Pharmacologically Active Sulfur-Containing Compounds

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Abstract: In addition to sulfur-containing compounds that form essential and indispensable constituents of all living cells, there is a plethora of less wide-spread sulfur-containing compounds with much less common and often unexpected properties, chemical structures and biological activities. Some of these compounds have long been known as efficient antibiotics. This review surveys these less known compounds with two and more sulfur atoms in their molecule. In particular, it deals with natural heterocyclic compounds isolated, e.g., from bacteria, fungi, lichens, plants, and animals but also from unusual and exotic sources such as sea invertebrates (coelenterates, sponges, tunicates, sea worms), etc. Compounds such as calicheamicins, thiazole-related compounds and epipolythiodioxopiperazines may serve as examples. Because of their vast variety, individual compounds are grouped according to their chemical structure (only compounds whose chemical structure has been identified are included). These compounds affect a huge array of organisms from microorganisms to man, and the pharmacological activities they exert range from antiviral, antibacterial, antifungal, insecticidal or antiprotozoan effects to regulation of gene expression, cytoskeleton disruption, immunomodulation, plant growth stimulation or inhibition, pro- or antioxidant action, effects on selected enzymes, receptors and signal transduction pathways, etc. The activities are however often known only sketchily, and this paucity of data, and the fact that the activities were tested on a diverse spectrum of test organisms, cell lines, etc., is of necessity reflected in our survey. Two seemingly trivial but important points deserve attention: (a) compounds with widely different structures and from widely different sources have often very similar pharmacological effects; (b) a slight modification in the molecule may bring vast changes in the biological effects of the compound, in terms of quality, quantity and targeting. The purpose of the review was to show both the unique and common features of these compounds and to point out their potential usefulness for mankind.

Keywords: Bithiazoles, Thiopeptides, Thiazole related compounds, Thiophenes, Thiolanes, Epipolythiodioxopiperazines, Six and more membered oligosulfides, Disulfides, Enediynes.

INTRODUCTION

Sulfur-containing natural compounds are highly varied in structure. We here present low-molecular compounds containing two or more sulfur atoms that have only rarely been described in the literature. We do not include common compounds such as penicillins [1] or cephalosporins [2,3] or oligopeptides [4] with disulfide bridges unless not formed by non-essential amino acids. The compounds we describe were found both in the plant and animal kingdom and also in microorganisms and usually exhibit antibiotic activity [5,6]. Their sources are as varied as is their chemical structure and they can be isolated from prokaryotic microorganisms, *e.g.* the genus *Streptomyces*, and from sponges or tunicates, as well as from higher plants of the family Asteraceae (Compositae). Some sulfur-containing natural compounds have interesting biological effects [7]. Unfortunately, the number of newly described compounds steadily increases, whereas that of compounds applied in the practice grows only relatively slowly and the scissors between the two groups of compounds are permanently gaped.

As far as the natural antibacterial drugs are concerned, the number of pharmaceutical companies engaged in this area seems to decrease. This is mainly due to the rapidly developing antibiotic resistance. The companies are therefore afraid that a financial return may not be made as high investments are required to bring an antibacterial drug through clinical trials to the market and that its viability will decrease with the occurrence of bacterial mutants resistant to the drug [8]. However, there is no doubt that natural products will still be used as leads for the development of new drugs. With the continuing research in genomics of microbial producers of natural compounds, it is hoped that genomic data will be successfully used for the discovery of new drugs or of new derivatives of known drugs that could not have been revealed without the genomic data. Similarly, a number of genomes of pathogenic bacteria have already been elucidated, and the research in this area, which rapidly develops, may reveal new targets for antibacterial drugs.

It should be noted that the compounds so far isolated from soil microorganisms represent at best but a tiny fraction of the vast variety of different compounds produced by these microorganisms in this environment. One of the main reasons is the unbelievably large diversity of soil organisms and the fact that, according to recent data [9], substantially less than 1 % of them are cultivatable.

BITHIAZOLES

Myxobacteria with their characteristic ability to glide in swarms, to feed cooperatively, and to form fruiting bodies upon starvation, have been shown to produce a wide variety of secondary metabolites with unique structures and biologi-

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cal activities – myxothiazoles, cystothiazoles, melithiazoles [10,11]. One of them is the electron transport inhibitor myxothiazole, which contains a thiazole ring that is formed by the incorporation of cysteine into the polyketide backbone. In addition to the bis-thiazole moiety, myxothiazole has some unique features: the unusual leucine-derived starter unit 3-methyl-butyryl-CoA and the linear polyketide backbone, which includes a β-methoxy-acrylate and a terminal amide structure [12].

Myxothiazole A (**1**) was first isolated from a gliding bacterium, *Myxococcus fulvus* [13] in 1980, and full details of its isolation, physical properties and gross structure were published later [14]. More recently, it was isolated from other gliding bacteria such as *Stigmatella aurantiaca* and *Angiococcus disciformis*. Myxothiazole A is highly active *in vitro* against a wide range of filamentous fungi and yeasts but, in addition, inhibits the growth of certain Gram-positive bacteria.

Other 23 myxothiazoles have been characterized. Myxothiazoles B to I (**2**) and K to O (**3**) (there is no myxothiazole J) are modifications of myxothiazole A in which the (*E*,*E*)-nonadienyl chain is truncated or oxidized. The substituent R^2 represents either a 1-hydroxyethyl or an acetyl group (myxothiazole N and O, respectively), or a branched 9-carbon chain incorporating olefinic, hydroxyl, ketone and/or epoxide functionality. The myxothiazoles Q, X and Y (**4**) are related compounds that contain an additional methyl substituent on the acrylamide unit, presumably the result of the incorporation of propionate instead of acetate during biosynthesis (see Fig. **1**); myxothiazole Q is the exact analog of myxothiazole A. Myxothiazole P is unique in that it contains only one thiazole ring. Finally, myxothiazoles R to W (**5**) contain no methoxyacrylamide unit, the group R representing an oxidized 3-, 5- or 6-carbon chain.

The antibiotic cystothiazole A (**6**) was isolated from the myxobacterium *Cystobacter fuscus* by using an inhibition assay against the phytopathogenic fungus, *Phytophthora capsici*. Cystothiazole A was the major and most active principal compound among the cystothiazole family [15]. Its structural features are the presence of β-methoxyacrylate and bithiazole moieties. As indicated by incorporation experiments this metabolite might be biosynthetically derived via a hybrid process by polyketide synthetases and non-ribosomal peptide synthetases. Other natural products recently discovered from myxobacteria include, *e.g.*, melithiazoles and myxothiazole A; these β-methoxyacrylate type molecules are thought to bind to the cytochrome bc_1 complex of the mitochondrial respiratory chain system to exhibit fungicidal but not bactericidal activity. The mode of action of cystothiazole A was demonstrated by an NADH oxidase inhibition experiment using sub-mitochondrial membrane. Further investigation [16] of a large-scale culture of *C. fuscus* resulted in the isolation of additional cystothiazole analogs, cystothiazoles B-F (**7**-**11**). These compounds showed also inhibitory activity against *P. capsici*, see below. Another analogue, *i.e.* cystothiazole G (**12**) was isolated more recently [15]. Disc diffusion test of antifungal activity against *P. capsici* of cystothiazoles A (0.09), B (2.3), C (12), D (12), E (14), F (0.5) and G (2.4) (nmol/disc) shows that the antifungal activity decreases in the order A > F > B = G > C = D = F. Hence the introduction of the 5-hydroxyl group into the most potent A diminishes the activity; the 13-methoxy, 13-unsaturated methyl ester also seems to be important for the activity; and introduction of a hydroxyl group to the isopropyl portion again diminishes the activity. Such a lower activity of C and D seems to be attributable to the instability of the 5-*O*-demethyl compounds. At concentrations from 0.2 to 14.9 nmol/ml cystothiazole A was inactive against bacteria. It was also tested for the *in vitro* cytotoxicity by using human colon carcinoma and leukaemia cell cultures. The IC_{50} values of cystothiazole A were 260-308 nmol/ml.

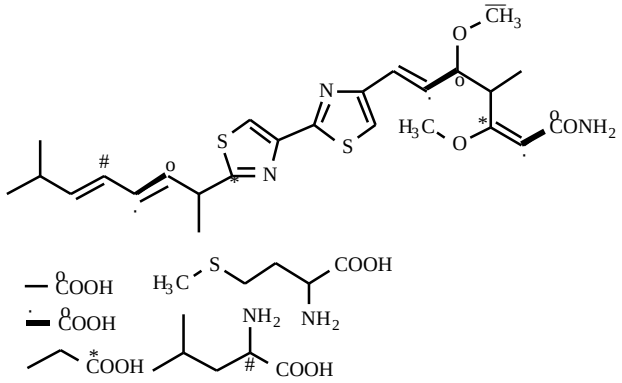
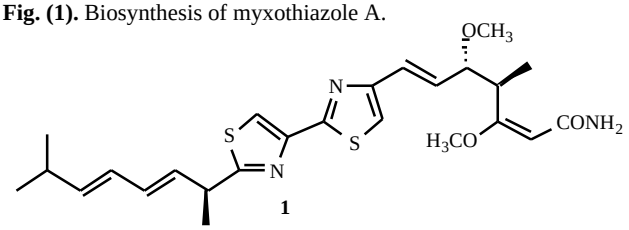
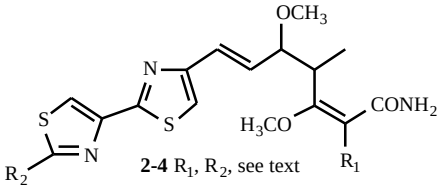


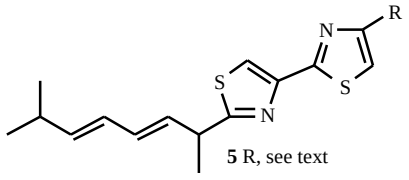
Fig. (1). Biosynthesis of myxothiazole A.



1



2-4 R_1, R_2, see text

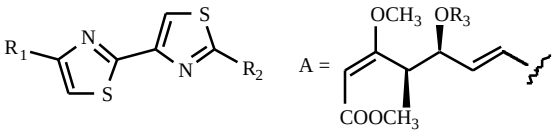


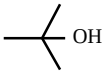
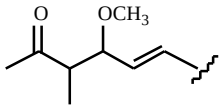
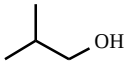
5 R, see text

Several related antibiotics from myxobacteria exhibit similar biological activities: myxothiazole from *Myxococcus fulvus*, and melithiazoles A and B from *Melittangium lichenicolum.*

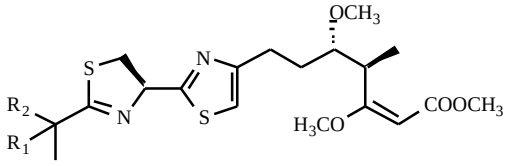
New antibiotic compounds, melithiazoles (**13**-**25**) [18], were isolated from the culture broth of strains of the myxobacteria *Melittangium lichenicolum*, *Archangium gephyra*, and *Myxococcus stipitatus*. These compounds belong to the group of β-methoxyacrylate inhibitors and are related to the myxothiazoles. The melithiazoles show a high antifungal activity (Table **1**) but are less toxic than myxothi-

azole A and its methyl ester in a growth inhibition assay with mouse cell cultures. The melithiazoles inhibit NADH oxidation by submitochondrial particles from beef heart. Like cystothiazole, melithiazole A blocks the electron transport within the bc (1)-segment (complex III) and causes a red shift in the reduced spectrum of cytochrome b.



