride (**163**), synthesized according to the procedures reported by Pirrung et al. [103] and Liu et al. [106] by the reaction of 4-methoxybenzaldehyde with hydroxylamine followed by further reaction of the resulting oxime with *N*-chlorosuccinimide, affords the diprotected hydroxamate (**164**). Deprotection of compound **164** using trifluoroacetic acid and triethylsilane gives rise to the desired isoxazoline hydroxamate (**165**) as a racemic mixture, as shown in Scheme 32.

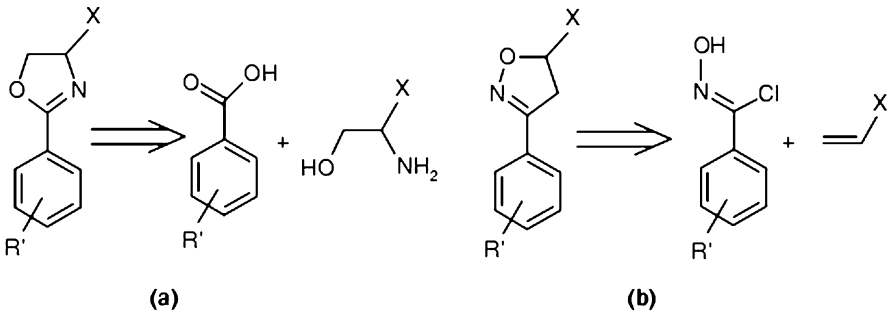
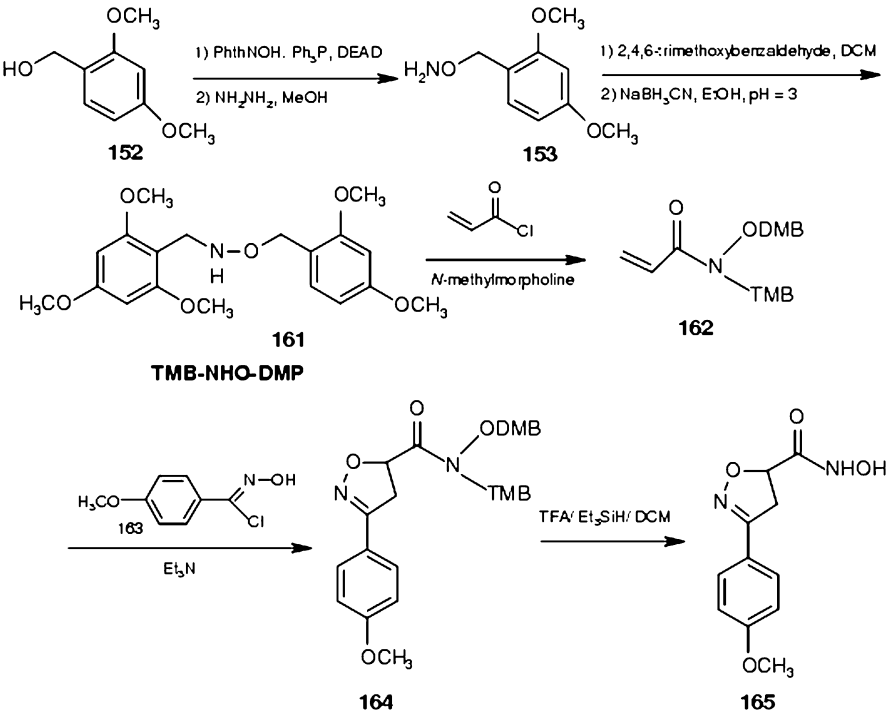


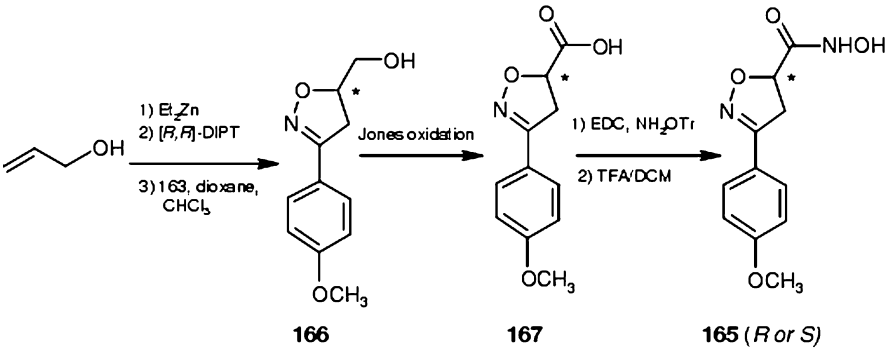
Fig. 10 Retrosynthesis of oxazolines (**a**) and isoxazolines (**b**)



Scheme 32 Synthesis of isoxazolone analogues of L-159,692

The results of enzymatic and antibacterial assays demonstrated that compound **165** (as a racemic mixture) exhibits activities similar to those of the parent oxazoline L-159,692 [103]. In order to identify the enantioselectivity of this compound, the corresponding *R* and *S* enantiomers were separately synthesized from (*R*)- or (*S*)-3-(4-methoxyphenyl)-5-hydroxymethyl-4,5-dihydroisoxazole (**166**) according to the method of Ukaji et al. [107, 108] and tested for LpxC inhibitory and antibacterial activities [103]. Interestingly, only the *S* enantiomer of **166** exhibited strong enzyme inhibitory and antibacterial activity, while the *R* enantiomer of L-159,692 was the active component. The enantiomeric synthesis of compound **165** is depicted in Scheme 33.

Namely, allyl alcohol is successively treated with diethylzinc, (*R*,*R*)-dipropyl tartrate, and 4-methoxybenzohydroximinoyl chloride (**163**) to afford the enantiomeric isoxazoline alcohol **166**, which under the Jones oxidation conditions affords the corresponding carboxylic acid derivative (**167**). Treatment of compound **167** with hydroxylamine-*O*-triflate followed by trifluoroacetic acid gives rise to the desired enantiomeric **165** in high excess enantiomeric yield. The synthesis of other isosteric analogues of **165** was reported in the same paper. None of the isosteric analogues exhibits LpxC inhibitory and antibacterial activities [103].



Scheme 33 Enantiomeric synthesis of compound **165**

5.3 Synthesis of a Carbohydrate-Derived Hydroxamic Acid Inhibitor of LpxC

The carbohydrate-derived hydroxamic acid derivatives were designed due to the resemblance of the carbohydrate part to the natural substrate of the LpxC enzyme: UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-GlcNAc. Jackman et al. [109] reported the LpxC inhibitory activity of TU-514, which structurally resembled UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-GlcNAc [Fig. 11], against a broad range of bacterial LpxCs.

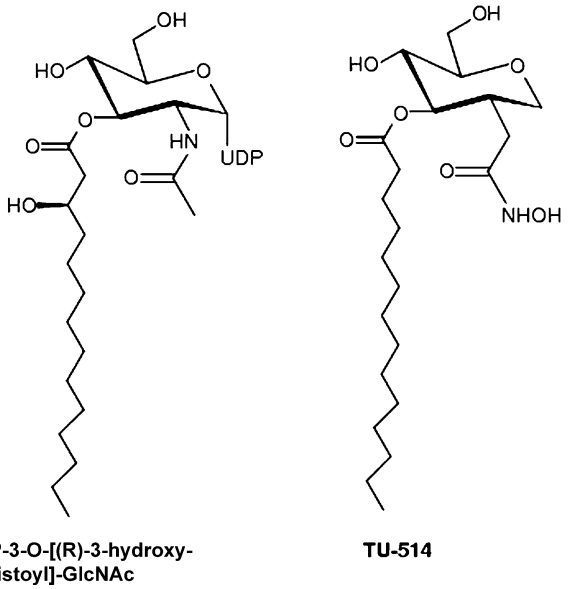
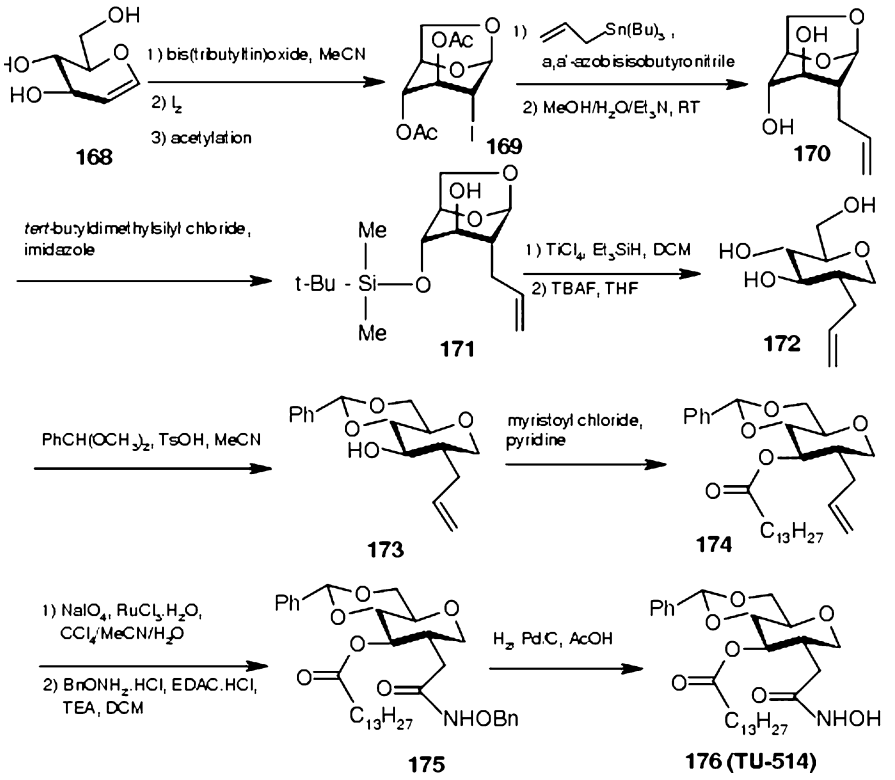


Fig. 11 Structure of UDP-3-*O*-[(*R*)-3-hydroxymyristoyl]-GlcNAc and TU-514

The synthesis of TU-514 was first reported by Li et al. [110]. The authors carried out two different synthetic procedures, and the one with the better yield and less complexity will be described in this section. The starting material for this procedure is compound **170**, which is prepared from D-glucal (**168**) according to the method of Leteux et al. [111]. Namely, *O*-stannylation of D-glucal followed by iodocyclization and acetylation affords compound **169**. Treatment of **169** with allyltributylstannane and α,α′-azobisisobutyronitrile followed by deacetylation yields the desired axially branched *C*-allyl derivative (**170**). Selective dimethylsilylation of **170** with *tert*-butyldimethylsilyl chloride and imidazole gives rise to compound **171**, which upon treatment with $TiCl_4$ and triethylsilane followed by desilylation yields compound **172**. Reaction of **172** with benzaldehyde dimethylacetal affords compound **173**, which upon acylation with myristoyl chloride gives rise to compound **174**. Oxidative cleavage of the double bond in **174** in the presence of ruthenium tetroxide gives the corresponding carboxylic acid intermediate, where the hydroxamic acid function could be introduced near the end of the synthetic route using *O*-benzylhydroxylamine/EDAC to give the



Scheme 34 Synthesis of TU-514

protected hydroxamic acid derivative (**175**). Finally, catalytic hydrogenation of **175** yields compound **176** (TU-514) in an overall yield of 27% (from **170**), as compared to the other procedure, which gives an overall yield of 12.5%. Scheme 34 describes the selected synthetic procedure for TU-514.

6 Concluding Remarks

The never-ending battle between humankind and pathogenic microorganisms has reached a climax. The smart biochemical machinery of the bacterium keeps mutating in response to any new therapeutic agent, providing a survival tool for the microorganism (resistance to antibacterial agents). This does not mean that we, as scientists and healthcare providers, should wave a white flag and give up the battle against pathogenic bacteria. There are still many unknown biochemical mechanisms involved in the bacterial life cycle that can be targeted and inactivated wisely using smart research tools. In the meantime, research on the understanding of the bacterial resistance mechanisms helps the design of chemicals that are able to target enzymes involved in specific resistance mechanisms, such as β-lactamases, and have the potential of being used in combination with strong, but resistance-prone, antibacterials to improve their therapeutic efficiency. The synthetic approaches and the design rationales for novel antibacterial agents (of nonfermented origin) discussed in this chapter will hopefully open an insightful window for nonmedicinal chemists.