No	R_1	R_2	R_3
6	A		CH_3
7	A		CH_3
8	A		H
9	A		H
10			-
11	A		CH_3
12	A		CH_3

Melithiazoles were also found to exhibit antifungal activity against *Hansenula anomala*, *Metschnikowia pulcherrima*, and *Pythium debaryanum* [19].



No.	R_1	R_2
13	-CH_2-	
16	CH_3	H
22	-CH_2O-	
23	CH_2OCH_3	H

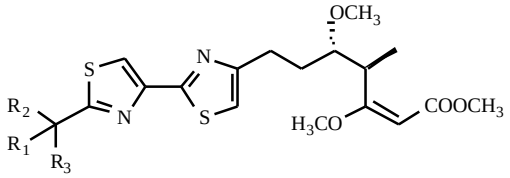
A new bithiazole [20], KR-025 (**26**), was isolated from *Myxococcus fulvus*. Its structure was elucidated by spectroscopic analysis. In addition to KR-025, the strain produced relatively large quantities of the closely related antibiotic, myxothiazole. KR-025 demonstrated potent cytotoxicity against human tumor cells, having IC_{50} values ranging from 0.02 to 19.3 pmol/ml. Against human tumor cells such as SK-OV-3, the activity of KR-025 was more than 100 000 times higher than that of antimycin A in terms of IC_{50}.

Two novel antibiotics, watasemycins A (**27**) and B (**28**) [21,22], were isolated from the fermentation broth of an actinomycete strain. The producing strain was isolated from a seawater sample collected in Toyama Bay, Japan, and identified as *Streptomyces* sp. based on a taxonomic study.

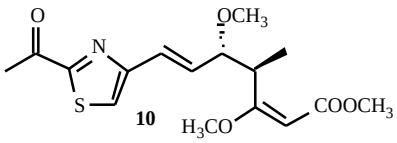
Table 1. Biological activities of melithiazoles

Compound	*Botrytis cinerea* IC_{50} [pmol/ml]	Inhibition of NADH oxidation IC_{50} [pmol/ml]	Cytotoxity [pmol/ml]
13	94	78	118
14	95	43	71
15	4419	4714	2062
16	352	164	70
17	188	108	118
18	635	311	53
19	684	93	137
20	365	195	1096
21	684	171	296
22	681	567	1475
23	175	66	110
24	94	71	118
25	342	137	342

The watasemycins A (**27**) B (**28**) exhibited activities against Gram-positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* at 35.5 and 71.0 nmol/ml, respectively, and potent activity against the Gram-negative bacterium *Proteus mirabilis* at 1.1 nmol/ml but they had no activity against *Escherichia coli.* Both compounds exhibited a low activity against yeasts. Other biological activities of watasemycins have not yet been tested. Watasemycin is structurally closely related to thiazostatin A (**29)** and B (**30**). The former was isolated from *Pseudomonas aeruginosa* as an endogenous growth promoter and the latter from *Streptomyces tolurosus* as an antioxidant.



No.	R_1	R_2	R_3
14		-CH_2-	CH_3
17	CH_3	CH_3	H
18	Ph	H	H
19	CH_2CH_3	CH_3	H
20	CH_3	H	H
21	i-Pr	H	H
24	CH_3		-O-
25		-CH_2O-	CH_3



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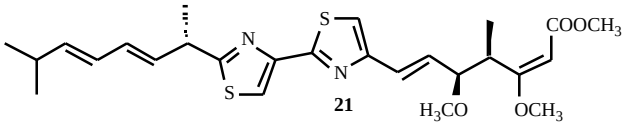

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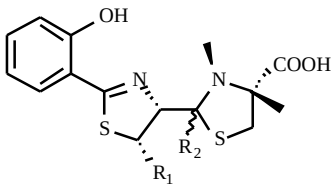
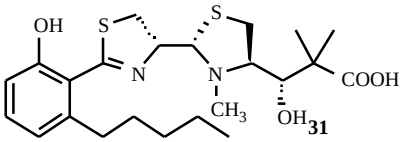


No	R_1	R_2
27	CH_3	····H
28	CH_3	H
29	H	····H
30	H	H

Agrochelin [23], a new alkaloid cytotoxic substance, was produced by the fermentation of *Agrobacterium* sp. Agrochelin (**31**) exhibited a cytotoxic activity, its IC_{50} value varying from 0.053 to 0.268 μM for P-388 and HT-29 cells, respectively.



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THIOPEPTIDE ANTIBIOTICS

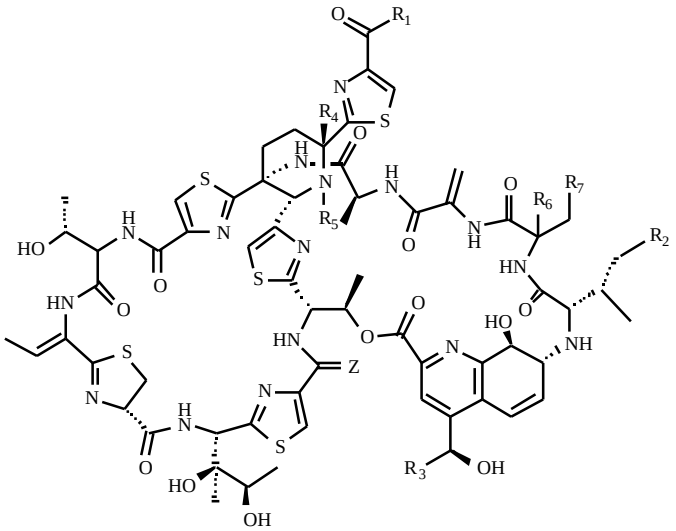
Thiopeptide antibiotics occur in nature as sulfur-containing macrocylic peptides composed of tri- or tetrasubstituted nitrogen heterocycles with thiazoles, oxazoles and dehydroamino acids as their structural core. All these compounds inhibit bacterial protein synthesis. They are produced primarily by actinomycetes, by the genus *Streptomyces* in particular, and were isolated from a number of different strains, predominantly from soil but also from marine sources. More than 75 compounds with a similar structure were described [24]. Irrespective of their chemical structure, similar mechanisms are involved in their biosynthesis and they exhibit similar biological effects - activity against Gram-positive, but not Gram-negative bacteria. A remarkable effect on methicillin-resistant *Staphylococcus aureus* was also observed.

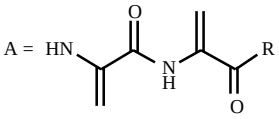
According to their chemical structure, thiopeptide antibiotics can be divided into five groups based on their central heterocyclic domain. Thus for instance antibiotics of the first and second group, *i.e.* thiostrepton or siomycin group, contain tetrasubstituted dehydropiperidine or saturated piperidine heterocycles, respectively. The third group comprises only Sch 40832 with an unusual imidazopiperidine cycle. Another group includes a trisubstituted pyridine cycle with the first isolated and identified antibiotic of this type, *viz.* micrococcin. The last group includes peptides, such as nosiheptide, with a tetrasubstituted hydroxypyridine.

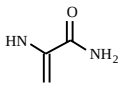
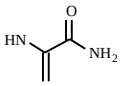
Piperidines and Dehydropiperidines

Compounds of this group have an excellent antibacterial activity and contain bis-macrocyclic peptide and quinaldic acid, thiazoline, dehydroalanine, dehydrodemethylvaline, and a number of thiazole heterocycles as structural units. The best known are thiostreptons (thiostrepton A **32** or B **33**) isolated from *Streptomyces azureus* in the middle of the last century [25]. Thiostrepton A was later also isolated from *Streptomyces laurentii* [26]. Siomycins **(34-37)** isolated from *Streptomyces sioyaensis* and active against Gram-positive bacteria and mycobacteria [28, 29] represent another group of compounds. The thiopeptins (**38-45**) produced by *Streptomyces tateyamensis* inhibit Gram-positive bacteria.

In mixtures, thiopeptin B is the main constituent; four minor components A_1, A_2, A_3, and A_4, that have later been demonstrated are mixture of the series **a** and **b**. Hence, thiopeptins A_{1a} **38**, A_{3a} **39**, A_{4a} **40**, B_a **41**, A_{1b} **42**, A_{3b} **43**, A_{4b} **44**, and B_b **45** are currently known [30,31]. Sch 18640, isolated from *Micromonospora arborensis* [32], may serve as a representative of compounds produced by a microorganism different from *Streptomyces*.





No	R_1	R_2	R_3	R_4, R_5	R_6, R_7	Z
32	A, R=NH_2	CH_3	CH_3	∏ bond	H	O
33	NH_2	CH_3	CH_3	∏ bond	H	O
34	A, R=NH_2	H	CH_3	∏ bond	$=CH_2$	O
35	NH_2	H	CH_3	∏ bond	$=CH_2$	O
36	A, R=OCH_3	H	CH_3	∏ bond	$=CH_2$	O
37	A, R=NH_2	H	H	∏ bond	$=CH_2$	O
38	A, R=OCH_3	H	CH_3	H	H	S
39	NH_2	H	CH_3	H	H	S
40		H	CH_3	H	H	S
41	A, R=OH	H	CH_3	H	H	S
42	A, R=OCH_3	H	CH_3	∏ bond	H	S
43	NH_2	H	CH_3	∏ bond	H	S
44		H	Me	∏ bond	H	S
45	A, R=OH	H	Me	∏ bond	H	S
46	A, R=NH_2	Me	Me	H	H	S

Sch 40832 **(46)** is the only known antibiotic of the third series, *i.e.* compound containing dihydroimidazopiperidine as the key structural unit. It was isolated from *Micromonospora carbonacea* var. *africana* [33] and is active against Gram-positive bacteria. Structurally, it is interesting that it contains two unusual saccharides, *viz.* β-D-chromose A and B.

Trisubstituted Pyridines

The chemical structure of this group of compounds differs from the previous ones. They are primarily compounds containing only a single ring and a trisubstituted pyridine connected with thiazole and/or oxazole heterocycles. This group of antibiotics is large and only some of almost 50 compounds will be mentioned here. Micrococcin was iso-

lated first in 1948 from a strain of *Micrococcus* found in sewage [34]. Later a mixture of two compounds, currently called micrococcin P_1 (**47**) and micrococcin P_2 (**48**), was isolated from the *Bacillus pumilus* group of spore-bearing bacilli collected from soil in East Africa [35] and also from a taxonomically completely different species *Staphylococcus equorum* isolated from French Raclette cheese [36].