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Overcoming Bacterial Resistance: Role of β-Lactamase Inhibitors

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Abstract Resistance to modern antibiotics is currently a major health concern in treating infectious diseases. Abuse, overuse, and misuse of antibiotics in treating human illness have caused the pathogens to develop resistance through a process known as natural selection. The most common mechanism of resistance to β-lactam antibiotics is the production of β-lactamases, which destroy β-lactam antibiotics before they reach the bacterial target. Over the last two decades, combination therapy involving treatment with a β-lactam antibiotic and a β-lactamase inhibitor has become very successful in controlling β-lactamase-mediated bacterial resistance. Currently available inhibitors like

clavulanate, sulbactam, and tazobactam are not suitable for use in combination with β-lactamase-susceptible antibiotics against the newly emerged β-lactamases, including class C enzymes. Over the last 10 years, X-ray crystallography, electrospray ionization mass spectrometry, and molecular modeling have become useful tools for determining the molecular basis for interactions between β-lactamases and β-lactamase inhibitors. This chapter describes some of the structure- and mechanism-based approaches that have been utilized in designing new broad-spectrum β-lactamase inhibitors of various β-lactam classes.

Keywords AmpC · β-Lactamase · β-Lactam · Cephem · Inhibitor · Metalloenzyme · Monobactam · Penem · TEM

1 Introduction

Many bacterial infections that used to be cured with specific antibiotics are now becoming resistant to them. Soon after penicillin was introduced into the market as a miraculous drug for treating bacterial infections, scientists began noticing the emergence of a penicillin-resistant strain of *Staphylococcus aureus*. Resistant strains of *Neisseria gonorrhoeae*, dysentery-causing *Shigella*, and *Salmonella* rapidly followed in the wake of *Staphylococcus*. Since then antibacterial resistance has become a serious public health problem with economic, social, and political implications that are global in scope. Multidrug-resistant tuberculosis (MDR-TB) is no longer confined to any one country, but has appeared in locations like Eastern Europe, Africa, and Asia. Likewise, penicillin-resistant pneumococci are spreading rapidly, while resistant malaria is killing millions of children and adults each year in the developing countries. In the industrialized countries, as many as 60% of hospital-acquired infections are caused by vancomycin-resistant *Enterococcus* (VRE) and methicillin-resistant *S. aureus* (MRSA). These infections are no longer confined to hospitals, but have crept into the community at large. Over the last 10 years one of the major challenges facing infectious disease physicians and microbiologists has been the control of hospital-acquired MRSA and VRE infections. The recent description of transferable VanA-mediated vancomycin resistance in MRSA is rather disturbing and may limit the use of a handful of newer agents such as linezolid and some novel cephalosporins. Infections caused by Gram-negative bacteria constitute about one-half of the cases of life-threatening nosocomial disease [1, 2]. The most widely used antibiotics are β-lactams such as penicillins and cephalosporins. This family of antibiotics is favored because of their broad spectrum of activity, good safety profile, and proven clinical efficacy. Their widespread use, however, has resulted in the emergence and rapid spread of resistance. The past decade has seen an increase in the frequency of resistance to modern antibiotics, including the "third-generation" cephalosporins [3, 4]. Resistance develops

wherever antibiotics are abused, overused, misused, and dispensed at levels lower than the suggested treatment guidelines. This means that instead of wiping out the infection altogether, antibiotics kill only the non-resistant organisms, while leaving behind their tougher counterparts to replicate and spread resistance genes. β-Lactam antibiotics interact with two main groups of bacterial enzymes. The first group consists of the cell wall synthesizing enzymes, which are inactivated by binding to β-lactam antibiotics. The second group of enzymes, β-lactamases, function to protect the cell by inactivating the β-lactam antibiotics. It is this latter group of enzymes with which we are concerned. This chapter is based on the efforts to identify effective inhibitors to inactivate these enzymes.

2
Resistance: Biochemical Aspects

An antibiotic inhibits bacterial growth if it is able to reach the site of its action, interacts, and substantially inhibits the function of an essential component necessary for its growth. A given bacterial cell becomes resistant to an antibiotic if at least one of these steps is no longer operative. This can result from one of the following four biochemical mechanisms.

2.1
Chemical Transformation of the Antibiotic

Both Gram-positive and Gram-negative bacteria can become resistant to β-lactam antibiotics via the production of β-lactamases. These enzymes catalyze the irreversible opening of the β-lactam ring, inactivating the antibiotic before it reaches its target penicillin binding proteins (PBPs). The β-lactamases produced by different resistant strains are not all identical. For example, the staphylococcal β-lactamase hydrolyzes penicillins but is not active against cephalosporins, in contrast to that of *Escherichia coli*, which is active against both classes.

2.2
Modification of the Target Site

In the case of β-lactam antibiotics, the bacterial targets are the so-called PBPs. Modification of PBPs can result in a decreased affinity for β-lactam antibiotics. In some cases the normal PBPs, through mutation, become less susceptible to acylation and inactivation by β-lactam antibiotics. So, the target protein is unable to bind the antibiotic effectively, and hence resistance to a particular antibiotic is acquired [5]. Frequently, this difference consists of substitution of a single amino acid in the protein chain. In *S. aureus*, ac-

quisition of a single new penicillin binding protein, PBP 2a, is responsible for resistance of this species to methicillin and other penicillinase-resistant penicillins. Since PBP 2a has low affinity for all β-lactam antibiotics, methicillin-resistant *S. aureus* are resistant to all drugs of this family. Resistance to penicillin via this mechanism among isolates of *Streptococcus pneumoniae* and *Neisseria gonorrhoeae* is becoming more common.

2.3 Modification of the Permeability

Changes in the outer membrane of Gram-negative bacteria may hinder the penetration of a β-lactam antibiotic, preventing access to the PBPs. This type of resistance is mostly attributed to loss or modification of porins, which are proteins that form hydrophilic channels in the outer membrane, allowing small water-soluble molecules to penetrate into the periplasmic space. This mechanism is particularly prevalent in organisms such as *Pseudomonas aeruginosa*. It has been shown that in modified bacteria (those exposed to antibiotics), the particular antibiotic is not able to use these porin channels effectively and hence these bacteria are resistant to this antibiotic [6]. Among the β-lactam antibiotics, resistance to imipenem via this mechanism is the single most common mechanism observed among isolates of *P. aeruginosa*. In this particular species, loss of a specific porin channel leads to resistance to imipenem and other carbapenems.

2.4 Efflux Action

Bacteria possess biological efflux pumps that involve an energy-dependent export of antibiotics, among other substances, from within either the periplasm or cytoplasm to the outside environment. This is essentially a detoxification process for the bacteria. The phenomenon decreases the concentration of antibiotic inside the cell [7].

3 Resistance: Genetic Aspects

Pathogens develop resistance to antibiotics through a process known as natural selection. During the process of duplication of genetic material, errors of replication may occur that lead to a change in the genetic information of the microorganism. Such modifications are called mutations. When a microbial population is exposed to an antibiotic, the susceptible organisms will succumb, leaving behind only those resistant to the antibacterial onslaught. Then the resistant organisms continue to multiply, and eventually the whole

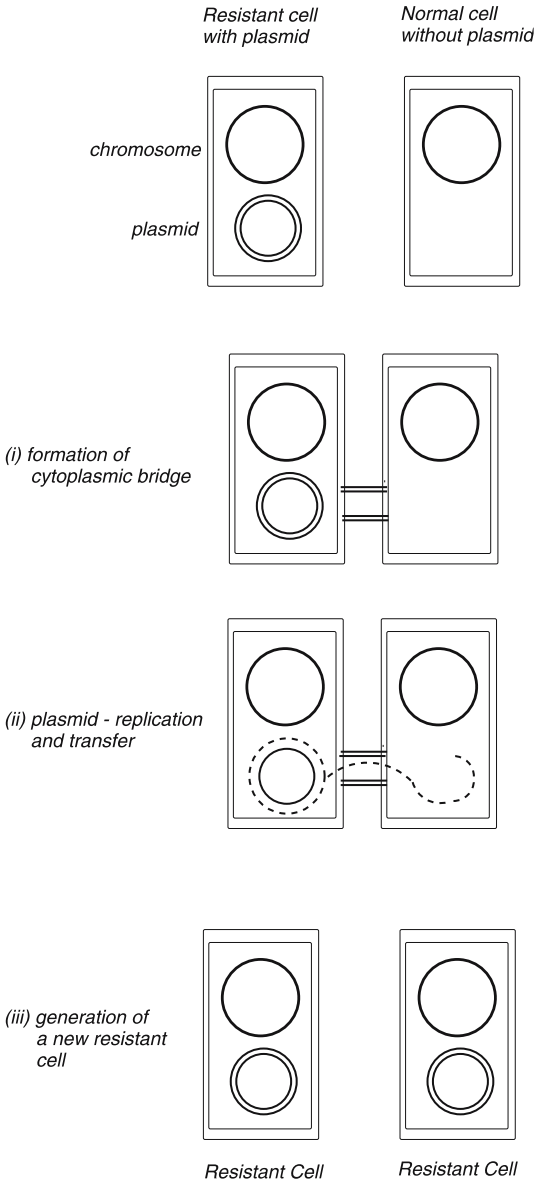


Fig. 1 Transfer of plasmid by conjugation and generation of a new resistant cell

population is composed of resistant bacteria. This action of the antibiotic is termed the selection of resistant mutants. The emergence and spread of bacterial populations resistant to antibiotics are the result of the combined effects of mutation and selection. These organisms can then either pass on their resistance genes to their offspring by replication (chromosomally medi-

ated resistance), or to other related bacteria through "conjugation" whereby plasmids carrying the genes "jump" from one organism to another (plasmid-mediated resistance).

3.1 Chromosomally Mediated Resistance

When bacteria are exposed to an antibiotic, only the cell with the resistant gene survives but it can then multiply, copying the resistant gene to its millions of progeny. This way, susceptible cells are obliterated and resistance becomes a stable feature of the bacterial population (Fig. 1).

3.2 Plasmid-Mediated Resistance

A resistant gene can also live on a plasmid which is extra-chromosomal. Plasmids can carry a string of genes for resistance to several different antibiotics. Plasmids can "jump" to another bacteria (recipient) and this way pass on the resistant genes without waiting for their host cell to divide. This is how multiresistance can spread in a population (Fig. 1).

4 Why β-Lactamase Inhibitors?

It was not until after ampicillin came into wide use following its release in 1961 that it was appreciated that bacterial β-lactamase might be of crucial importance in determining resistance to β-lactam antibiotics in Gram-nagative bacteria, as well as in staphylococci. Initial approaches to overcoming β-lactamase-mediated resistance involved the development of semisynthetic β-lactam agents with minor variations in the structure of the existing antibiotics. The goal was to develop new agents that would resist hydrolysis by the common β-lactamases. Using this approach several third generation cephalosporins, carrying a sterically bulky oxyimino aminothiazolyl substituent at C-7 position, were introduced into the market. Unfortunately, resistant strains possessing extended spectrum β-lactamases (ESBLs), including the most rapidly growing new families of ESBLs named CTX-M (cefotaximase), appeared immediately after their introduction and are now capable of hydrolyzing these new cephalosporins [8]. In many cases, the resistance to these antibiotics is due to a high level of expression of class C β-lactamases [9–11]. These enzymes are mostly chromosomally encoded, but recently class C enzymes have appeared on plasmids, thus crossing interspecies boundaries [12]. An attractive strategy has been to protect a potent β-lactam antibiotic by another β-lactam that is a specific β-lactamase antago-

nist. The ultimate aspiration is, however, the β-lactam that retains its affinity for the membrane peptidases while remaining impervious to the potent hydrolytic capability of the β-lactamase.