Thiocillins I-III **(49-51)** [37,38] were isolated from different species of bacilli (*Bacillus badius*, *megaterium*, *pumilus*). Quite recently, structurally highly similar polypeptides called YM-266183 (**52**) and YM-266184 (**53**) were described in *B. cereus* isolated from the marine sponge *Halichondria japonica*. Both compounds are highly active against staphylococci and enterococci including the highly resistant strain of *Staphylococcus aureus* with MIC reaching values of 0.67 and 0.33 nmol/ml, respectively but as usually, inactive against Gram-negative bacteria [39,40]. Berninamycins (A-D) **(54-57)** and sulfomycins were identified in *Streptomyces bernensis* and found to specifically inhibit bacterial protein synthesis [41,42]. As well as showing the antibacterial activity, the berninamycins also act as regulators of gene expression by exhibiting *tipA* promoter activity at nanomolar concentrations [43].

Sulfomycins (I-III) **(58-60)** were isolated from *Streptomyces viridochromogenes*, with sulfomycin I being obtained from *Streptomyces viridochromogenes* subsp. *sulfomycini* [44,45]. All sulfomycins exhibit a high activity against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* and, as expected, do not inhibit Gram-negative species (Table **2**).

The isolated strain *Streptomyces* sp. produces two thiopeptides; one of them is methylsulfomycin I **(61)**, which shows potent antibiotic activity against several Gram-positive bacteria such as *Micrococcus luteus*, and *Staphylococcus aureus* [46] and a good *in vitro* activity against a wide range of Gram-positive bacteria. It was more than 800 times more active than vancomycin against vancomycin- and teicoplanin-resistant strains. The MIC values against several *Staphylococcus* and *Enterococcus* species are in the range of 0.05-0.099 nmol/ml. The other is a new thiopeptide named radamycin **(62)** with no antibiotic activity detected in the assays. However, it has a very strong capacity as an inducer of the *tipA*. Induction of the *tipA* promoter also occurs with methylsulfomycin I [47,48].

A mixture of antibiotics designated A10255 **(63-66)** (with B, E, G, and J as majority compounds) was isolated from *Streptomyces gardneri* and found to exhibit a strong activity against Gram-positive bacteria as well as promote growth and alleviate acidosis in ruminants. It is worth mentioning that cobalt or cobalamin must be present in the cultivation medium. The addition of these compounds strongly affects the ratio of individual antibiotics, due mainly to the fact that it increases methionine biosynthesis and the incorporation of methyl groups into the antibiotic [49-51]. Geninthiocin **(67)** was isolated from *Streptomyces* sp. using the technique of insertion of the promoter (*ptip A*) of *tipA* gene into a promoter probe vector making it possible to identify thiostrepton-like compounds according to their ability to initiate *ptipA* transcription [52].

The producing strain *Streptomyces* sp. produces a thiazolyl peptide promoinducin **(68)** exhibiting activity against Gram-positive bacteria including *Micrococcus luteus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and methicillin-resistant *Staphylococcus aureus*, with MIC of 0.30, 0.08, 0.08, and 1.18 nmol/ml, respectively, and acts as a *tipA* promoter at 30 pmol/ml [53]. Thiotipin **(69)** was also isolated from the mycelium of *Streptomyces* sp. as a *tipA* promoter inducing substance [54]. Thiotipin has antibacterial activity against *S. pneumoniae*, *S. pyogenes*, and *M. luteus* within the range of nmol/ml and a minimum induction concentration of 63 pmol/ml for *tipA* promoter inducing activity.

Two other antibiotics, thioactin **(70)** and thioxamycin **(71)**, were also isolated from *Streptomyces* sp. with the aid of the screening program utilizing the *tipA* promoter inducing activity [55]. The former was isolated from another species of the genus *Streptomyces* [56]. Both compounds exhibit activity against Gram-positive bacteria. Minimum induction concentrations of thioxamycin and thioactin for *tipA* promoter activity were 72 and 30 pmol/ml, respectively, and thioactin exhibited activity against *S. pyogenes* (0.62 pmol/ml), *S. pneumoniae* (1.23 pmol/ml) and *Streptococcus constellatus* (9.86 pmol/ml).

One of the largest groups of thiopeptide antibiotics are compounds designated GE2270 **(72-81)**. Ten compounds of

Table 2. Antibiotic Activity of Sulfomycins

Test organisms	Sulfomycin (MIC, nmol/ml)		
	58	**59**	**60**
Staphylococcus aureus	0.08	0.16	0.63
Staphylococcus epidermidis	0.16	0.32	1.26
Enterococcus faecalis	0.08	0.16	1.26
Enterococcus faecium	0.04	0.08	1.26
Escherichia coli	>80	>81	>81
Klebsiella pneumonie	>80	>81	>81
Pseudomonas aeruginosa	>80	>81	>81

this type have so far been discovered [57]. Compound A **(72)** isolated from the fermentation broth of *Planobispora rosea* is the majority component. Similarly to all other thiopeptides, these compounds inhibit Gram-positive microorganisms and anaerobes [58]. However, their main effect is on *Propionibacterium acnes* (MIC for A < 0.003 nmol/ml) and *Mycobacterium tuberculosis* (MIC for A < 0.8 nmol/ml) [59]. These antibiotics inhibit bacterial protein synthesis by specifically binding to the GTP-bound form of EfTu [60], the elongation factor required for binding of aminoacyl-tRNA to the ribosomal A site.

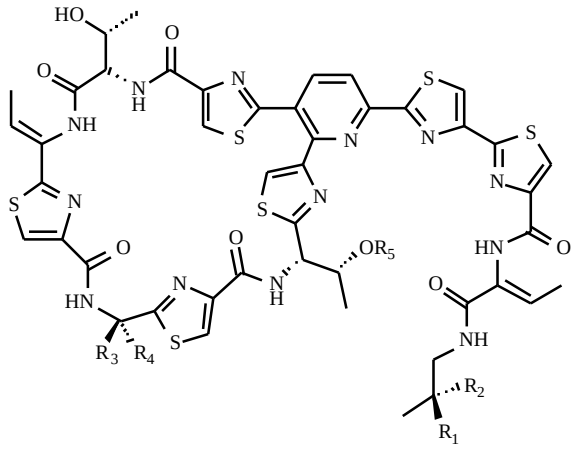
Using the *S. aureus* screening method, new antibiotics amythiamicins **(82-85)** [61-63] were discovered in the fermentation broth of the fungus *Amycolatopsis* sp., isolated from soil in Japan. They are active against Gram-positive bacteria including methicillin- and multi-drug resistant *S. aureus* [64]. However, they exhibit *in vivo* toxicity in mice (100 mg/kg) and have an unusual structure, with the dehydroalanine moiety missing, which leads to binding to the protein elongation factor Tu (Ef-Tu).

Cyclothiazomycin **(86)** was isolated from a *Streptomyces* sp. [65] identified in the Japanese soil. Unfortunately, its antibacterial activity has not yet been tested. However, it was found to exert the *tipA* promoter action with a selective activity (IC_{50} 1.7 μmol/ml) against human plasma rennin.

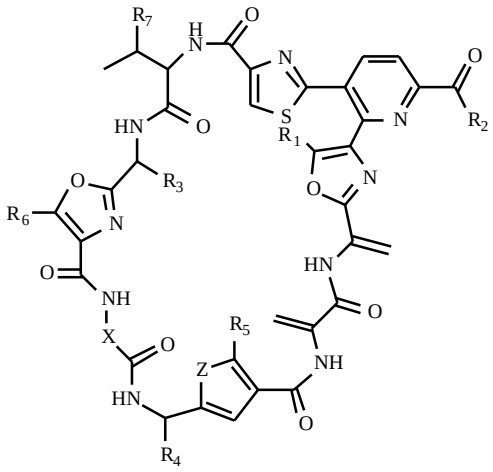
Hydroxypyridine Thiopeptides

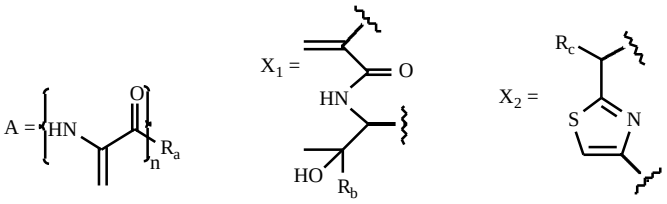
The fifth group of thiopeptide antibiotics is characterized by tetrasubstituted pyridine in the central chain. Compounds of this group also contain 5-alkoxy substituents, a tryptophan residue and occasionally glycosidic units. Nosipeptide **(87)** isolated from *Streptomyces actuosus* [66] is one of long-time known peptides. It was used as an additive of pig and poultry fodder. It is highly active against Gram-positive bacteria (*e.g.* MIC in *S. aureus* is 0.7 pmol/ml) and binds selectively to ribosomes inhibiting protein synthesis in *Bacillus subtilis* and *E. coli*. Antibiotic designated S54832 A-I **(88)** [67] was obtained from *Micromonospora globosa* isolated from soil in Spain. It inhibits growth of Gram-positive bacteria including staphylococci, streptococci, corynebacteria, and mycobacteria *in vitro* and is *in vivo* active against *S. pyogenes* and *pneumoniae* and *S. aureus*. Two structurally related antibiotics LL-14E605β **(89)** and its O-methyl derivative **(90)** containing an unusual saccharide, were isolated from the fermentation broth of *Sebekia benihana* [68,69].

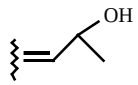
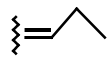
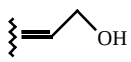
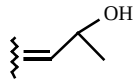
The fungus *Amycolatopsis* sp. from Japanese soil was found to produce two compounds called simply MJ347-81F4A **(91)** and B **(92)** [70]. Both exhibit activity against Gram-positive bacteria including resistant *S. aureus* and *Enterococcus faecalis* (MIC 0.005 - 0.08 nmol/ml). Activity against thiostrepton-resistant *B. subtilis* varies significantly within the range of 0.15 - 79 nmol/ml. The results indicate that bacterial 50S ribosomal subunit could be the target. Nocathiacins **(93-96)** obtained from cultures of the genus *Nocardia* but interestingly also from *Amycolaptosis* sp. are the last antibiotics of this group presented in this review. Nocathiacin I **(93)** exhibits activity against Gram-positive bacteria including many antibiotic-resistant strains. The mechanism of action is its interaction with the 23S RNA complex of the bacterial ribosome [71]. Nocathiacins II-IV **(94-96)** were also described.

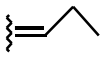
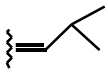
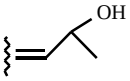
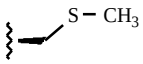
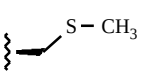


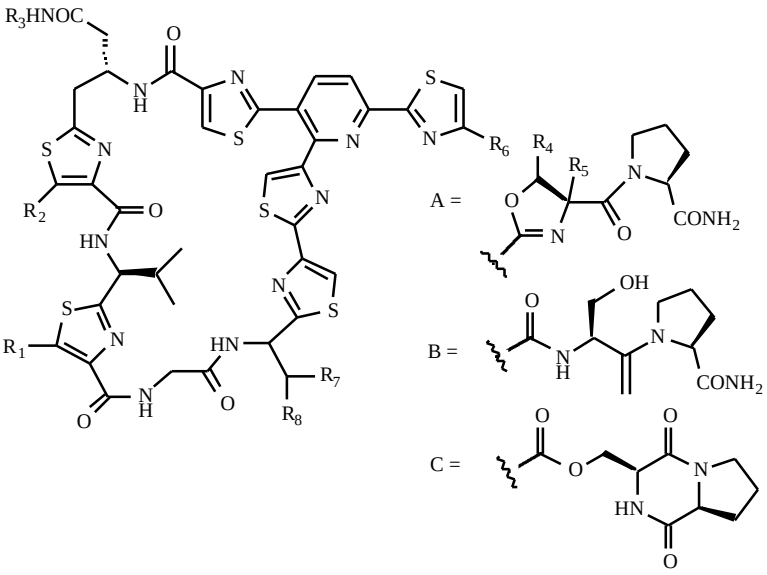
No	R_1	R_2	R_3	R_4	R_5
47	OH	H	H	$CH(CH_3)_2$	H
48	=O		H	$CH(CH_3)_2$	H
49	H	OH	$C(CH_3)_2OH$	H	H
50	H	OH	$C(CH_3)_2OH$	H	CH_3
51	H	OH	$CH(CH_3)_2$	H	CH_3
52	=O		$C(CH_3)_2OH$	H	H
53	=O		$C(CH_3)_2OH$	H	CH_3





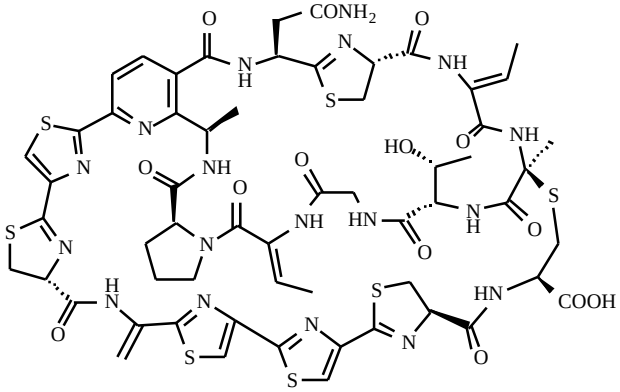
No	R_1	R_2	R_3	R_4	R_5	R_6	R_7	X	Z
54	H	A, n = 2, $R_a = NH_2$			CH_3	CH_3	OH	X_1, R_b = OH	O
55	H	A, n = 2, $R_a = NH_2$			CH_3	CH_3	OH	X_1, R_b = H	O
56	H	A, n = 1, $R_a = NH_2$			CH_3	CH_3	OH	X_1, R_b = OH	O
57	H	A, n = 0, $R_a = NH_2$			CH_3	CH_3	OH	X_1, R_b = OH	O
58	H	A, n = 3, $R_a = NH_2$			CH_3	CH_3	OH	X_2, R_c = OCH_3	O
59	H	A, n = 3, $R_a = NH_2$			CH_3	CH_3	OH	X_2, R_c = OCH_3	O
60	H	A, n = 3, $R_a = NH_2$			CH_3	CH_3	OH	X_2, R_c = OCH_3	O
61	CH_3	A, n = 3, $R_a = NH_2$			CH_3	CH_3	OH	X_2, R_c = OCH_3	O
62	CH_3	A, n = 2, $R_a = NH_2$			H	CH_3	Me	X_2, R_c = CH_3	S

63	H	A, n = 4, R_a = OH			H	H	OH	X_2, R_c = H	S
64	H	A, n = 4, R_a = OH			H	H	OH	X_2, R_c = H	S
65	H	A, n = 4, R_a = OH			H	H	OH	X_2 , R_c = H	S
66	H	A, n = 1, R_a = NH_2			H	H	OH	X_2, R_c = H	S
67	H	A, n = 2, R_a = NH_2			CH_3	H	OH	X_1, R_b = OCH_3	O
68	H	A, n = 4, R_a = OH			CH_3	CH_3	OH	X_2, R_c = OCH_3	O
69	H	A, n = 3, R_a = OH			CH_3	CH_3	OH	X_2, R_c = H	O
70	H	A, n = 1, R_a = NH_2			H	H	OH	X_2, R_c = H	S
71	H	A, n = 4, R_a = OH			H	H	OH	X_2, R_c = H	S

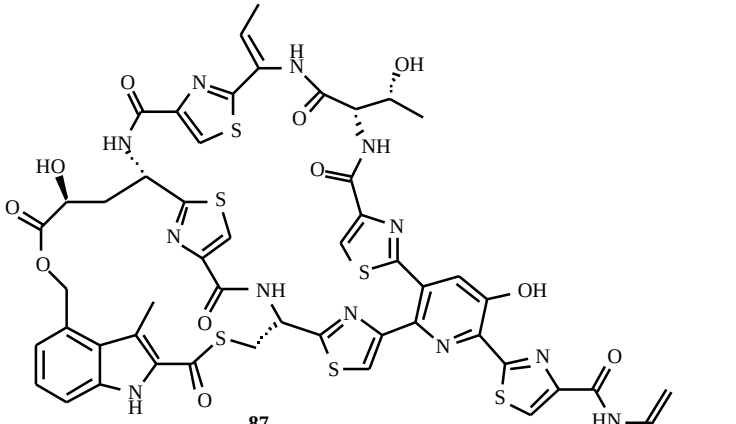


No	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
72	CH_2OCH_3	CH_3	CH_3	H	H	A	OH	Ph
73	CH_2OCH_3	CH_3	H	H	H	A	OH	Ph
74	CH_3	CH_3	CH_3	H	H	A	OH	Ph
75	H	CH_3	CH_3	H	H	A	OH	Ph
76	CH_2OCH_3	CH_2OH	CH_3	H	H	A	OH	Ph

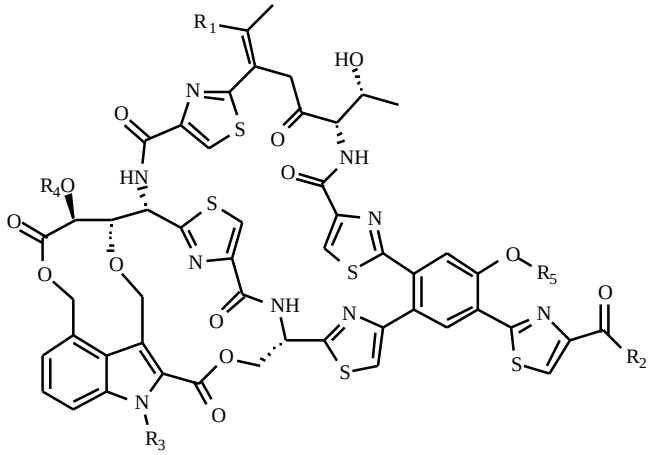
77	CH_2OCH_3	H	CH_3	H	H	A	OH	Ph
78	H	CH_3	H	H	H	A	OH	Ph
79	CH_2OH	CH_3	CH_3	H	H	A	OH	Ph
80	CH_2OH	CH_3	H	H	H	A	OH	Ph
81	CH_2OCH_3	CH_3	CH_3		Π-bond	A	OH	Ph
82	H	CH_3	CH_3	H	H	A	CH_3	CH_3
83	H	CH_3	CH_3	-	-	B	CH_3	CH_3
84	H	CH_3	CH_3	-	-	C	CH_3	CH_3
85	H	CH_3	CH_3	-	-	$COOCH_3$	CH_3	CH_3

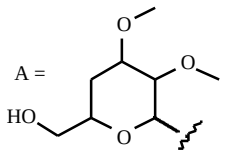


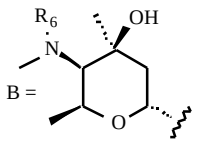
86

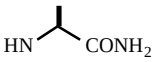
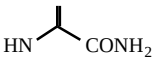
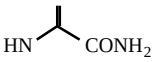
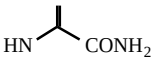
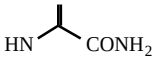
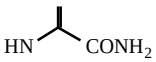


87







No.	R_1	R_2	R_3	R_4	R_5	R_6
88	H		OH	A	H	-
89	CH_3O	NH_2	H	B	CH_3	CH_3
90	CH_3O	NH_2	H	B	H	CH_3
91	CH_3O		OH	B	H	CH_3
92	CH_3O		OH	B	H	H
93	CH_3O		OH	B	H	CH_3
94	CH_3O		H	B	H	CH_3
95	CH_3O		OH	H	H	CH_3
96	CH_3O	NH_2	OH	B	H	CH_3

Biosynthesis of thiopeptide antibiotics was investigated with the aid of isotopically labeled precursors including cysteine, serine, isoleucine, threonine, methionine, and tryptophan. In various strains of the genus *Streptomyces* these amino acids were incorporated into the antibiotic, followed by determination by NMR and chemical degradation. All the data obtained so far indicate that the biosynthesis of the thiopeptide antibiotics occurs non-ribosomally.

General Mechanism of Action of Thiopeptide Antibiotics

Thiopeptide antibiotics inhibit the growth of Gram-positive bacteria, although it has been reported that some of them are also active as antifungal or anticancer agents or exceptionally act on Gram-negative bacteria, as renin inhibitors, or on *Plasmodium falciparum* (antimalarial). According to their mechanism of action they can be divided into two basic classes: 1. Antibiotics that bind to a region of the 23S ribosomal RNA called L11 binding domain and 2. Antibiotics that bind to the protein (Ef-Tu) complex and thus affect the elongation cycle.

Thiopeptide antibiotics of class 1 resemble penicillins in having practically no toxic effects on mammalian cells, and inhibiting protein synthesis in the bacterial ribosome rather than in the mammalian one. Thiostrepton, which was most thoroughly studied, was found to inhibit the binding of the aminoacyl-tRNA-containing ternary complex to the ribosomal A site *in vivo* [72]. Thiostrepton and micrococcin inhibit the growth of *Plasmodium falciparum*, a protozoan causing malaria. They inhibit organellar protein synthesis by targeting the large subunit encoded by a 35-kb organelle, one of two extrachromosomal DNAs of the parasite [73]. Two types of resistance to thiostrepton have been observed in Gram-positive bacteria, whereas Gram-negative bacteria are completely resistant to its action due to the fact that thiostrepton does not penetrate the bacterial cell. In Gram-positive bacteria, resistance mechanism includes the loss of ribosomal proteins BM-L11 [74] in *Bacillus megaterium* and BS-L11 [75] in *B. subtilis* resulting in deficient ribosomes with a reduced affinity for thiostrepton *in vitro* but retaining a considerable protein synthetic activity. The genus *Streptomyces* is generally highly sensitive to thiostrepton. In spite of that, *S. azureus*, its producer, is completely unaffected by the antibiotic. This is due to the production of RNA-pentose methylase enzyme produced constitutively and responsible for the autoimmunity. The enzyme introduces a methyl group into the A1067 residue of 23S rRNA in *E. coli,* yielding a modified ribosome that is fully resistant to the antibiotic.