5
The β-Lactamases

5.1
Microbiology

The bacterial β-lactamases are a continuously evolving and diverse group of enzymes involved in protecting bacteria from the effect of β-lactam antibiotics. Abraham and Chain first reported β-lactamase activity in 1940, noting that the extract of an *E. coli* strain destroyed penicillin [13]. They called the enzyme penicillinase. The age of penicillin saw the rapid emergence of resistance in *S. aureus* due to plasmid-encoded penicillinase. This β-lactamase quickly spread to most clinical isolates of *S. aureus* as well as to other species of staphylococci. Among the Gram-positive bacteria, *S. aureus* is the only important pathogen that is routinely β-lactam resistant. More than 90% of *S. aureus* strains worldwide now produce β-lactamases, which is liberated extracellularly and hydrolyzes all penicillins other than those in the methicillin group. Similar spread of β-lactamases occurred in Gram-negative bacteria following the introduction and widespread clinical use in the 1960s of the early broad spectrum penicillins (e.g., ampicillin and carbenicillin) and cephalosporins (e.g., cephalothin and cephaloridine). Most importantly, plasmid-mediated β-lactamases spread in *Enterobacteriaceae* and other Gram-negative bacteria. The first such β-lactamase, TEM-1, was found in 1965 in an ampicillin-resistant strain of *E. coli* isolated from the blood culture of a young Greek girl named Temoniera; hence the designation TEM [14]. The TEM-group remains most widely distributed, accounting for 70–80% of plasmid-mediated β-lactamases from *Enterobacteriaceae*, and almost all those from *Haemophilus influenzae* and *N. gonorrhoeae*. The broad spectrum TEM-1, its single amino acid variant TEM-2, and the functionally similar SHV-1 enzyme, together with the oxacillin hydrolyzing OXA-1 enzyme are the most common plasmid-encoded β-lactamases in Gram-negative bacteria. Chromosomal β-lactamases have also proved a source of resistance to broad spectrum cephalosporins, cephamycins, and monobactams in *P. aeruginosa*, *Enterobacter spp*., *Citrobacter spp*., *Serratia spp*., *Morganella spp*. and *Providencia spp*. These species have inducible class C "AmpC" β-lactamases, and the cephalosporins are prone to select mutants that hyperproduce these enzymes independently of induction. With the aim of overcoming resistance to β-lactam antibiotics mediated by the above mentioned β-lactamases, new β-lactam antibiotics were introduced to the market in the

1980s. Development of the third generation cephalosporins in the early 1980s was based heavily on the ability of these agents to escape hydrolysis by all the common β-lactamases in both Gram-positive and Gram-negative bacteria. One of these new classes had the oxyimino aminothiazolyl side chain at the 7-position of the cephalosporin nucleus. These new cephalosporins (e.g., cefotaxime, ceftazidime, and ceftriaxone) and the monobactam aztreonam exhibited good antibacterial activity against Gram-negative organisms, in part because of their exceptional stability to the infamous TEM, SHV, and OXA enzymes. However, with each new class that has been used to treat patients, new β-lactamases have emerged that caused resistance to the new class of drug. Presumably, the selective pressure of the use and overuse of new antibiotics in the treatment of patients has selected for new variants of β-lactamase. Thus the newer third generation cephalosporins were challenged by an unexpected set of mutational events shortly after their introduction into clinics. Some of these enzymes represent stepwise selection of variants of the common plasmid-encoded TEM, SHV, and OXA broad spectrum β-lactamases, or the inhibitor-resistant TEM enzymes. The first extended spectrum β-lactamase (ESBL) was reported from Germany in 1983 with the description of three independent *K. pneumoniae* isolates from the same hospital exhibiting transferable cefotaxime resistance [15]. The name ESBL originates because of their increased spectrum of activity, especially against the oxyimino aminothiazolyl cephalosporins. Major outbreaks of ESBL producing *Enterobacteriaceae* were first reported from France, where 283 cefotaxime-resistant *K. pneumoniae* isolates were detected from 1984 to June 1987, in addition to another 200 isolates of *E. coli*, *Enterobacter spp*., *Serratia marcescens*, *K. oxytoca*, and *Citrobacter freundii* that produced the same ESBL [16]. One of the most rapidly growing new families of ESBLs is the CTX-M family. CTX-M-1 was first identified in cefotaxime-resistant *K. pneumoniae* isolates from Western Europe; CTX-M-2 was then found in several South American isolates and differed by 16% in its amino acid sequence from CTX-M-1 [17, 18]. These enzymes, called cefotaximases, strongly prefer cefotaxime as a substrate and hydrolyze ceftazidime poorly. The combined contributions of porin mutations, quantity of enzyme activity, and number of β-lactamases per strain result in elevated minimum inhibitory concentrations (MICs) for these cephalosporins. Over the past six decades, a wide variety of β-lactamases have been identified. The number of unique β-lactamases now approaches nearly 470, and this number continues to grow.

5.2 Classification

β-Lactamases have been classified according to either molecular characteristics or according to their functional properties. Using amino acid sequencing, Ambler [19] categorized these enzymes into four molecular classes, desig-

nated as A, B, C, and D. Class A, C, and D enzymes are remote in evolutionary terms, but share the same catalytic mechanism in which a serine residue at the active site forms an acyl ester with the opened β-lactam ring. Hydrolysis of this ester reliberates the active enzyme and inactivated drug. Class B β-lactamases have a different mechanism based on zinc ion located at the active site, and are designated metallo-β-lactamases. With the discovery of more β-lactamases, and the availability of additional sequence data and biochemical information, Bush et al. [20] introduced a new and more elaborate classification for these enzymes. However, for simplicity, Ambler's classification will be used throughout this chapter. The most clinically important β-lactamases are those of class A, whose members include the plasmid-based TEM penicillinases, and class C, represented by chromosomal cephalosporinases.

5.3 Location

In Gram-positive organisms, the only clinically important β-lactamase is the staphylococcal penicillinase, which is encoded by a plasmid and its expression is inducible. Since staphylococci, like all Gram-positive organisms,

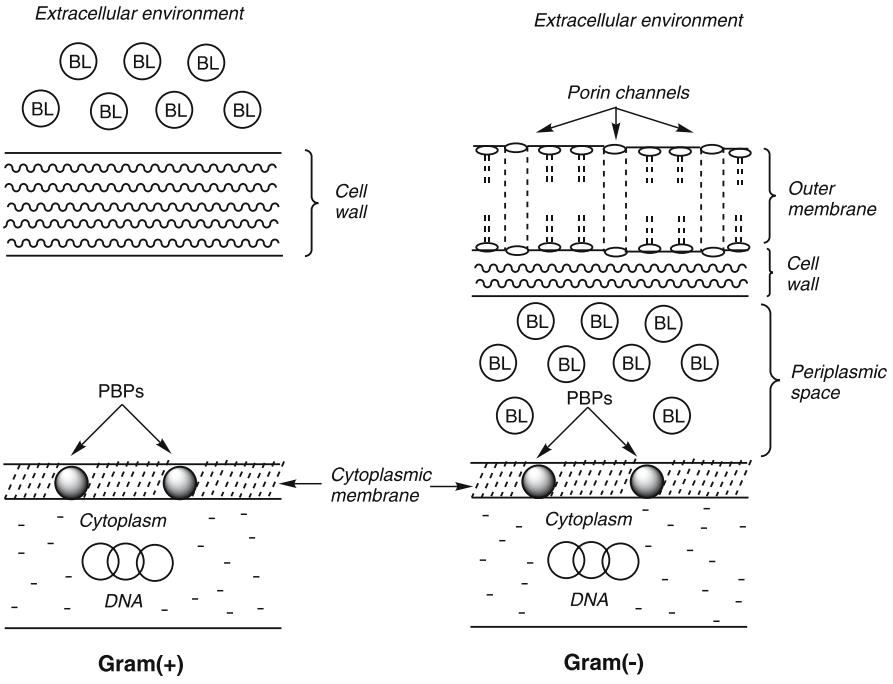


Fig. 2 Location of β-lactamase in Gram-positive and Gram-negative bacteria

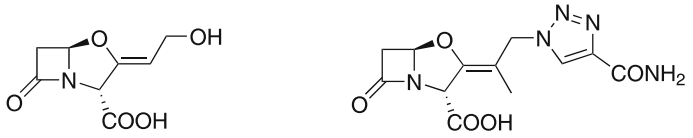
do not have a permeability barrier between penicillin binding proteins and the extracellular environment, staphylococci produce very large quantities of penicillinase and secrete it extracellularly. In Gram-negative bacteria, there are many different types of β-lactamases. Some are encoded by genes on the chromosome while others are encoded by genes on plasmids. Expression of some of the chromosomal enzymes is inducible while expression of plasmid-mediated enzymes is always constitutive. Since the outer membrane of Gram-negative bacteria acts as a permeability barrier for β-lactam antibiotics, the amount of drug entering through the outer membrane into the periplasmic space is much less than the concentration in the extracellular environment. So, the Gram-negative bacteria do not need to produce a large amount of β-lactamase. Besides, it is not necessary for Gram-negative bacteria to secrete their β-lactamases into the extracellular environment since the enzymes need only inactivation of those drug molecules that pass through the outer membrane. Therefore in Gram-negative bacteria, β-lactamases are located intracellularly in the periplasmic space (Fig. 2).

6
β-Lactamase Inhibitors

Beginning in the late 1980s, three β-lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam) have been used against serine enzymes, usually in combination with penicillins more susceptible to β-lactamase hydrolysis. This therapeutic strategy has been effective over two decades. The following section provides a brief overview on various classes of β-lactam-based inhibitors.

6.1
Clavulanic Acid and its Derivatives

The real breakthrough in identifying a β-lactamase inhibitor came in 1976 with the isolation of a bicyclic β-lactam, called clavulanic acid **1a** (Fig. 3), produced by a strain of *Streptomyces clavuligerus* [21, 22]. In itself, this bicyclic β-lactam is a weak antibiotic, but a powerful inhibitor of most class A en-



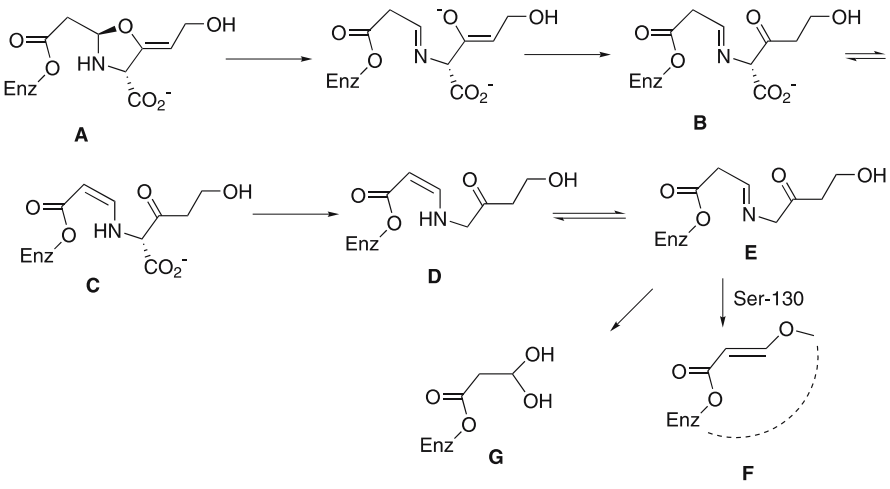
1a **1b**

Fig. 3 Clavulanic acid and its derivative

zymes. In combination with a β-lactam antibiotic, it increases the concentration and lifetime of the antibiotic to the point that the antibiotic becomes lethal, causing the cell death of the bacterium.

The detailed mechanism of inhibition of TEM-2 (class A) enzyme with clavulanate has been established (Scheme 1) [23, 24]. The inhibition is a consequence of the instability of the acyl enzyme formed between the β-lactam of clavulanate and the active site Ser-70 of the enzyme. In competition with deacylation, the clavulanate acyl-enzyme complex **A** undergoes an intramolecular fragmentation. This fragmentation initially provides the new acyl enzyme species **B**, which is at once capable of further reaction, including tautomerization to an entity **C** that is much less chemically reactive to deacylation. This species **C** then undergoes decarboxylation to give another key intermediate enamine **D**, which is in equilibrium with imine **E**. The imine **E** either forms stable cross-linked vinyl ether **F**, by interacting with Ser-130 or is converted to the hydrated aldehyde **G** to complete the inactivation.

The clinical success of clavulanate stimulated extensive research to modify this bicyclic β-lactam chemically with the hope of getting a clinical successor to clavulanic acid. Mearman et al. [25] replaced the hydroxyl group of clavulanic acid with a variety of substituted triazolyl groups. Most of these derivatives showed a slight improvement in activity over clavulanic acid. Compound **1b** displayed the best activity against several β-lactamases from *S. aureus*, *E. coli*, and *K. aerogenes*. A number of clavulanic acid analogs lacking a carboxyl group at C-3 (clavam) have also been reported [26–28]. Although chemical modification of clavulanate has been investigated extensively [29], it has failed to provide a second generation β-lactamase inhibitor for clinical use.

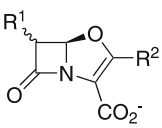


Scheme 1 Mechanism of inhibition of TEM-2 with clavulanate

6.2 Oxapenems

The main difference between clavulanic acid and the oxapenems is the presence of a double bond between C-2 and C-3 in the oxazolidine ring system (Fig. 4). The biological activity and chemical stability of this class of compounds are greatly influenced by the stereochemistry at the ring junction of the bicyclic β-lactam ring. The oxapenem derivative **2a** showed inhibitory activity against cell-free β-lactamases extracted from Gram-negative organisms, and showed better activity than clavulanic acid against Staphylococcal penicillinase PC1 and AmpC enzyme from *E. cloacae* P99 [30]. Several other oxapenem derivatives **2b** with a hydroxyethyl side chain at C-6, as in penems and carbapenems that have been developed for their antibiotic activity, displayed excellent activity against cephalosporinases produced by *E. coli* SR6, *Morganella morganii* SR7, *Providencia stuarti* SR1031, and *E. cloacae* SR92, but weak activity against the class A penicillinases of *Klebsiella oxytoca* SR 696, *E. coli* W3110, and *E. coli* ML 1410 [31]. In another attempt, Pfaendler and coworkers [32] prepared 5*R*- and 5*S*-*trans*-2-*tert*-butyl-6-hydroxymethyl oxapenem-3-carboxylate (**2c** and **2d**, respectively) that showed significant inhibitory profile against a selected group of β-lactamase-producing strains.