Antibiotics of class 2 act by binding to the elongation factor (Ef-Tu), the most abundant protein of the bacterial cell; this either prevents the dissociation of Ef-Tu-GDP from the ribosomal complex or inhibits the binding of aminoacyl-tRNA to Ef-Tu-GTP. It was shown that some actinomycetes have more *tuf* genes encoding Ef-Tu translation factors. Some of the factors are fully resistant to some thiopeptides, *e.g.* kirromycin. For instance, Ef-Tu-mutants of *Bacillus subtilis* resistant to Ef-Tu-binding thiopeptide antibiotics showed considerable changes of conserved amino acids, which may account for their similar resistance behavior [76,77].

The goadsporin **(97)** [78,79], an active substance from the culture broth of *Streptomyces* sp., promoted the forma-

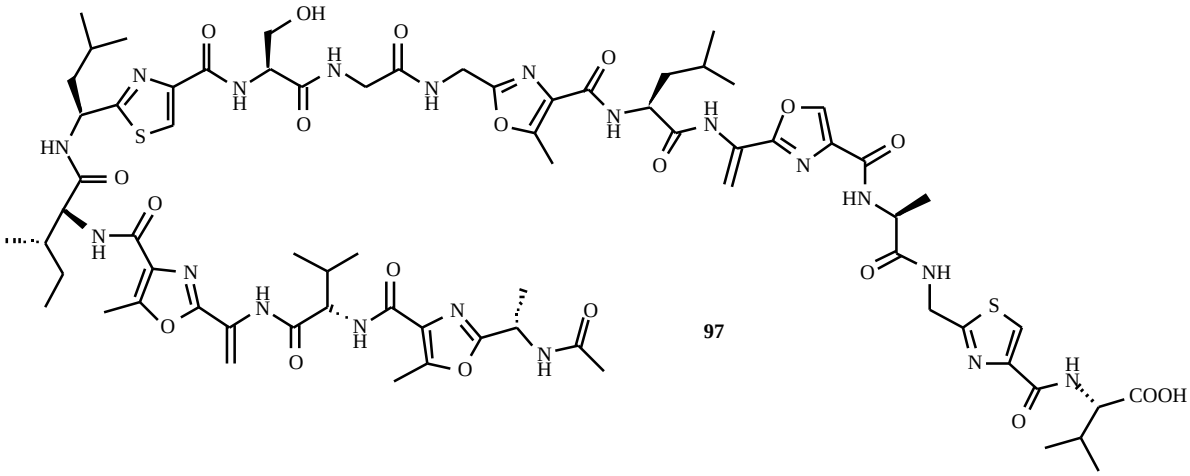
tion of red pigment and sporulation at a concentration of 1 μM in *Streptomyces lividans* which does not produce the pigment under normal growth conditions. Goadsporin is an oligopeptide consisting of 19 amino acids. Sporulation and/or secondary metabolite production was induced in 36 streptomycete strains among 42 strains tested. These results suggest that goadsporin acts on a common regulatory pathway for sporulation and secondary metabolism in streptomycetes and can be a powerful tool to analyze the regulatory mechanism. The antibiotic activity (Table **3**) of goadsporin showed growth inhibition of *Streptomyces but* not of other microorganisms at 62 μM. Goadsporin apparently can be useful in agrochemistry by inhibiting *Streptomyces scabies* causing potato scab.

The marine tunicate *Lissoclinum patella* produces a family of lipophilic cyclic peptides, all of which contain an unusual fused oxazoline-thiazole unit. They are predominatly patellamides A-G (**98**-**104**) and ulithiacyclamides A (**105**) and B (**106**). The patellamides A-C are cytotoxic and patellamide A is further active against the human cell line CEM [80]. Ulithiacyclamide A possesses a powerful cytotoxic activity and suppresses the growth of leukemia cells L 1210 and cells of human cancer CEM line [81-85], see Table **4**. Pure patellamide B and ulithiacyclamide A showed only a modest general cytotoxicity in the NCI's 60 human tumor cell line panel [86].

Dendroamide A (**107**), one of three new bistratamide-type cyclic hexapeptides (dendroamides B (**108**) and C (**109**)) from the terrestrial blue-green alga *Stigonema dendroideum*, exhibits multidrug-resistance reversing activity [87-89]. The physiological relevance of dendroamide A's interaction with P-glycoprotein rests mostly in its abilities to

Table 3. ***In vitro*** **Antibacterial Activities of Goadsporin (97)**

Organism	Relevant characteristics	MIC (nmol/ml)
Streptomyces lividans TK23	Gram(+)	4.0
Streptomyces coelicolor A3(2)	Gram(+)	2.0
Streptomyces scabies JCM7914	Gram(+)	0.12
Bacillus subtilis ATCC6633	Gram(+)	>62
Staphylococcus aureus 209P JC-1	Gram(+)	>62
Pseudomonas aeruginosa TP-B0270	Gram(-)	>62
Escherichia coli NIHJ JC-2	Gram(-)	>62
Proteus mirabilis ATCC21100	Gram(-)	>62
Saccharomyces cerevisiae TP-F0176	yeast	>62
Candida albicans TP-F0594	yeast	>62
Cryptococcus neoformans ATCC 90112	yeast	>62
Torulopsis glabrata IFO0622	yeast	>62
Aspergillus fumigatus IFO8866	filamentous fungus	>62



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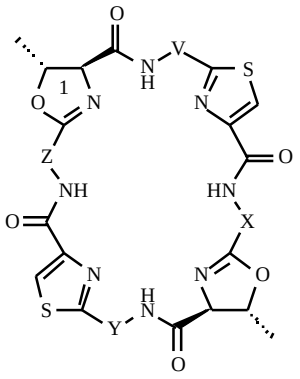
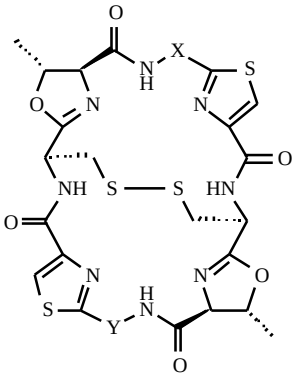
selectively potentiate the cytotoxicity of P-glycoprotein-transported drugs, increase the intracellular accumulation of vinblastine, and compete with [^{3}H]azidopine for binding to P-glycoprotein. These properties are characteristic for multidrug resistance reversing agents which act as antagonists for drug transport by P-glycoprotein.



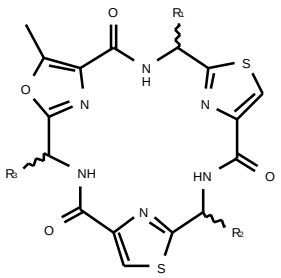
No.	V	X	Y	Z	Abbrev
98	D-Val	L-Ile	D-Val	L-Ile	ring 1 has no CH_3
99	D-Phe	L-Leu	D-Ala	L-Ile	
100	D-Phe	L-Val	D-Ala	L-Ile	
101	D-Phe	L-Ile	D-Ala	L-Ile	
102	D-Phe	L-Val	L-Val	D-Ile	
103	D-Phe	L-Val	L-Val	L-Ile	
104	D-Phe	L-Ile	D-Ala	L-Leu	

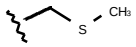
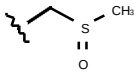
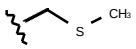
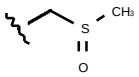


105 X = Y = D-Leu, R = i-Pr
106 X = D-Ile, Y = D-Phe, R = Ph

Table 4. Cytotoxicity of Marine Metabolites

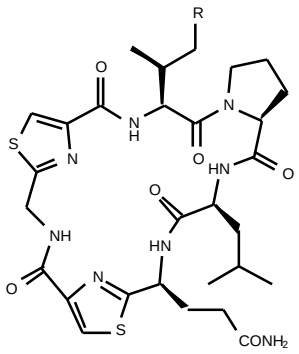
No.	IC_{50} nmol/ml		
	L1210	**CEM**	**NCI's 60**
98	4.3	0.031	-
99	2.1	-	48000
100	3.4	-	-
105	0.41	0.014	3000



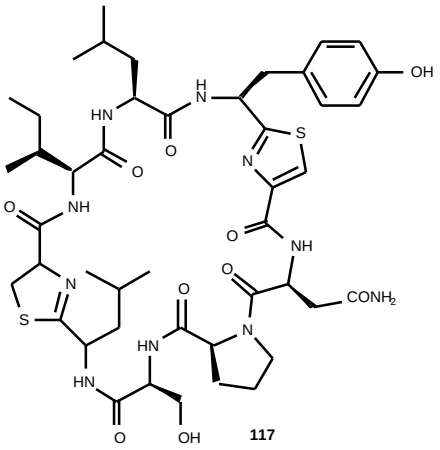
No.	R_1	R_2	R_3
107	(*R*)-i-Pr	(*R*)-CH_3	(*R*)-CH_3
108	(*R*)-CH_3	(*R*)- 	(*R*)-CH_3
109	(*R*)-CH_3	(*R*)- 	(*R*)-CH_3
110	(*R*)-CH_3	(*R*)-CH_3	(*S*)-CH_3
111	(*R*)-CH_3	(*S*)-CH_3	(*S*)-CH_3
112	(*S*)-CH_3	(*R*)- 	(*S*)-CH_3
113	(*S*)-CH_3	(*S*)- 	(*S*)-CH_3
114	(*S*)-CH_3	(*R*)-i-Pr	H

Four modified cyclic hexapeptides, tenuecyclamides A-D (**110-113**), were isolated from the methanol extract of *Nostoc spongiaeforme* var. *tenue* [90]. Tenuecyclamide A (**110**) was found to inhibit the division of sea urchin embryos with ED_{100} of 10.8 μmol/ml, tenuecyclamide C (**112**) with ED_{100} of 9.0 μmol/ml, and tenuecyclamide D (**113**) with ED_{100} of 19.1 μmol/ml. Tenuecyclamide B (**111**) was not tested. A new anticyanobacterial and antialgal secondary metabolite, nostocyclamide (**114**), that is also toxic against *Brachionus calyciflorus* has been isolated as a major component from the dinitrogen-fixing cyanobacterium *Nostoc* [91]. Nostocyclamide strongly inhibited the growth of cyanobacteria *(Anabaena* and *Synechocystis*), diatoms *(Navicula minima)*, and chlorophyceae *(Nannochloris coccoides*) but did not show antifungal activity when tested against *Saccharomyces cerevisiae.* In liquid cultures, nostocyclamide was inhibitory to the growth of *Anabaena* at a concentration of 0.1 μM (1-week incubation in the light at 25 °C). Considerable toxicity was observed (LC_{50} 12 μM) in a freshwater rotifer *(Brachionus calyciflorus)* bioassay.

A collection of the marine cyanobacterium *Lyngbya majuscula* from Palau contained the peptides dolastatin 3 (**115**), and new peptides homodolastatin 3 (**116**), and kororamide (**117**) [92]. Homodolastatin 3 (**116**), which only differs from dolastatin 3 (**115**) by replacement of a valine residue by isoleucine, showed no activity at 151 μmol/ml. The significance of Val/Ile substitution for SAR lies in two directions. a) Ile has a larger side chain than Val, which brings about lower



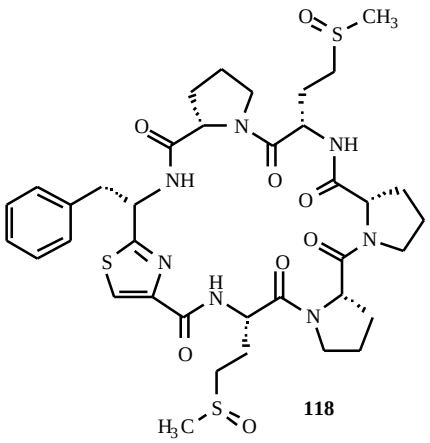
115 R = H
116 R = Me



117

polarity and higher steric requirements, b) In contract to Val, Ile has an optically active carbon in the side chain; the result of this is that one of the diastereoisomers has a larger affinity for the enzyme (protein).