Not only the synthetic challenge, but also the chemical instability of the oxapenem skeleton are major obstacles in actively pursuing this class of compounds for commercial use. It would certainly be true to say that the design and chemical modification of the oxapenem core structure was dictated primarily by academic interest rather than by the clinical success. In comparison to the modifications of oxapenem, the chemical modifications of clavulanic acid have been far greater. This extensive modification has helped gather more knowledge about the inhibitory profile and the mode of action of the oxapenam skeleton, particularly of clavulanic acid. First, it seems that the oxapenem molecule is intrinsically incapable of providing the desired β-lactamase inhibitory profile. It was also soon discovered that these compounds were slowly degraded during biological testing or even upon long storage, and thus developments in the oxapenem field have been in a decidedly minor key.



2a: R^1 = H, R^2 = Et

2b: R^1 =  , R^2 = Me, Pr^i, Ph

2c: R^1 =  , R^2 = Bu^t

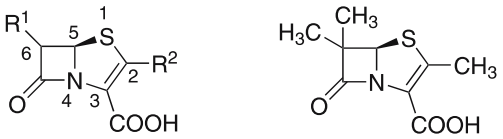
2d: R^1 =  , R^2 = Bu^t

Fig. 4 Oxapenem derivatives

6.3 Penems

To combine both structural features that appear to be associated with the biological activity of penicillins and cephalosporins (i.e., the five-membered ring and the double bond) in one single structure was an open challenge for synthetic chemists working in the β-lactam field. The idea of synthesizing such compounds became realistic in 1975, when a team at the Woodward Research Institute in Basel announced the first successful synthesis of the novel penem **3** (Fig. 5) at a lecture given to the Swedish Chemical Society in Stockholm. The discovery of clavulanic acid as a potent β-lactamase inhibitor and of thienamycin as a powerful broad-spectrum antibiotic raised hopes that interesting biological activity might be found in the penem skeleton. The synthesized penems, although very labile, proved sufficiently stable to permit isolation, and although not very active suggested antibiotic activity in the new β-lactam system. Within a short time, great effort was expended at several pharmaceutical companies in the search for a practical useable penem antibiotic. A delicate balance between reactivity of the β-lactam system on the one hand and reasonable stability on the other seems to be important for high biological activity in this class of compounds. To find such a fortunate balance in a penem, the possibility of modifying both positions C-2 and C-6 offers chemists an inexhaustible number of combinations to test.

The occurrence in nature of highly potent antibiotics with an alkyl or α-hydroxyalkyl substituent in position 6 of the 1-carbapenem nucleus gave an impetus to attempts to modify the closely related penem system structurally at the same position by synthesizing various 6-alkyl- and 6,6-dialkyl-substituted penem acids. Although the 6,6-dialkylpenem acid **4** was found to be devoid of antibacterial activity, the penem **4**, however, exhibited inhibition of β-lactamases from *P. aeruginosa* 18SA and *Enterobacter* P99 (Regos J, CIBA-Geigy, unpublished results, 1977). In the search for penems with increased biological activity, the α-hydroxyethyl group, which is the natural side chain of the highly active thienamycin, was thought to be an interesting substituent to examine. Pfaendler [33] reported that the penem **5** (Fig. 6) showed β-lactamase inhibitory activity against enzymes produced by *E. cloacae* and *E. coli*. A significant practical contribution was made by the scientists from the Beecham Research Laboratories, whose patents and publications describe



3 **4**

Fig. 5 Penem derivatives

the preparation of a large number of 6-ethylidene penem-3-carboxylic acids (Fig. 6).

These penems are very potent inhibitors of bacterial β-lactamases, with a broader spectrum of activity than any clinically available inhibitor (i.e., clavulanic acid, sulbactam, or tazobactam) and inhibit both class A and class C enzymes. From structure–activity relationship studies, it was apparent that replacement of a C-8 methyl group with either a thiophene or furan ring resulted in a considerable increase in synergistic activity, in combination with amoxicillin or cephaloridine, against β-lactamase-producing strains including members of the species *E. cloacae*, *P. mirabilis*, *E. coli*, *K. pneumoniae*, and *S. aureus* [34]. Secondly, the (*Z*)-isomers were more potent than the corresponding (*E*)-isomers. Thirdly, introduction of substituent at the C-2 position resulted in the reduction of inhibitory activity for both class A and C enzymes. Further modification with various heterocyclic substituent at C-8 led to a series of triazolylmethylene penem derivatives that showed excellent activity in vitro as well as in vivo, as compared with clavulanic acid, sulbactam, and tazobactam. Of the many derivatives prepared, (5*R*)-(*Z*)-6-(1-methyl-1,2,3-triazol-4-yl methylene) penem-3-carboxylic acid, **6a** (BRL-42715) was the best, with stability and overall inhibitory activity against plasmid-mediated class A TEM, SHV, and staphylococcal enzymes, as

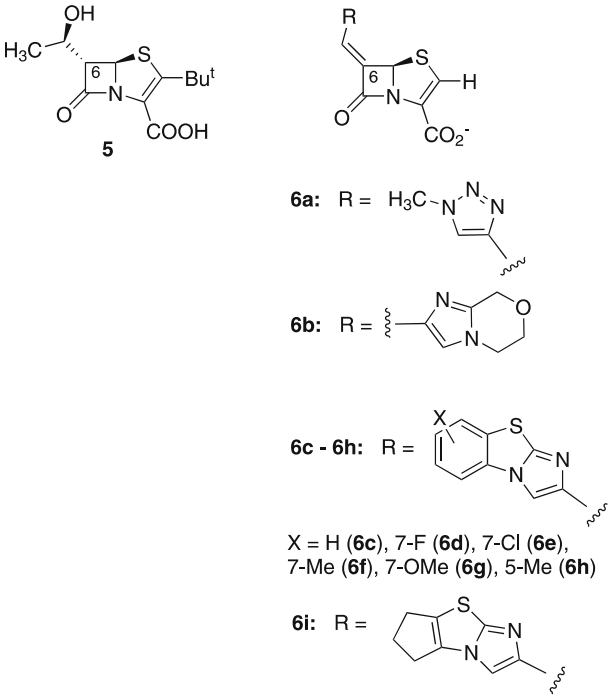
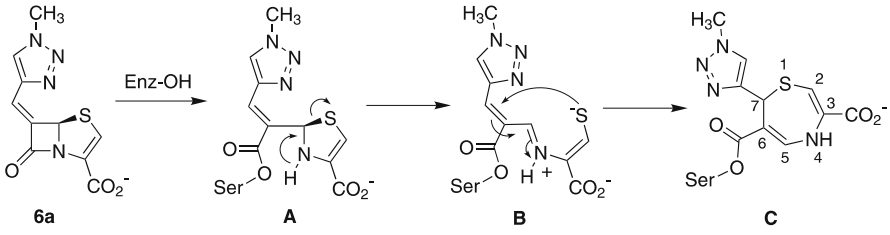


Fig. 6 C-6 substituted penems

well as against the chromosomal AmpC enzymes of *E. cloacae*, *C. freundii*, *S. marcescens*, *M. morganii*, *E. coli*, and *P. aeruginosa*.

Based on chemical evidence and mass spectroscopy studies, the mechanism of inhibition shown in Scheme 2 has been proposed. The interactions of **6a** with β-lactamases of classes A, B, C, and D were investigated kinetically [35]. A crystal structure of **6a** in complex with *E. cloacae* 908R β-lactamase has been reported recently [36].

Acylation of the active site Ser-64 by the penem **6a** resulted in the formation of a stable covalent adduct, a cyclic β-aminoacrylate-enzyme complex **A**. This was followed by the opening of the five-membered thiazole ring system at the C5-S bond. Finally, the rearrangement, via a Michael addition, formed a seven-membered dihydrothiazepine ring system **C** (Scheme 2). This structure is in good agreement with spectral properties of the product, which are identical to those of the dihydrothiazepine obtained after sodium hydroxide hydrolysis [37], and with mass spectrometry results [35]. Another methylidene penem **6b** (Fig. 6), having a 5,6-dihydro-8*H*-imidazo[2,1-*c*]-[1,4]oxazine heterocyclic substituent at the C-6 position with a *Z*-configuration, irreversibly inhibited both class A and class C serine β-lactamases with IC_{50} values of 0.4 and 9.0 nM for TEM-1 and SHV-1 respectively, and 4.8 nM against AmpC β-lactamases. High-resolution crystal structures [38] of its reaction intermediate with the SHV-1 and GC1 β-lactamases gave further insight into its mode of action. The crystallographic studies reveal that in class A SHV-1 complex, the stability of the serine ester bond to hydrolysis is due to the low occupancy or disorder of the hydrolytic water molecule and, in part, to the conjugation of the ester with the dihydrothiazepine ring system. Further, for the class C complex, the orientation of the dihydrothiazepine ring does not permit its C-3 carboxylic acid group and N-4 atom to aid the enzyme's Tyr 150 in activating the hydrolytic water molecule, providing support for the hypothesis of substrate-assisted deacylation in the class C β-lactamases. In another study, Mansour's group [39] synthesized a series of tricyclic penems, **6c–i** (Fig. 6), and evaluated their inhibitory properties against various enzymes. These compounds were potent inhibitors like BRL-42715 with IC_{50} values of 1–18 nM and 1–3 nM, respectively. Within this series of new inhibitors, comparison of their potencies revealed that any sub-



Scheme 2 Mechanism of inhibition of class A enzyme with penem **6a**

stitution on the aromatic ring did not alter their potency against the AmpC enzyme significantly. In combination with piperacillin, their in vitro activities enhanced susceptibility of all class C-resistant strains from various bacteria.

A unique complication for the clinical development of the penem class of compounds is their metabolic instability towards renal peptidase via the hydrolytic opening of the β-lactam ring, resulting in suboptimal serum concentration and lifetime. Thus, although, **6a** (BRL-42715) proved to be a very potent broad spectrum β-lactamase inhibitor, it was not suitable for commercial development because of chemical instability and a short half-life in humans. Because of their toxicity, and instability or poor transportability, not a single member from this class of compounds has been introduced into clinical use. Nevertheless, there is no reason to believe that the metabolic instability of the penem class of compounds could not be altered by proper chemical modifications. The only barriers to this approach are those relating to chemical reactivity, and desirable changes in its structure may require synthetic conditions too stringent for its survival.

6.4 Penicillanic Acid Derivatives

After clavulanic acid, the penicillanic acid derivatives (particularly the corresponding sulfone analogs) have been the subject of intense research in the β-lactamase inhibitor area. From this extensive investigation, two compounds (sulbactam and tazobactam) from this class have been successfully introduced into clinical use. The penicillanic acid sulfones are β-lactamase inhibitors that are quite homologous to clavulanate in both their mechanism of action and in the spectrum of β-lactamases susceptible to their action. The first notable success in this field was the discovery of sulbactam **7** (Fig. 7), which was reported by Pfizer chemists in 1978 and shown to possess potent inhibitory activity, principally for class A β-lactamases. It had greater affinity for class C types than clavulanate. From careful comparison of its structure to clavulanate, a rational basis for the similarities between the two is apparent. Both lack a C-6 substituent. Since the absence (or presence) of this substituent is an important, but not exclusive, factor in β-lactamase recogni-

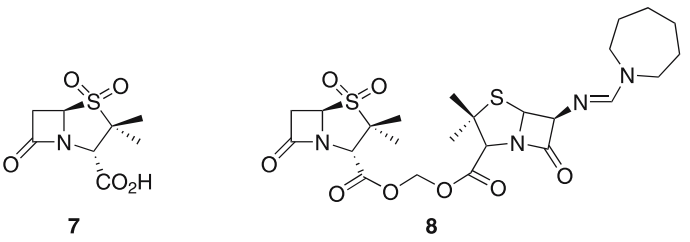


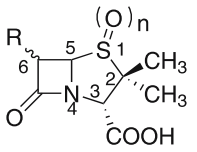
Fig. 7 Sulbactam and its derivatives

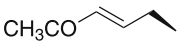
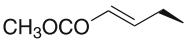
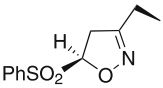
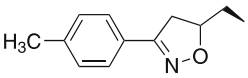
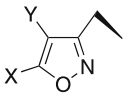
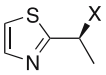
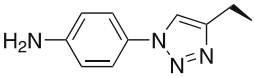
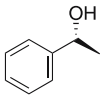
tion, as a first approximation sulbactam and clavulanate will be recognized by and will acylate the same β-lactamases. Following acylation, the oxidized sulfur of sulbactam promotes cleavage of the analogous C-5 to a heteroatom bond, in competition with deacylation. The enolate leaving group of clavulanate is, in this guise, a sulfinate anion.

While sulbactam and clavulanate share strong parallels, they are not identical. Against the class A plasmid enzymes, sulbactam is generally several to 100-fold less potent as a β-lactamase inhibitor; against the class C chromosomal enzymes, sulbactam is generally several to 100-fold better as an inhibitor than clavulanate. There are several additional contrasts between sulbactam and clavulanate. An important decision regarding both of these β-lactamase inhibitors is the choice of the β-lactam antibiotic to which it is paired. In the case of clavulanate, a decision to formulate it with amoxicillin was dictated by the oral absorption of both partners. With sulbactam, however, the salt is poorly absorbed following oral administration. Methanediol-linked diesters **8** (Fig. 7) of sulbactam provide excellent oral absorption, resulting in high serum levels of the two β-lactams in balanced proportion.