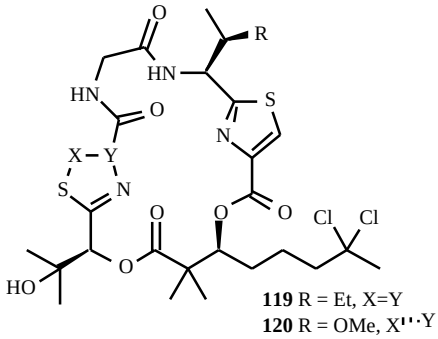
A new cyclic hexapeptide, waiakeamide (**118**) was isolated from the sponge, *Ircinia dendroides*, collected in Indonesia. Bioassay data of this compound showed a strong activity against P388 cells, with an IC_{50} of 0.066 nmol/ml [93].



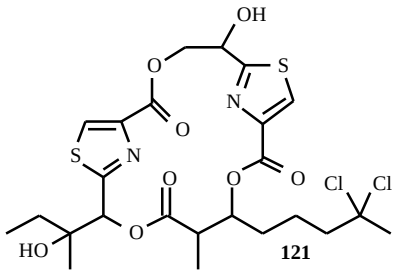
118

Lyngbyabellin A (**119**), a significantly cytotoxic cyclic depsipeptide with unusual structural features, was isolated from a Guamanian strain of the marine cyanobacterium *Lyngbya majuscula*. It was shown to be a potent disrupter of the cellular microfilament network [94]. Lyngbyabellin B (**120**) was isolated from *Lyngbya majuscula* collected in Florida [95]. It was toxic to brine shrimp (*Artemia salina*), with an LD_{50} value of 3.0 ppm, and to the pathogenic yeast *Candida albicans* in a disc diffusion assay, giving a 10.5-mm zone of inhibition at 125 nmol/disc and a slight halo at 12.5 nmol/disc. It exhibited IC_{50} values of 0.13 and 1.04 nmol/ml against KB and LoVo cells, respectively [96].

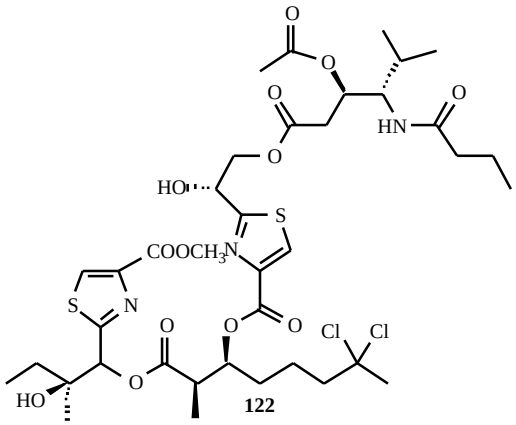
Re-collections of the cyanobacterium *Lyngbya* sp. yielded other members of the lyngbyabellin families, *viz.* lyngbyabellin C (**121**) [97], and D (**122**) [98] that displayed an IC_{50} value of 0.1 μmol/ml against the KB cell line.



119 R = Et, X=Y
120 R = OMe, X···Y



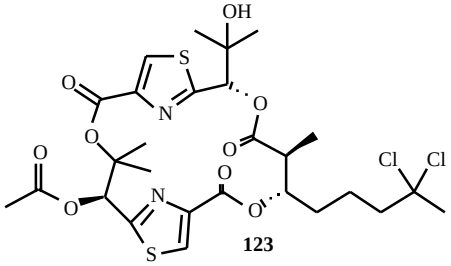
121



122

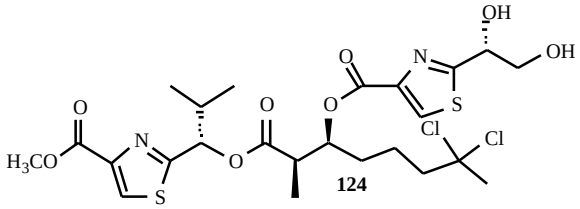
Hectochlorin (**123**) was isolated from isolates of *Lyngbya majuscula* collected in Panama [99]. Hectochlorin has the ability to promote actin polymerization but is unable to displace a fluorescent phalloidin analogue from polymerized

actin. In addition, it shows potent inhibitory activity toward *Candida albicans*. Hectochlorin inhibited the growth of CA46 cells (IC_{50} 0.09 μmol/ml) and PtK2 cells (IC_{50} 0.42 μmol/ml).



123

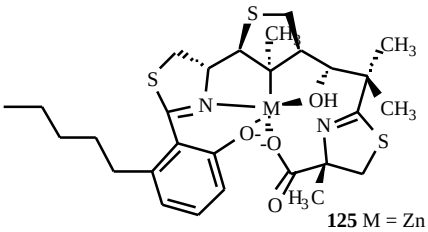
Dolabellin (**124**), a novel cytotoxic metabolite (IC_{50} = 0.01 pmol/ml (HeLa-S_3 cells)), which consists of two thiazole hydroxy acids and a new dichlorinated β-hydroxy acid, was isolated from the Japanese sea hare *Dolabella auricularia* [100].



124

THIAZOLE-RELATED COMPOUNDS

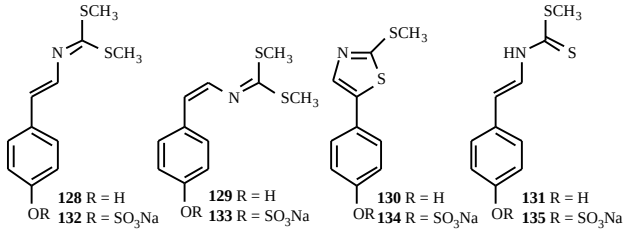
A Gram-negative bacterium *Pseudomonas* sp. was found to produce new zinc-containing antibiotics, micacocidin A (**125**), and related compounds containing Cu or Fe, micacocidin B (**126**) and C (**127**), respectively [101]. These new antibiotics exhibited an excellent activity against *Mycoplasma* species (MIC against *M. pneumoniae* in nmol/ml was ≤ 0.01 for all three micacocidins). Micacocidin (**127**) displayed also high activity against fungi such as *Candida*, *Aspergillus* and *Trichophyton* species [102].



125 M = Zn
126 M = Cu
127 M = Fe

Three novel phenolic metabolites, tridentatols A-C (**128-130**), with an uncommon sulfur-containing functional group, have been isolated from the marine hydroid *Tridentata marginata* [103]. Ecological studies showed that tridentatol A deters feeding by a common hydroid predator. These metabolites may also function to protect *T. marginata* from damaging solar ultraviolet radiation.

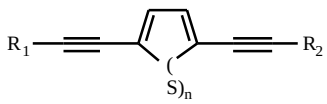
Five other secondary metabolites, tridentatols D-H (**131-135**), have been isolated from this hydroid [104]. When hydroid tissue is crushed, as attacking predators would do, tridentatols E-H (**132-135**), which do not deter fish feeding, are rapidly converted to tridentatols A-D (**128-131**), thereby repelling potential predators. Additionally, the tridentatols were isolated from this hydroid's nematocysts (*i.e.*, stinging organelles), representing the first report of a nonprotein venom produced by cnidarian nematocysts.



128 R = H
132 R = SO_3Na
129 R = H
133 R = SO_3Na
130 R = H
134 R = SO_3Na
131 R = H
135 R = SO_3Na

THIOPHENES

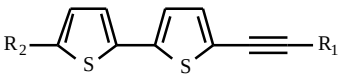
An increasing attention is devoted to a new class of sulfur-containing heterocyclic compounds, 1,2-dithia-3,5-cyclohexadienes or thiophenes - in connection with their unusual structure and a wide spectrum of biological activities. Red thiarubrines A (**136**) and B (**137**) are produced by many plants used as medical products by the peoples of Africa. For example, leaves of African plant *Aspilia africana*, containing thiarubrines A and B, serve as medicines for abdominal infection. It has been noticed that chimpanzees chew leaves of this plant [105]. Compound (**136**) possesses antiviral, fungicidal (*Candida albicans*) and antibacterial activity (in concentration 10^{-7} μmol/ml) [106]. The following plants also serve as sources of thiarubrines: *Aspilia mossambicensic*, *A. plurisetta* [107], *Ambrosia artemisiifolia* [108], *Pegolettia senegalensis* [109], *Wedelia hookeriana* [110], *Schkhuria miltiflora* [111], *S. senecioides* [112], *Chaenactis douglasii*, *Eriophyllum lanatum* [113], and *E. caespitosum* [112,114]. Compounds **138** and **139** were also identified in the different genera [111-114].

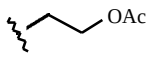
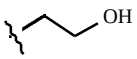
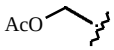
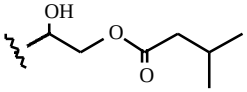
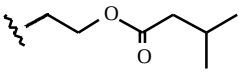
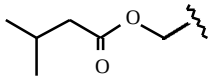


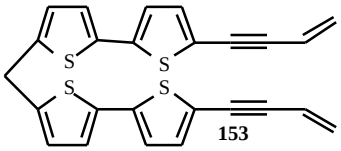
No.	R_1	R_2	n
136	CH_3	$C{\equiv}C\text{-}CH{=}CH_2$	2
137	$C{\equiv}C\text{-}CH_3$	$CH{=}CH_2$	2
138	CH_3	$C{\equiv}C\text{-}CH{=}CH_2$	1
139	$C{\equiv}C\text{-}CH_3$	$CH{=}CH_2$	1
140	$CH{=}CH\text{-}CH_3$	$CH{=}CH_2$	2
141	CH_3	$CH{=}CH\text{-}CH{=}CH_2$	2
142	$CH{=}CH\text{-}CH_3$	$CH{=}CH_2$	1
143	CH_3	$CH{=}CH\text{-}CH{=}CH_2$	1

A number of plants of the family Compositae produced dihydrothiarubrines (**140, 141**) that are biosynthetically connected with thiophenes **142** and **143**. For example, dithiine (**140)** was isolated from *Picradeniopsis woodhousei*, *Lasthenia* sp. [115] or *Ogedaea boliviana* [116], and 2,5-disubstituted thiophene **143** was found in *Melampodium divaricatum* [117]. The acetylenic bithiophene constituents from the aerial parts of *Blumea obliqua* yielded novel

dithienyl acetylenes (**144-153**) [118-120]. The compounds (**148** and **149**) showed antifungal activity against *Epidermophyton floccosum* and *Pleurotus ostreatus* (both at MIC 600 nmol/ml).



No.	R_2	R_1
144	CH_3	
145	CH_3	
146		
147	CH_3	
148	CH_3	
149		
150	CH_2OH	
151	H	
152	CHO	

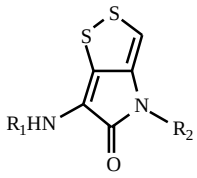


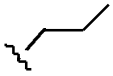
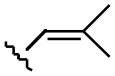
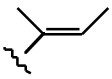
153

THIOLANES

Holomycin (**154**), which has been known for a long time, is a member of the pyrrothine class of antibiotics [121]. It was isolated from *Streptomyces griseus.* It displayed a broad-spectrum antibacterial activity, inhibiting a variety of Gram-positive and Gram-negative bacteria, with the exception of *Enterobacter cloacae*, *Morganella morganii*, and *Pseudomonas aeruginosa*. It lacked antibiotic activity against the eukaryotic microorganisms such as *Saccharomyces cerevisiae* and *Candida kefyr*. Holomycin exhibited a bacteriostatic response against *Escherichia coli* that is associated with a rapid inhibition of RNA synthesis in whole cells. Inhibition of RNA synthesis could have been a secondary consequence of inhibiting tRNA aminoacylation, thereby inducing the stringent response. However, the levels of inhibition of RNA synthesis by holomycin were similar in a stringent and relaxed pair of *E. coli* strains that were isogenic except for the deletion of the *relA* gene. This suggests that inhibition of RNA synthesis by holomycin could reflect direct inhibition of DNA-dependent RNA polymerase. Furthermore, inhibition of RNA polymerase was observed only at holomycin concentrations in excess of those required to inhibit the growth of *E. coli*. It is possible that holomycin is a prodrug, requiring conversion in the cell to an active species that inhibits RNA polymerase [122].

Three new natural antibacterial and antifungal dithiolopyrrolone antibiotics PSA (**155**), PSB (**156**), and PSC (**157**) were isolated [123] along with the known isobutyropyrrothine (**158**) and thiolutine (**159**) [124] from the fermentation broth of *Saccharothrix* sp. which was isolated from a Saharian palm grove soil. They showed a high activity against Gram-positive bacteria such as *Bacillus coagulans, B. subtilis* and *Micrococcus luteus*, except for *Staphylococcus aureus* (MIC 100-375 nmol/ml). PSA (**155**) and PSB (**156**) showed activity against *Saccharomyces cerevisiae, Mucor ramanniamus* and the phytopathogenic fungi *Fusarium oxysporum* sp. *albedinis, F. lini,* and *F. culmorum.* All antibiotics had no or weak activity against Gram-negative bacteria except for *Klebsiella pneumoniae*, which was strongly inhibited.



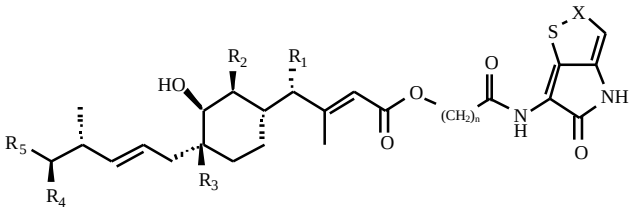
No.	R_1	R_2	No.	R_1	R_2
154	CH_3	H	**157**		CH_3
155		CH_3	**158**		CH_3
156		CH_3	**159**	CH_3	CH_3

Thiomarinol A (**160**) was produced by a marine bacterium, *Alteromonas rava*, and found to exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria [125]. Thiomarinol B (**161**) showed [126] excellent *in vitro* antimicrobial activity against Gram-positive and Gram-negative bacteria (Table **5**). Thiomarinol C (**162**) was slightly less active than thiomarinol A.

Thiomarinols D, E, F, and G (**163-166**) exhibited [126,127] antimicrobial activities against Gram-positive and Gram-negative bacteria. They were especially active against Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus*. Among them, thiomarinol E (**164**) was the most potent, being as active as thiomarinol A (**160**), while thiomarinol G (**166**) showed the lowest activity. Thiomarinols strongly inhibited isoleucyl-transfer RNA synthetase.

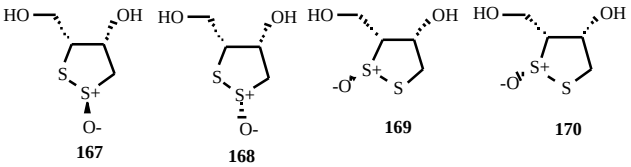
Table 5. Antimicrobial Activities of Thiomarinol B (161) - G (166) (MIC nmol/ml)

Test organism	161	162	163	164	165	166
Staphylococcus aureus	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015
Enterococcus faecalis	0.07	1.3	0.3	0.1	0.3	4.7
Escherichia coli	1.2	5.0	2.3	1.2	4.5	75.1
Salmonella enteritidis	0.6	2.4	1.2	1.2	4.5	37.5
Klebsiella pneumoniae	1.2	2.4	1.2	1.2	4.5	18.8
Enterobacter cloacae	1.2	5.0	2.3	2.2	18.3	>300
Serratia marcescens	4.6	10.0	9.5	9.3	36.7	>300
Proteus vulgaris	0.07	0.3	0.2	0.3	2.2	4.7
Morganella morganii	9.2	20.0	19.1	18.7	146.6	>300
Pseudomonas aeruginosa	0.3	1.3	0.6	0.6	9.1	18.8



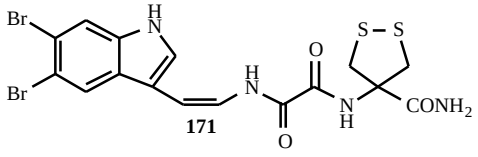
No.	n	R_1	R_2	R_3	R_4	R_5	X
160	7	OH	OH	H	OH	CH_3	S
161	7	OH	OH	H	OH	CH_3	SO_2
162	7	H	OH	H	OH	CH_3	S
163	7	OH	OH	H	OH	C_2H_5	S
164	9	OH	OH	H	OH	CH_3	S
165	7	OH	OH	H	=O	CH_3	S
166	7	H	H	OH	OH	CH_3	S

Four plant growth inhibitors were isolated from the tropical weed *Sphenoclea zeylanica*, showing allelopathic properties [128]. Those compounds hitherto not reported from any plant source were the isomers of cyclic thiosulfinate, and were named zeylanoxide A (**167**), epi-zeylanoxide A (**168**), zeylanoxide B (**169**), and epi-zeylanoxide B (**170**), respectively. The cyclic thiosulfinates completely inhibit the root growth of rice seedlings at 3.0 mM. While the specific activity of the inhibitors was not high, since they accumulated to roughly 0.61% *S. zeylanica* by dry weight, this suggests that the inhibitors are nevertheless potent allelochemicals in this weed.



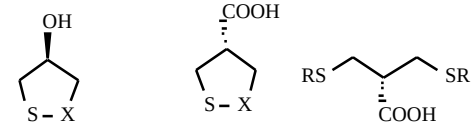
167 **168** **169** **170**

Kottamide E (**171**), a novel alkaloid, has been isolated from the New Zealand ascidian *Pycnoclavella kottae* [129].



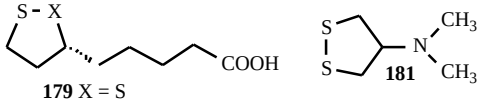
171

The methanolic bark extract of the tree *Brugiera conjugata* yielded diastereoisomeric 1,2-dithiolan-1-oxides - brugierol (**172**) and isobrugierol (**173**), affecting plant growth [130,131]. 1,2-Dithiolan-4-carboxylic acid (**174**) isolated from young sprouts of *Asparagus officinalis* with a mechanism of action similar to that of abscisic acid has been described as inhibitor of plant development [132]. Sulfoxides of acids (**175,176**), obtained from methanolic extract of asparagus [133] exhibit a potent plant growth inhibition. Asparagusic acid (**177**) takes part in an important biochemical process - the transport of acetyl groups, probably as compound (**178**).



172 X=S⁺···O⁻
173 X=S⁺–O⁻
174 X=S
175 X=S⁺···O⁻
176 X=S⁺–O⁻
177 R=H
178 R=Ac

(*R*)-Lipoic acid (**179**) and sulfoxide-lipoic acid (**180**) are present both in animals and in plant organisms. They are bound to amidic nitrogen in the pyruvate dehydrogenase- and 2-oxoglutarate dehydrogenase complex. Lipoic acid plays a role in the transfer of the acetyl groups, and is an important growth factor in both bacteria and protozoans [134-136].



179 X = S
180 X = SO
181

4,4-Dimethylamino-1,2-dithiolan-nereistoxin (**181**), was isolated from the ringlet sea worm *Lumbriconereis heteropoda*. This simple molecule possesses a unique insecticidal activity [137,138] and it served as a model for the synthesis of a highly effective insecticide [139].

1,2-Dithiolic derivatives include the antibiotic leinamycin (**182**), which is produced by *Streptomyces* sp. and possesses an essential antineoplastic activity [140]. Leinamycin (**182**) [141, 142], thought to derive its antitumor activity from reactions with DNA, is of interest because of its potent biological activity and because it represents a new chemical class of DNA-damaging agents. It is a thiol-dependent DNA-cleaving agent suggesting that the 1,2-dithiolan-3-one 1-oxide heterocycle of the natural product plays an integral role in DNA cleavage. Both leinamycin and enediynes are nucleophilically attacked by SH-containing compounds (mostly glutathione in living cells). The subsequent mechanism, however, is completely different. Enediynes yield a stable benzene ring; its formation is the driving force producing a biradical which is then responsible for DNA cleavage. Leinamycin was found to have at least two unprecedented chemical mechanisms involving (A) thiol-triggered release of hydrodisulfide and polysulfide species that cause oxidative DNA damage and (B) thiol-triggered generation of a DNA-alkylating episulfonium ion. The different DNA cleavage mechanisms are clearly seen on comparing Fig. **2** and Fig. **3**.

The bark of the Brazilian tree *Cassipourea guianensis* contains a group of alkaloids of 1,2-dithiolic type. The main component of the extract - gerrardine (**183**) - is a strong fungicidal agent. Guinesines A, B, C and G (**184-187**) also possess a high insecticidal activity [143-145]. The bark of *C. guianensis* yielded, in addition to gerrardine, two new minor alkaloids **(187** and **188)**. Antimicrobial testing indicated some effects (MIC, 150 nmol/ml) against *Candida albicans* and a weak activity (MIC, 300 nmol/ml) against other microorganisms *(Escherichia coli* and *Klebsiella pneumoniae*). New sulfur-containing amides, cassipoureamide-A (**189**) and -B (**190**), have been isolated from the stem wood of *C. guianensis* [146]. Cassipourine (**191**) was isolated from *C. gummiflua* [147].