A second contrast is the greater solution stability of sulbactam, which may confer a longer lifetime in physiological fluids. It is probable, though, that the chemical features that render sulbactam more stable also render it less efficient in acyl-enzyme fragmentation. The nature of the interaction of this inhibitor with class A β-lactamases has been thoroughly investigated [40, 41]. The clinical success of sulbactam stimulated extensive research by exploring the effect of structural variations within the penam sulfones, making this class of compounds of intense interest and investigation. In this context, while much effort was devoted to modification at the 6-position of the 6-amino penicillanic acid (6-APA) nucleus, greatest success was achieved by modification of the 2β-methyl group. Within the first category of derivatives, the traditional substituents (Table 1) have been electron-withdrawing groups like halogens [42, 43], allyls [44], α,β-unsaturated systems via a methylene bridge [45], electron-withdrawing functionality via a methylene bridge [46], and heterocyclic systems via a methylene bridge, like isoxazolinylmethyl [47, 48], isoxazolylmethyl [47], thiazolylmethyl [49], triazolylmethyl [45], hydroxymethyl [50, 51], 1-hydroxyethyl [50], hydroxyarylalkyl [52], and mercaptomethyl [53]. Although, each compound listed in Table 1 has its unique β-lactamase inhibitory profile, few compounds deserve additional remarks. The orientation of the allyl group in compound **9d** played a crucial role in the interaction with the enzyme. The 6β-isomer was a potent inhibitor, whereas the corresponding 6α-isomer was inactive. The substituted allyl derivatives, **9e** and **9f**, were prepared based on the assumption that, following initial acylation, nucleophilic residues in the active site might interact with the α,β-unsaturated system leading to an enzyme-bound species that might irreversibly inactivate the enzyme. Both the compounds were inactive against TEM-1 (class A) or AmpC (class C) enzymes. The 6β-isomers **9e** and

Table 1 C-6 substituted penam sulfones



Compound	R	*n*	Refs.
9a	α-Cl	2	[42]
9b	α-F	2	[43]
9c	β-F	2	[43]
9d		2	[44]
9e		2	[45]
9f		2	[45]
9g	CH_2CN	2	[46]
9h	CH_2COCH_3	2	[46]
9i		2	[47]
9j		2	[48]
9k		2	[47]
9l		2	[49]
9m		2	[45]
9n		2	[50]
9o		0	[51]
9p		2	[50]
9q		2	[52]
9r	 (α&β)	0	[53]
9s	 (α&β)	2	[53]

9f showed potent activity against CcrA metalloenzyme (class B, IC_{50} of 3.0 and 1.4 μM, respectively), compared to sulbactam or tazobactam. Molecular modeling studies of tazobactam with several β-lactamases revealed that there is ample space in the active site to accommodate larger substituents at the C-6 position of the penam sulfone. It was thought that introduction of heterocyclic rings like isoxazole **9k**, isoxazoline **9i**, or triazole ring **9m** might improve the initial binding of the molecule, and perhaps the binding of the subsequent acylated species, thus slowing the deacylation process. Overall, all the compounds showed better activity against CcrA and AmpC enzymes than sulbactam and tazobactam. The aniline-substituted triazole derivative **9m** had the best activity against all three enzymes (IC_{50}s of 0.6, 1.8, and 1.4 μM versus TEM-1, CcrA, and AmpC, respectively). Further in vitro evaluation of compound **9m** against β-lactamase-producing organisms indicated that this compound was able to restore the activity of piperacillin to a certain degree, but was less active than tazobactam.

The design of the new mechanism-based inhibitor 6α-hydroxymethyl penicillanic acid **9o** was aided by computer modeling using high resolution crystal structure for the TEM-1 β-lactamase. The molecule is believed to displace the hydrolytic water molecule (Wat-712) with its α-oriented hydroxymethyl function so that hydrolysis of the acyl-enzyme intermediate is retarded, leading to longevity of the inhibitor enzyme intermediate to effectively inhibiting the enzyme. In fact, compound **9o** inactivated the TEM-1 enzyme rapidly in a process which is saturable. The retarded rate of hydrolysis of the acyl-enzyme intermediate supports the idea that Glu-166 plays a critical role as a general base in activating Wat-712 for the deacylation step of the hydrolytic reaction.

Mobashery's group [54] showed that the hydrolytic water of class C β-lactamases approaches the acyl-enzyme intermediate from the β-face of the ester (opposite of that for class A β-lactamases). Introduction of a 6β-hydroxymethyl group will crowd the β-face of the ester in the acyl-enzyme intermediate. Thus the approach of the hydrolytic water in class C β-lactamases to the ester will be impaired. This would impart relative longevity to the acyl-enzyme intermediate in class C β-lactamases, accounting for the onset of inhibition. Based on this mechanistic reasoning, a large number of 6-hydroxyalkylpenam sulfones varying the stereochemistry at C-6 position, as well as the alcohol of the hydroxyalkyl group, were synthesized to explore the substituent effects and the stereochemical requirements. Of these substituents, only compound **9n** was active against both TEM-1 and AmpC β-lactamases and was able to restore the activity of piperacillin in vitro and in vivo against various β-lactamase-producing microorganisms.

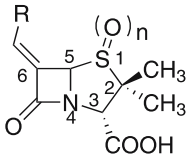
Presumably, 6β-hydroxymethyl sulbactam **9n** works in the same way that sulbactam and tazobactam work against class A β-lactamases.

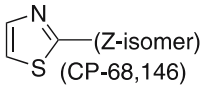
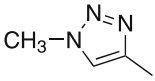
Since many natural substrates (β-lactam antibiotics) of β-lactamases contain pendant aromatic groups, it was postulated that a hydroxybenzyl deriva-

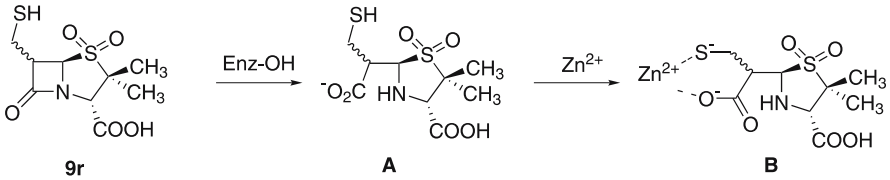
tive **9q** of sulbactam might show greater selectivity and affinity towards the enzymes than those of sulbactam. Compound **9q** has proven to be a highly effective inhibitor against class C β-lactamases, including that of *P. aeruginosa*. Kinetic studies suggest that the inhibitory action is accompanied by a small amount of turnover product and that compound **9q** acts as a high-affinity substrate. The inhibition is also irreversible with no recovery of enzyme activity over several hours at pH 8.0. The benzylidene sulfone **10d** (Table 2) prepared by dehydrating the alcohol **9q** also behaved as an inactivator of the β-lactamases from *P. aeruginosa*, but was only half as potent as **9q**.

Buynak et al. [53] synthesized several 6-(mercaptomethyl) penicillanates (**9r** and **9s**, Table 1) that include both C-6 stereoisomers as well as the sulfide and sulfone oxidation states of the penam thiazolidine sulfur. Selected mercaptomethyl penicillanates inactivated both metallo- and serine β-lactamases, and displayed synergism with piperacillin against various β-lactamase-producing strains, including metallo-β-lactamase-producing *P. aeruginosa* strain. Compound **9r** would be capable of bidentate chelation of zinc subsequent to enzymatic hydrolysis of the β-lactam (Scheme 3).

Table 2 C-6 vinylidene substituted penam sulfones



Compound	R	*n*	Refs.
10a	CH_3O (*Z*-isomer)	2	[55]
10b	CH_3CO (*Z*-isomer) (Ro 15-1903)	0	[56]
10c	CN (*Z*-isomer)	0	[58]
10d	Ph (*Z*-isomer)	2	[52]
10e	2′-py (*Z*-isomer) (CP-65,372)	2	[59–61]
10f	 (Z-isomer) (CP-68,146)	2	[59–61]
10g		2	[62]
10h		2	[63]

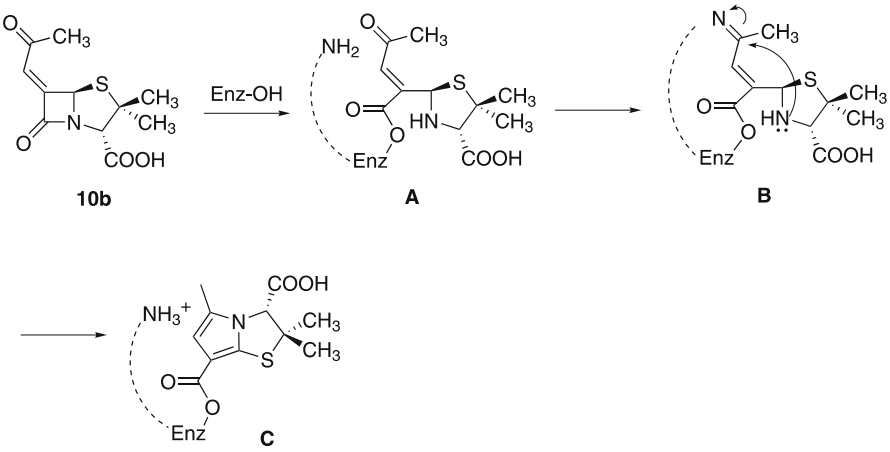


Scheme 3 Mechanism of inhibition of metalloenzyme with **9r**

The mechanism suggests that the presence of an α,β-unsaturated system at the C-6 position of sulbactam would be an essential component for enhancing the inhibitory activity towards various β-lactamases. Thus, several compounds with an exocyclic double bond at the C-6 position of the penicillanic acid, with and without sulfone, were prepared (Table 2).

Knowles et al. [55] designed 6-(methoxymethylene) penam sulfone **10a** as a potential β-lactamase inhibitor by exploiting the fact that this compound already has a β-aminoacrylate system in the acyl-enzyme intermediate. This compound had shown the predicted behavior of slower turnover and stronger inhibition of β-lactamases compared to sulbactam, but did not show good in vivo activity, presumably due to poor penetration into the bacterial cell wall. Ro 15-1903, **10b** (Table 2), a penicillanic acid derivative without a typical sulfone moiety (a requirement for the enhancement of the β-lactamase inhibitory activity) was discovered in the laboratory of Hoffmann-La Roche. Interestingly, this compound was a potent β-lactamase inhibitor against TEM-1 (IC_{50} 1.4 nM). On the basis of IC_{50} values it was 60 and 1350 times more active than clavulanic acid and sulbactam, respectively, against TEM-1 enzyme. However, its in vitro combination with ampicillin had only slightly greater activity against β-lactamase producers that did ampicillin/sulbactam or ampicillin/clavulanic acid. Chemical instability in serum and the inability to penetrate the bacterial cell envelope were the major reasons for these disappointing results. The chemical reactions of compound **10b** and TEM-1 were studied [57] using spectrophotometric methods. It was found that the ketone functionality was essential for the inhibitory activity against TEM-1 enzyme. After the initial acylation reaction (Scheme 4), a nucleophilic amine residue in the active site of the enzyme can further react with the keto group to give a cross-linked iminium acyl-enzyme **B**, leading to a rather stable inactivated enzyme **C**.

Although replacement of the acetyl group with a cyano group gave compound **10c** a similar inhibitory profile as **10b** against isolated enzymes, the cyano derivative again failed to show good in vivo activity due to chemical instability. The chemical stability of these compounds, **10b** and **10c**, was improved by reducing the 6-exocyclic double bond, but these reduced compounds failed to retain β-lactamase inhibitory activity. The lost activity could be regained by converting the sulfide to sulfone. Thus, although compounds **9g** and **9h** (Table 1) were potent β-lactamase inhibitors, their advantage was



Scheme 4 Mechanism of inhibition of class A enzyme with **10b**

lost in vitro and in vivo and the synergistic spectrum and potency became comparable to that of clavulanate and sulbactam.

Several π-deficient 2-heteroaryl substituents attached to the C-6 methylene position of the penam sulfone nucleus (e.g., compounds **10e**, **f**, and **g**, see Table 2) were reported to possess potent β-lactamase inhibitory properties. In each case, the *Z*-isomer was better than the corresponding *E*-isomer. The compounds **10e** and **10f** were equivalent to, or better than, clavulanic acid and sulbactam in potentiating ampicillin against the penicillinase-producing strains *S. aureus* 01A400, *K. pneumoniae* 53A079, and *H. influenzae* 54A042. The compounds also demonstrated a synergistic effect in combination with cefazolin against *E. coli* 51A129, which has TEM-1 penicillinase, and against AmpC-producing cephalosporinase-producing organisms such as *E. cloacae* 67B009, *S. marcescens* 63A095, and *M. morganii* 97A001. In spite of the synergistic activities of these inhibitors, the level of activity was not sufficient to warrant further development of these sulfones for clinical use.

In recognition of a number of existing studies on allenes functioning as irreversible inhibitors of various enzymes, Buynak [63] was intrigued by the idea that incorporation of such a functional group into a β-lactamase substrate might produce a useful inhibitor. Depending on the specific substitution pattern, the α-vinylidene-derived penicillin sulfones showed some promise. One compound, **10h** (Table 2), from this series was able to inhibit the AmpC β-lactamase of *E. cloacae* P99. Additional substituents on the 6-alkylidene penam sulfone produced compounds that exhibited synergy with piperacillin in the treatment of Gram-negative organisms, including ceftazidime-resistant *P. aeruginosa* [64]. Bycroft et al. [65, 66] discovered that 6-spiro epoxide **11** (Fig. 8) was a good mechanism-based inhibitor of β-lactamases. However, when the chlorine was replaced by hydrogen, the resulting epoxide had diminished activity, indicating the importance of the chloro group.

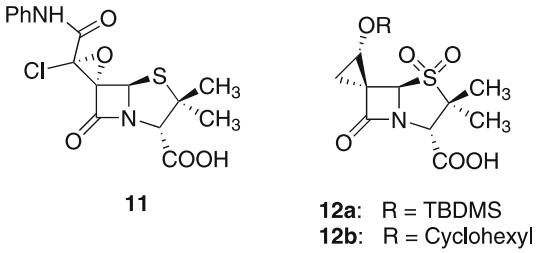
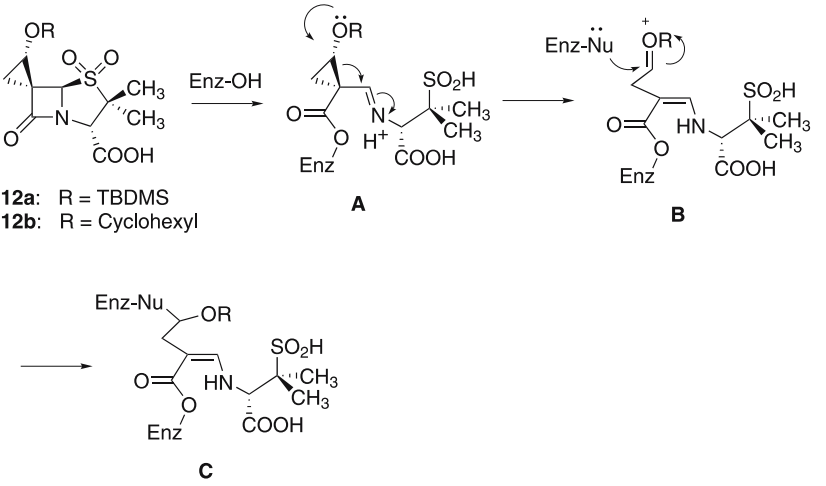


Fig. 8 C-6 modified sulbactams

In a recent report [67], the spirocyclopropyloxy structural motif was incorporated in sulbactam, and the compounds **12a** and **12b** had good activity against various β-lactamases. The mechanism of inhibition of β-lactamase by **12a** or **12b** is unique. After the initial acylation, the cyclopropyloxy group can promote the subsequent chemical events to form the aldehyde or the oxycarbenium moiety for further cross-linking with other active site residues of the enzyme (Scheme 5).

In comparison to sulbactam, penam sulfones **12a** and **b** exhibited excellent activity against TEM-1 and AmpC enzymes, respectively, with over 2500-fold improvement against the class C enzyme. Within the same series, **12a** and **12b** are appreciably more potent than their corresponding diastereomers. In particular, they are potent against the AmpC enzyme suggesting the stereochemical preference of the alkoxy substituent of the cyclopropyl ring, which plays a critical role in binding with the enzymes. Further, in vitro evaluations in a cell-based assay (MIC) established the effectiveness of **12a.** In

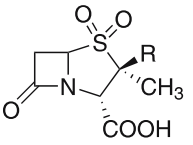


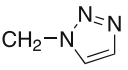
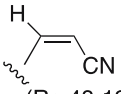
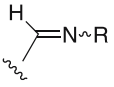
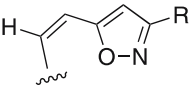
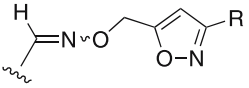
Scheme 5 Mechanism of inhibition of class A enzyme with **12a** or **b**

combination with piperacillin at a ratio of 1 : 1, the activity was two dilutions better than tazobactam against class C-producing organisms, consistent with its more potent AmpC enzyme activity. Moreover, the combination of **12a** with cefotaxime increases the antimicrobial activity of cefotaxime against Gram-positive and Gram-negative organisms [68].

Other major modifications of the penam nucleus have been performed at the C-2 methyl group. In 1981, Gottstein et al. [69] first described the synthesis of 2β-halomethyl penam sulfones, **13a** as β-lactamase inhibitors. Following Gottstein's work, 2β-halomethyl was converted to the corresponding 2β-azidomethyl analog [70]. On reaction with a variety of acetylenes this gave a series of 2β-(1,2,3-triazol)methyl penam sulfones. The most successful molecule derived from these efforts was tazobactam **13b** (Table 3),

Table 3 2β-substituted penam sulfones

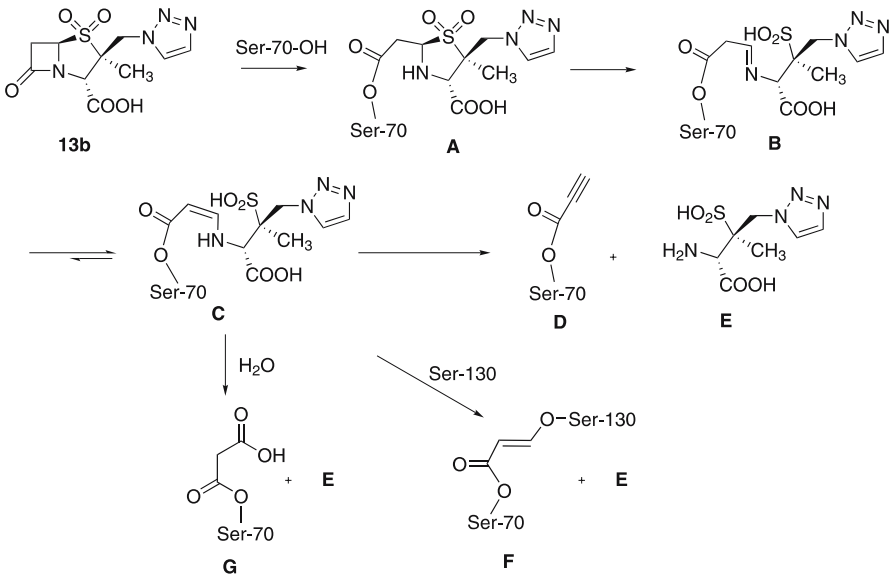


Compound	R	Refs.
13a	CH_2X (X = Cl, Br, OH)	[69]
13b	 (YTR-830)	[71]
13c	COOR	[75]
13d	CONR′R″	[76]
13e	$COCH_3$	[77]
13f	 (Ro 48-1220)	[78]
13g	 (R = OR', NR'R")	[79]
13h		[80]
13i		[80]

a commercially available inhibitor used in conjunction with piperacillin under the name of Zosyn or Tazocin [71]. When combined with piperacillin, **13b** displayed a spectrum of activity that was superior to that of amoxicillin/clavulanate or ampicillin/sulbactam. Recently, the mechanism of inactivation of tazobactam has been elucidated [72] using electrospray ionization mass spectrometry (Scheme 6). Initial acylation of Ser-70 by tazobactam and opening of the lactam ring are followed by one of several different events:

1. Rapid decomposition of tazobactam with loss of the enamine moiety to form the propiolylated enzyme **D**
2. Intramolecular nucleophilic displacement of the imine or enamine moiety by Ser-130 to form a cross-linked vinyl ether **F** as the inactivated species
3. Hydrolysis of the imine or enamine to form a Ser-70 linked aldehyde **G**

Further, using a combination of X-ray crystallography and mass spectroscopy, Knox et al. [73] has firmly established a central role for Ser-130 in the inhibition of SHV-1 β-lactamase (class A) by tazobactam. Many additional modifications (Table 3) were carried out on tazobactam with the aim of increasing inhibitory activity against AmpC enzymes, but none of these derivatives (e.g., **13c**, **13d**, and **13e**) had any advantage over tazobactam [74–77]. Renewed interest in the modification at the C-2 position of sulbactam was developed when scientists from Hoffmann-La Roche disclosed a series of 2β-alkenyl penam sulfones that possess the ability to simultaneously inactivate both class A penicillinase as well as class C cephalosporinase. Compound

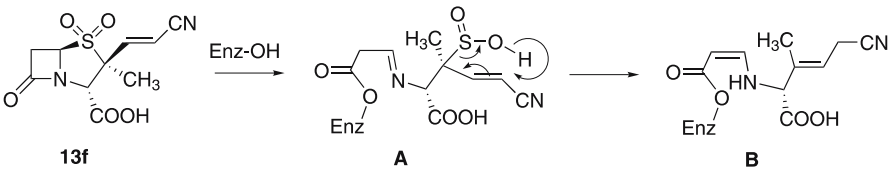


Scheme 6 Mechanism of inhibition of class A enzyme with tazobactam **13b**

13f (Table 3) from this series showed best synergy in combination with ceftriaxone against resistant microorganisms. The increased spectrum of activity of compound **13f** was not associated with the increased affinity or enhanced reactivity with the inhibited enzyme, but rather correlated to the slow deacylation of the enzyme–inhibitor complex **B**. The mechanism of inactivation is supposedly via an allylsulfinic acid rearrangement as shown in Scheme 7.

An independent approach to inhibitor design in the 2β-substituted penam sulfone series was the incorporation of an oxime or hydrazone functionality into the sulbactam nucleus. Surprisingly enough, these compounds were equally as active as compound **13f**, Ro 48-1220. In particular, compounds **13g** (R = OCH_3 and R = $NHCH_3$, Table 3) showed improved synergy in combination with piperacillin and ceftazidime against class C cephalosporinase-producing organisms, except *P. aeruginosa*.

In penicillins, the C-3 carboxylate group, or in cephalosporins the C-4 carboxylate group, plays a critical role in recognizing these antibiotics in a positively charged pocket of the active site of the penicillin binding proteins (PBPs) and in their descendants, the β-lactamases. In the case of the roughly planar bicyclic cephalosporin system, the carboxylate group is attached to an sp^2-hybridized carbon and lies in approximately the same plane as both the six- and four-membered ring of this bicyclic system. On the other hand, in the more strained and bowed bicyclic penam, the carboxylate group is attached to an sp^3 carbon and skewed to the convex (i.e., to the α) face. This geometric difference may partially account for the difference in substrate specificity between class A and class C enzymes. A second structural feature, the endocyclic double bond of the cephalosporins, penems, and carbapenems, is an important contributor to the enhanced acylation capability of the antibiotic to acylate the active site serine. Perhaps, it is logical to assume that inhibitors like sulbactam and tazobactam are selective for class A enzymes since they structurally resemble penicillins more closely than they resemble cephalosporins. On the basis of these mechanistic reasons, it was hypothesized that a C-3 homologated penam-derived β-lactamase inhibitor might have a broader specificity for both class A and class C enzymes than do current commercial inhibitors. The enhanced conformational flexibility of the carboxylate of the homologated penam derivative could enable the molecule to fulfill the geometric requirements of both A and C classes of serine β-lactamases. The longer chain might also enable the carboxylate



Scheme 7 Mechanism of inhibition of class A enzyme with **13f**

to penetrate deeper into the positively charged pocket. This hypothesis was confirmed by the fact that the homologated sulbactam analog **14** (Fig. 9) had a tenfold improved inhibitory activity against the class C P99 enzyme as compared with the sulbactam itself [81]. Yet another rationale is that placing a double bond in direct conjugation with the nitrogen, but exocyclic to the five-membered ring might also enhance acylation efficacy. Unfortunately, the incorporation of exocyclic unsaturation, as in compound **15**, did not display significant ability to inactivate either class A or class C serine β-lactamases [81].

In the penam sulfone series, a general trend is that C-6 substituted penam sulfones exhibit inhibitory activity against class C enzymes, while 2β-substituted penam sulfones exhibit better activity against class A enzymes. It would be expected that a combination of both structural features in a single compound might give an inhibitor that can inhibit both class A and class C β-lactamases. To prove this hypothesis, several analogs (**16a–h**, Table 4) were prepared by functionalizing both C-6 and 2β-methyl positions of sulbactam.

As expected, compounds **16d–f** demonstrated good IC_{50}s against both TEM-1 and AmpC β-lactamase. Further, they were able to restore in vivo activity of piperacillin at a ratio of 1 : 1 of piperacillin to the inhibitor against TEM-1 and AmpC β-lactamase-producing organisms. In addition, at a 2 : 1 ratio of piperacillin and compound **16f**, the ED_{50} values for piperacillin were reduced from 256–612 mg/kg and 128–256 mg/kg to 4–8 mg/kg and 8–32 mg/kg, respectively, against TEM-1 and AmpC-expressing bacterial isolates. Substituents that improve transport across the bacterial cell membrane have also been incorporated. In this regard, compound **16g** deserves further remarks. It is well established that the presence of an iron chelating functionality like catechol in an antibiotic allows the molecule to enter the cell of Gram-negative bacteria (e.g., *P. aeruginosa* and *S. marcescens*) via an iron transport mechanism. It was noted that a combination of piperacillin and compound **16g** having a catechol functionality improved the synergistic activity against Gram-negative strains, including *P. aeruginosa* and *S. marcescens*.

Over the past decade the essential goal in the modification of sulbactam or tazobactam has been to extend their activity towards the class C cephalosporinases. In spite of an enormous amount of effort, there has not been much success in achieving this goal. The penicillanic acid derivatives, as a class, show a good β-lactamase inhibitory profile against class A

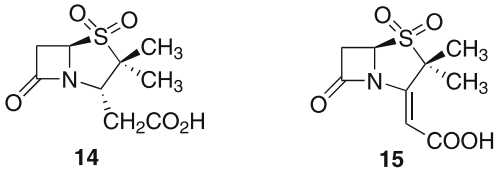
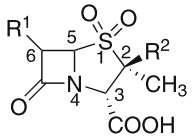
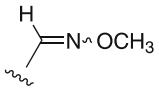
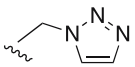
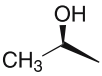
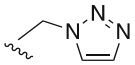
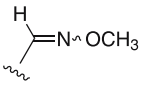
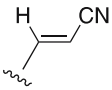
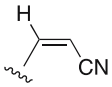
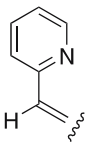
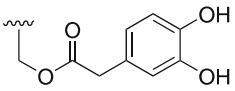
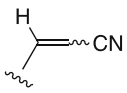


Fig. 9 C-3 modified sulbactams

Table 4 C-6 and 2β-substituted penam sulfones



Compound	R^1	R^2	Refs.
16a			[77]
16b			[82]
16c			[82]
16d			[82]
16e			[82]
16f			[82]
16g			[64]
16h			[64]

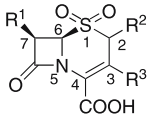
β-lactamases. Few simple generalizations are possible, although several trends may be discerned. The 2β-substituted penicillanic acid derivatives tend to be better inhibitors than the C-6 substituted analogs. Although, in many respects, the penicillanic acid sulfones offer the most versatile template for the manipulation of specificity for β-lactamase inactivation, unfortunately, there is as yet no indication that these newly synthesized additional penam sulfone derivatives possess any advantage over the sulbactam or tazobactam.

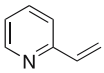
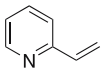
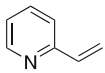
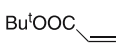
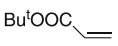
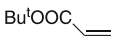
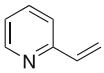
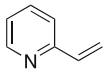
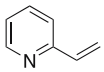
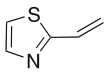
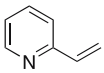
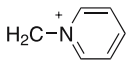
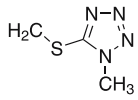
6.5 Cephem Derivatives

In comparison to the penicillin series, relatively very few cephalosporin-based β-lactamase inhibitors have been reported in the literature, although the cephalosporin nucleus provides an excellent opportunity for creating modifications at both C-2 and C-3 positions of the six-membered ring. The success of clavulanic acid and the announcement of two penicillanic acid sulfones (sulbactam and tazobactam) as the two clinical successors spurred efforts in several laboratories to modify the readily available cephem nucleus (7-amino cephalosporanic acid, 7-ACA) by introducing a wide variety of substituents at C-3 and C-7 positions, and to evaluate them as β-lactamase inhibitors. Although a large number of pharmaceutical companies directed their efforts at developing this class of compounds as powerful broad-spectrum antibiotics, attempts to modify the cephem nucleus have been rather limited and thus far have not provided a single useful β-lactamase inhibitor. Not until 1970 were research efforts directed towards the preparation and testing of cephalosporins as antibiotics, with an electron-withdrawing group directly attached to the C-3 position of the cephem ring system. Exploration of this chemistry has led to two members of this class of β-lactams that have achieved clinical importance because of their marked antibacterial activity. On the other hand, because of the recognition of the potential of the 6α-hydroxyethyl side chain for broadening the antibacterial spectrum in the resulting compound, this functionality continued to be of great interest as a favorite side chain of many β-lactam agents during 1970s and 1980s. Quite possibly, Nishimura et al. [83] were intrigued by the idea that the incorporation of such a functionality, i.e., a α-hydroxyethyl group at C-7 position, and an electron withdrawing group like CN or CHO at C-3 position of the cephem nucleus might produce a useful β-lactamase inhibitor. In fact, the prepared cephem analogs **17a** and **b** (Table 5) exhibited β-lactamase inhibitory activity against AmpC cephalosporinases and showed synergistic activity in a 1 : 1 combination with ceftizoxime against AmpC-hyperproducing strains of *M. morganii*, *C. freundii*, *E. cloacae*, and *P. aeruginosa.*

The discovery of the ethylidenecarbapenems, the asparenomycins, as naturally occurring β-lactamase inactivators in the early 1980s was another striking point in β-lactamase inhibitor research. The substituted exomethylene function in asparenomycins is a distinctive feature of this class of compounds, which many scientists recognized could be a key factor for β-lactamase inhibition. The exocyclic methylene is expected to increase the acylation ability, and form an α,β-unsaturated ester of the active site serine residue as an acyl-enzyme complex. This ester will be similar in structure to the acyl-enzymes formed from clavulanate and sulfone fragmentation, and will be quite resistant to hydrolytic deacylation. Thus, the exocyclic methylene promotes acylation by the enzyme and subsequently represses deacylation. Based on

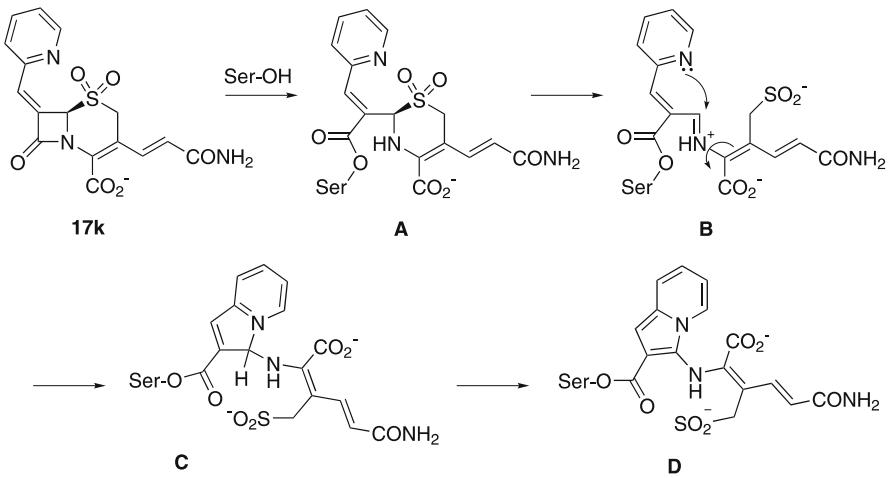
Table 5 Substituted cephem sulfones



Compound	R^1	R^2	R^3	Refs.
17a		H	CN	[83]
17b		H	CHO	[84]
17c		H	CH_2OAc	[85]
17d		$=CH_2$	CH_2OAc	[86]
17e		$=CH_2$	CH_3	[86]
17f		H	CH_2OAc	[85]
17g		$=CH_2$	CH_2OAc	[86]
17h		$=CH_2$	CH_3	[86]
17i		H	CN	[87]
17j		H	E – CH = CH – CN	[87]
17k		H	E – CH = CH – $CONH_2$	[87]
17l		H	E – CH = CH – $CONH_2$	[87]
17m		H	SO_2CH_3, SO_2Ph	[88]
17n		H	CH_2OAc	[89]
17o	H	H		[90]
17p	H	H		[90]

this rationale, an attractive and ultimate aspiration has been to introduce an exocyclic double bond in a similar position to other β-lactam agents. Thus, Buynak et al. [85–88] prepared a series of cephan sulfones (Table 5) that have a substituted methylidene group at C-7 position of the cephem nucleus. The preferred one was (2′-pyridyl) methylidene group, exclusively in the *Z*-form. As mentioned earlier, in contrast to the penicillin nucleus, the cephalosporin nucleus allows facile synthetic modification at C-3. The initially synthesized cephalosporin-derived inhibitor **17c** was selective against class C enzyme [85]. However, through modification at C-3 it was possible to achieve the goal of dual inhibition of class A and class C enzymes. Appropriate substituents at the C-3 position resulted in a 1000-fold improvement in the inhibition of the class A enzymes and a simultaneous 20-fold improvement in the inhibition of class C. Within this cephem series, some generalizations regarding structure–activity relationships can be made:

1. In general, the inhibitory activity of 7-(2′-pyridylmethylidene) cepham sulfones remains high, but the 7-position carboxylate was low. Similarly, 7-(2′-thiazolidinyl) methylidene cephalosporin **17l** lacks significant inhibitory activity.
2. In comparison with the parent compound, 3-vinyl substituted cepham sulfones containing an electron-withdrawing group displayed excellent inhibitory activity against both class A and class C β-lactamases. Although increasing the electronegativity at C-3 seems to improve inhibition of both serine classes, other factors like inhibitor–enzyme recognition at C-3 probably plays a significant role in determining activity.
3. Incorporation of an exocyclic methylidene at C-2 decreased inhibition of class A enzyme.



Scheme 8 Mechanism of inhibition of class A enzyme with **17k**

Compound **17j** from this series had potent activity with IC_{50}s of 0.01 nM, 0.014 nM, and 0.72 nM against P99, TEM-1, and PC-1, respectively. The proposed mechanism of inhibition of compound **17k** was based on X-ray crystallography studies (Scheme 8) [91]. It is logical to assume that each of this class of compounds inactivates the enzymes in a similar manner.

6.6 Bridged Lactams

In many respects, bridged monobactams represent an evolutionary end point of our understanding of β-lactamase inhibitor design within the monobactam framework. The thought process behind their design has been lucidly described [92]. They are potent inhibitors of class C β-lactamases, but are less effective against class A and class B enzymes, because of the different hydrolysis mechanisms in these classes of β-lactamases. The activity arises from the formation of a stable acyl-enzyme complex that blocks the access of water to the enzyme–inhibitor ester bond. The information obtained from the crystal structure of the acyl-enzyme complex formed with aztreonam **18** (Fig. 10), a monobactam, and *C. freundii* 1203 β-lactamase (class C) was very helpful in designing this class of inhibitors. The modeling studies revealed that when aztreonam forms the acyl-enzyme complex with the class C enzyme, there is nearly 70° rotation about the $C_3 - C_4$ bond, in comparison to the intact aztreonam molecule.

The eclipsed conformation **A** of the intact aztreonam molecule becomes relaxed, through the counterclockwise rotation about the $C_3 - C_4$ bond to the

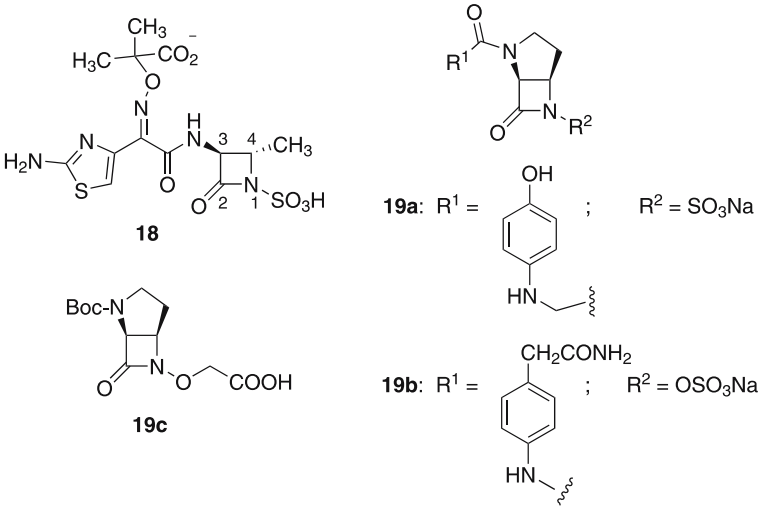
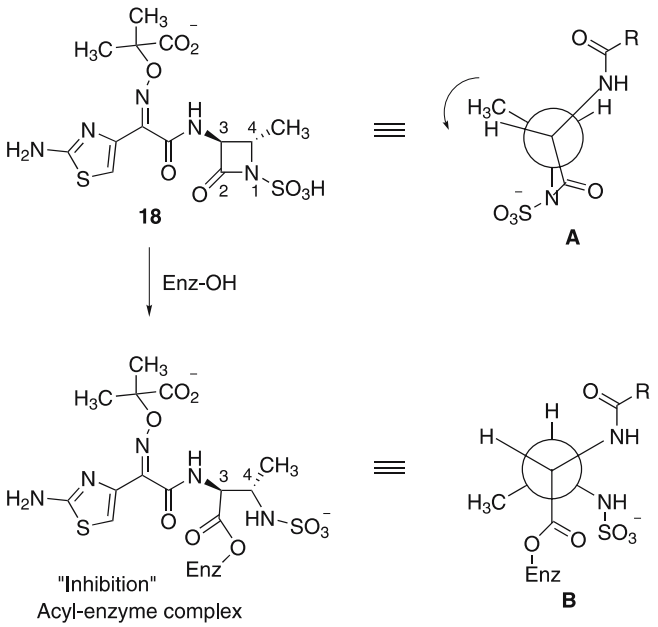


Fig. 10 Aztreonam (**18**), bridged lactam (**19a**), bridged sulfactam (**19b**), and oxamazin (**19c**)

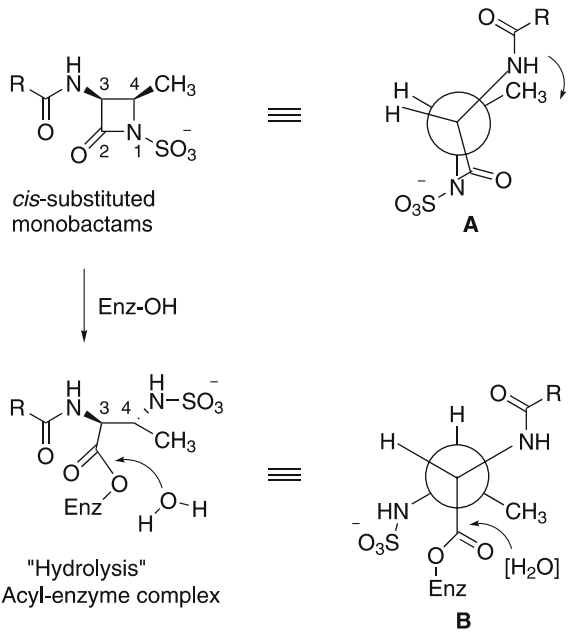
energetically more favored gauche conformation **B**. To adopt the alternative gauche conformation, the clockwise rotation is less favorable because it would bring the C-4 methyl substituent into steric conflict with the side chains of tyrosine-150 and leucine-119. The rearrangement in the aztreonam complex **B** leaves the $NH - SO_3^-$ moiety in a position where they block one face of the ester bond formed with Ser-64. They also displace a water molecule (Wat-192) that is essential for the catalytic mechanism of class C enzymes (Scheme 9).

In contrast, with penicillins, cephalosporins, and monobactams where the substituents are *cis* to each other across the $C_3 - C_4$ bond, clockwise rotation can occur without conflict with protein side chains, and will leave the path open for the water molecule to attack and hydrolyze the ester group in **B** (Scheme 10). Thus, *cis*-substituted monobactam, as well as penicillins and cephalosporins are rapidly hydrolyzed by class C enzymes (Scheme 10). If this rotation could be prevented by a suitable structural modification, the access of the water molecule to the ester bond will be blocked, which would result in increased stability of the acyl-enzyme complex.

As expected, most of these compounds exhibited good inhibitory activity against class C enzymes. The compound **19a** (Fig. 10) showed the best activity in both cell-free and whole cell assays (IC_{50} 3 nM and 25 nM against β-lactamases from *C. freundii* and *P. aeruginosa*, respectively). Synergy between the bridged monobactams and ceftriaxone, a third-generation



Scheme 9 Mechanism of inhibition of class C enzyme with aztreonam **18**



Scheme 10 Mechanism of inhibition of class C enzyme with *cis*-substituted monobactams

cephalosporin antibiotic, could be observed when such combinations were tested against strains of *Enterobacteriaceae* that produce large amounts of class C β-lactamases. However, the synergy was less pronounced than expected against *P. aeruginosa*, which could be partly attributed to a reduced penetration of the inhibitors through the outer membrane of *P. aeruginosa*.

Two other series of bridged monobactams (i.e., sulfactams **19b** and oxamazins **19c**) have also been studied [93]. Oxamazins were not β-lactamase inhibitors, perhaps due to insufficient activation of the β-lactam ring. On the other hand, the bridged sulfactams **19b** were potent inhibitors (IC_{50} 20–165 nM), and were more active than the corresponding bridged monobactams. Kinetic studies revealed that the low IC_{50} values of sulfactams occur primarily as a result of a high affinity and a high rate of acylation. These compounds, however, showed weak synergistic activity in combination with ceftriaxone.

6.7 Monobactams

In monocyclic β-lactams (termed monobactams) the activation is achieved solely by electronic effects. X-ray crystallographic data indicate that there is no steric activation since the azetidinone ring is planar with the sulfonate residue lying within the plane. Electron withdrawal by the sulfonate group is

responsible for activation of the β-lactam in the monobactams. Not only does the charge of the sulfonate residue function in enzyme–substrate recognition, but it also serves to modulate activation of the β-lactam ring by decreasing the inductive effect of the SO_2 group. The sulfonate group therefore plays a multiple role in balancing activation and stability of the β-lactam ring while positioning the anionic charge for recognition by the enzyme. Aztreonam **18** and its closest congener carumonam **20** (Fig. 11) are available clinically to treat Gram-negative infections including that caused by *P. aeruginosa*. Drugs like penicillins and cephalosporins inhibit cell wall biosynthesis by binding with the penicillin binding proteins (PBPs). As described earlier, aztreonam is a potent inhibitor of AmpC cephalosporinases exhibiting the highest affinity for the enzymes from *E. cloacae*, *C. freundii*, and *E. coli*. Its mode of inhibition against the class C enzyme is described in the bridged-lactam section. It is logical to think that proper modification of the aztreonam molecule could lead to a potent inhibitor specifically against class C enzymes. On this basis, two series of novel 2-oxo-1-azetidine sulfonic acids [94, 95] were synthesized and evaluated against various class C-producing bacterial isolates.

In this series, compound **21a** (also called Syn 2190), a monobactam containing 1,5-dihydroxy-4-pyridone as the C-3 side chain, was found to be a strong inhibitor of class C β-lactamases. It showed strong synergy with ceftazidime against strains producing class C β-lactamases including *P. aeruginosa*, which is a clinically significant organism. Also, compound **21b** (also called Syn 2161), having an aminoethyl functionality in the oxime component, was equally active and showed strong synergy in combination with the β-lactamase-susceptible antibiotic ceftazidime against various clinical isolates, except *P. aeruginosa*. This suggests that the presence of 1,5-dihydroxy-4-pyridone in compound **21a** may assist in penetrating the inhibitor through the outer membrane of *P. aeruginosa* by a ton-B dependent iron transport

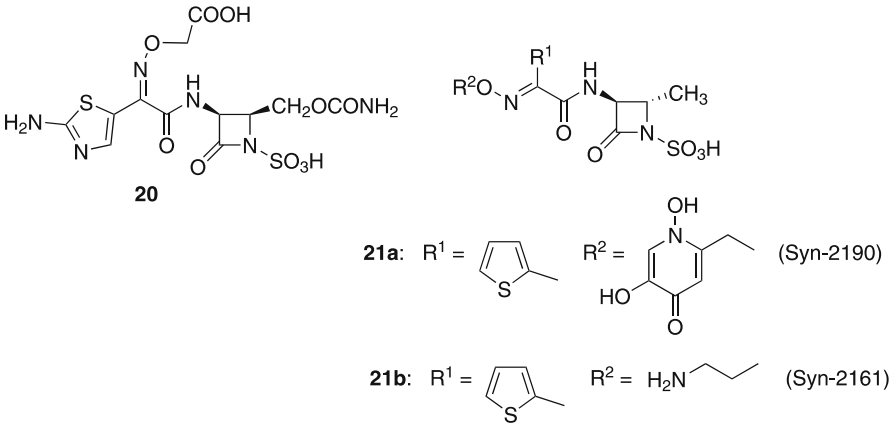


Fig. 11 Monobactam derivatives

mechanism. From structure–activity relationship studies, several points can be made:

1. A sulfonic acid substituent at the N-1 position shows higher cephalosporinase inhibitory activity than a phosphoric acid group.
2. α-Methyl substituent is preferred over hydrogen and other substituents at C-4 position.
3. The presence of a substituted oxime in the amide side chain at the C-3 position is the important criteria for cephalosporinase inhibitory activity.
4. The substituted oxime in the amide side chain should be in the *anti* orientation for higher cephalosporinase inhibitory activity
5. Thiophene, furan, and isothiazole are the preferred heterocycles for cephalosporinase inhibition. However, thiophene is the preferred heterocycle.
6. An aminoalkyl group or a 1,5-dihydroxy-4-pyridone group is the preferred substituent in the oxime for strong synergy with various cephalosporin antibiotics, particularly ceftazidime.
7. Replacement of the N-OH group in the 4-pyridone ring with other groups reduces synergy.
8. A combination of thiophene or isothiazole as the heterocyclic ring, the presence of a 1,5-dihydroxy-4-pyridone group in the *anti*-oxime, an α-methyl in the 4-position, and a SO_3H group at N-1 exhibits a better cephalosporinase inhibition and synergistic activity against *Enterobacter*, *Citrobacter*, *Pseudomonas*, and *Morganella* strains, including resistant *Pseudomonas* strains.

Compound **21a** in combination with ceftazidime or cefpirome at a ratio of 1 : 1 was observed to have efficacy in vivo [96]. It improved the efficacy of both the cephalosporins in both a murine systemic infection model with cephalosporinase-resistant rods and in urinary tract infection models with cephalosporin-resistant *P. aeruginosa*. Further developments in this class of compounds would be rewarding.

7 Conclusion

The past decade has seen an increase in the frequency of resistance to modern antibiotics, which is currently a major health concern in treating infectious diseases. Many currently effective drugs continue to be underused by patients who do not complete courses, and misused through indiscriminate and over-prescribing. Resistance increases clinical complications, lengthens hospital stays, and adds cost. With more resistance and fewer new antibiotics, modern medicine will undergo significant setbacks. Major drug manufacturers turned away from intensive antibacterial research and concentrated their

efforts on seeking cures for heart disease, Alzheimer's disease, HIV infection, and other chronic diseases – thus effectively closing the door on further research into new drugs designed to combat bacterial infections. In many cases, the production of β-lactamase enzyme is the main cause of bacterial resistance to β-lactam antibiotics, and seems likely to remain so. β-Lactamase inhibitors provide one strategy to overcome this resistance. Success depends on the inhibitor being able to bind and inactivate the β-lactamase molecules; but also on the amount of enzyme, the partner drug, the permeability of the target organism, and growth conditions. Clavulanate, sulbactam, and tazobactam are irreversible inhibitors of many β-lactamases, forming covalent complexes that resist hydrolysis. The clinical efficacy of combinations such as amoxicillin/clavulanate (called Augmentin), ticarcillin/clavulanate (called Timentin), ampicillin/sulbactam (called Unasyn for injectable or Sultamicillin for oral), and piperacillin/tazobactam (called Zosyn) has proven the validity of the strategy. These available inhibitors are active mostly against class A β-lactamases but, unfortunately, none is very effective against class C (AmpC) cephalosporinases, which are the other important family of β-lactamases in clinical isolates. Hyperproduction of chromosomal AmpC β-lactamases has long been a source of cephalosporin resistance in *Enterobacter* and *Citrobacter spp*. Genes for AmpC β-lactamases have now found their way onto plasmids, paving the way for their further dissemination among Gram-negative bacteria. This highlights the importance of finding β-lactamase inhibitors with high activity against these AmpC enzymes. Although effective inhibitors of AmpC enzymes are known in the penem, bridged lactam, and monobactam classes, none has yet proved suitable for clinical development. Further research and developments in this area should be rewarding.

A new and final threat comes from zinc metallo-β-lactamases of molecular class B. The mechanism of hydrolysis of β-lactam rings by metalloenzymes is quite different from that of serine enzymes (class A, C, or D). The metallo-β-lactamases are resistant to virtually all inhibitors possessing a β-lactam ring. They are ubiquitous in *Stenotrophomonas maltophilia* and a few other clinically infrequent organisms. Disturbingly, however, they are now appearing as plasmid-mediated types in enterobacteria and non-fermenters in Japan. These developments challenge the researchers to seek potent new β-lactamase inhibitors in the coming years. There is a potentially large market for a newer generation of β-lactamase inhibitors that would extend the effectiveness and life of the later generation of cephalosporins and carbapenems. In this context, X-ray crystallography, molecular modeling, and electrospray ionization mass spectrometry studies over the past decade have been very useful in gaining new information about the structural and mechanistic details of β-lactamases and β-lactamase inhibitors, and will hopefully assist in the discovery of new clinically important broad-spectrum β-lactamase inhibitors.

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