EPIPOLYTHIODIOXOPIPERAZINES

An internal disulfide (oligosulfide) bridge and a highly substituted piperazine ring with amino acids (Phe, Ser, Tyr, Ala, Trp, Val, etc.) as precursors characterize the group of epipolythiodioxopiperazines (ETPs). This arrangement is responsible for the particular pharmacological activities of ETPs. The epipolythiodioxopiperazines have immunosuppressive properties and have been implicated in human and animal mycotoxicoses. The bridged disulfide moiety is thought to be generally essential for biological activity. All ETPs can thus also be considered as potential anti-cancer agents, *e.g.* gliotoxin exhibits a pronounced *in vivo* activity

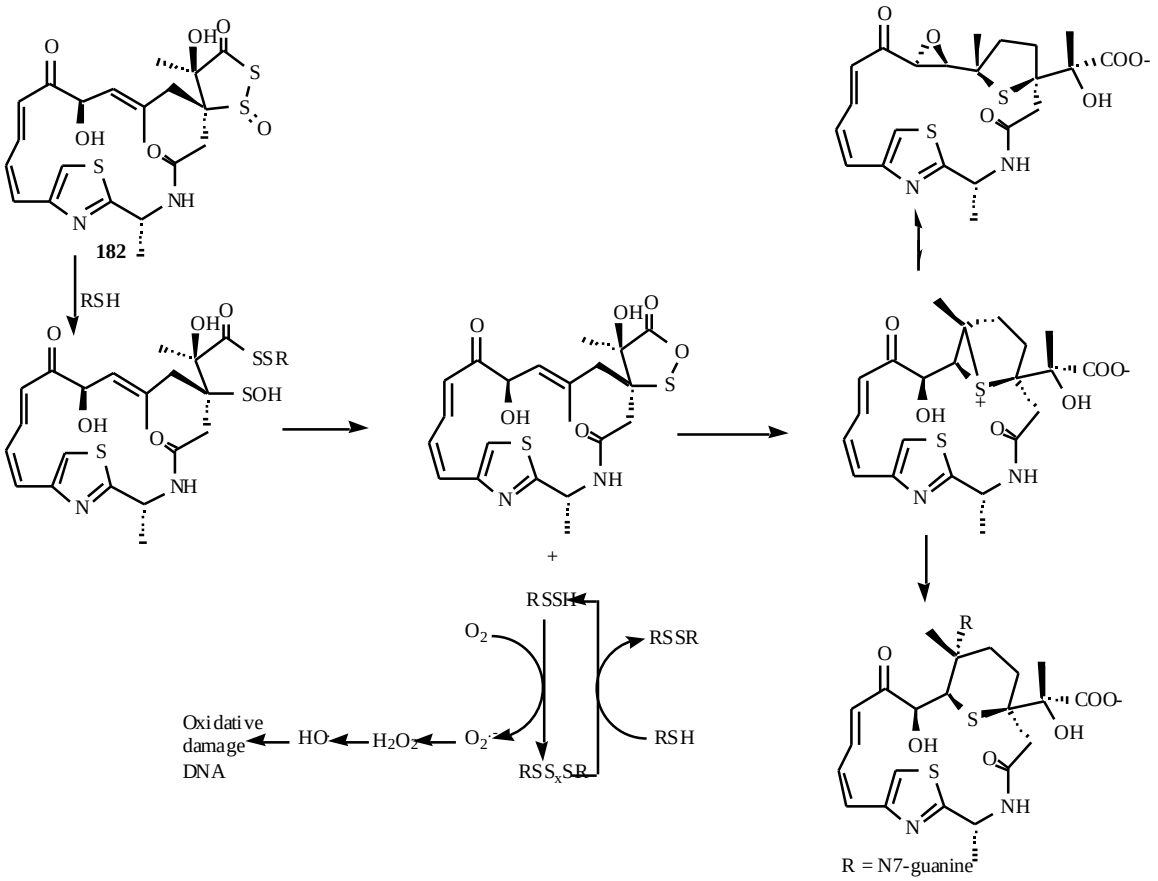
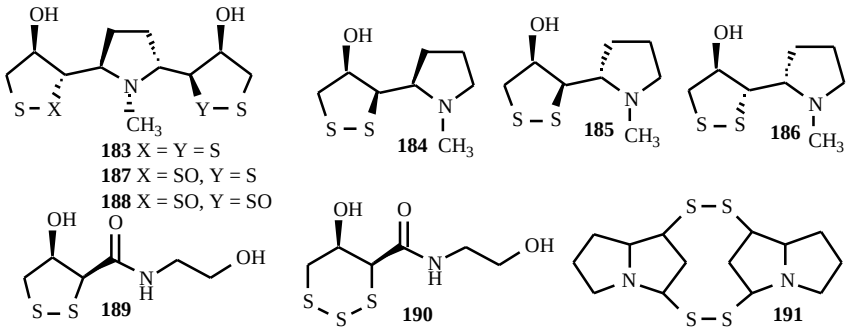
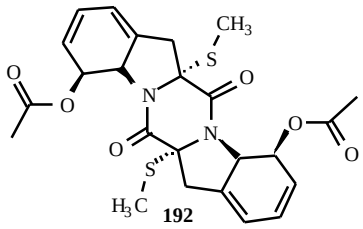


Fig. (2). Triggered damage of DNA by leinamycin.



against rat mammary carcinomas. The nature of the side groups of ETPs does not appear to affect the toxicity [148].



Until now, several tens of ETPs have been described. The structures are highly varied due to the biosynthesis of these compounds from different amino acids and to their further modifications including methylations, esterifications, chlorinations, oxidations, rearrangements, dimerizations or skeleton modifications (*e.g.* the presence of more sulfur atoms in the bridge). The introduction of the sulfur atoms into the core ETP moiety has not yet been fully clarified. Labeling experiments have shown that methionine, cysteine and sodium sulfate can all act as sources of sulfur, although cysteine is thought to be the direct donor. ETPs have so far been detected in fungi and lichens. It has not yet been clarified why certain species, *e.g. Aspergillus fumigatus*, produce gliotoxin, whereas other *Aspergillus* spp. do not. On the other hand, gliotoxin is produced by many taxonomically distant genera such as *Aspergillus fumigatus, Penicillium* spp., *Candida* spp., and *Trichoderma* spp.

Two mechanisms of action have been considered, *viz.* either conjugation with proteins carrying susceptible thiol residues and subsequent inactivation, and/or generation of reactive oxygen species *via* redox cycling [148]. Unfortunately, opposite effects have been described in other studies [149].

A number of ETPs have been specifically associated with mammalian or plant disease. For instance, gliotoxin (see below) is immunosuppressive, with selective effects on mature lymphocytes and macrophages; it contributes to invasive aspergillosis of mammals, is virulent against larvae of the insect *Galleria mellonella* and disrupts cellular responses to fungal infections involving the NADPH oxidase enzyme complex in human polymorphonuclear leukocytes.

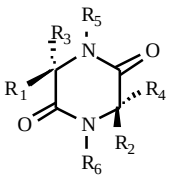
A new antifungal diketopiperazine named haematocin (**192**) was isolated from the culture broth of *Nectria haematococca* [150,151] causing blight disease in ornamental plants, *Phalaenopsis* spp., and *Doritanopsis* spp. Haematocin inhibited the germ-tube elongation and spore germination of *Pyricularia oryzae,* with the ED_{50} values of 60 nmol/ml and 320 nmol/ml, respectively.

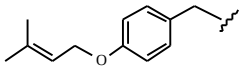
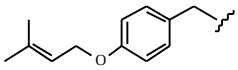
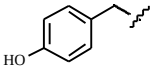
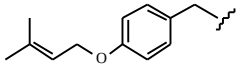
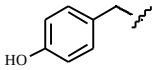
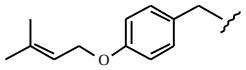
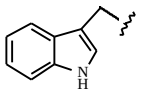
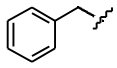
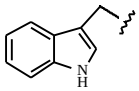
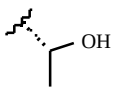
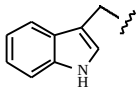
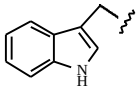
Two new compounds, Sch 54794 (**193**) and Sch 54796 (**194**), were isolated from the fermentation of a fungal culture, *Tolypocladium* sp. [152]. The *trans* isomer, which is similar to other diketopiperazines reported as PAF (platelet-activating factor) inhibitors, displayed a weak inhibitory activity in PAF assay with an IC_{50} value at 50 μM. However, the *cis* isomer was found to be inactive (IC_{50} >100 μM).

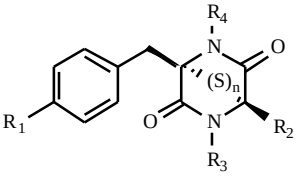
A strain of *Fusarium chlamydosporum* was described to produce two new sulfur-containing dioxopiperazine derivatives, fusaperazines A (**195)** and B (**196**), in addition to four related compounds (**193, 194, 197, 198**) [153]. The compounds were identified as Sch 54794 (**193**), Sch 54796 (**194**), 3-[(4-hydroxypheny1)-methyl]-1,4-dimethyl-3,6-bis(methylthio)-2,5-piperazinedione (**197**), and *cis*-bis(methylthio) silvatin (**198**). Among the compounds, **193**, **195**, and **198** exhibited weak cytotoxic activities (ED_{50} 56.6, 72.8, and 18.9 nmol/ml, respectively) against P388 lymphocytic leukemia cells, whereas others were inactive (ED_{50} > 270 nmol/ml). Biogenetically, it is very interesting that the same fungal strain produced two kinds of derivatives with an opposite chirality (**195** and **197**, or **193** and **198**). Polanrazine B (**199**), a new dioxopiperazine, was produced by isolates of the blackleg fungus (*Phoma lingam*, perfect stage *Leptosphaeria maculans*) [154,155]. The phytotoxicity assays of polanrazines B on plants resistant and susceptible to *P. lingam* indicated that it has moderate but selective toxicity, causing necrotic and chlorotic lesions (1-3 mm diameter) on brown mustard leaves ($5x10^{-4}$ M), whereas no damage was observed on canola or white mustard leaves. Further, dithiosilvatin (**200**) was isolated from *Aspergillus silvaticus* [156].

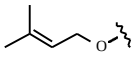
Two new diketopiperazines, pyrenocine A (**201**) and pyrenocine B (**202**), were isolated from a culture broth of the entomopathogenic fungus *Verticillium hemipterigenum* [157]. The compounds were tested for *in vitro* antimalarial activity and cytotoxicity against human epidermoid carcinoma (KB) and human breast cancer (BC-1) cell lines and African green monkey kidney fibroblasts (Vero cells). The compound **202** shows antimalarial activity (IC_{50} 6.7 nmol/ml).

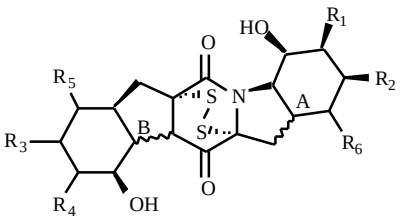
The epicorazines A (**203**), B (**204**), C (**205**), and 3822-A (**206**) were isolated from fermentations of *Stereum hirsutum* [158] and from the saprophyte *Epicoccum nigrum* (epicorazine A and B), *E. purpurascens* (epicorazine B), and



No.	R_1	R_2	R_3	R_4	R_5	R_6
193		H	SCH_3	SCH_3	H	H
194		SCH_3	SCH_3	H	H	H
195		H	SCH_3	SCH_3	H	H
196		SCH_3	OCH_3	H	H	H
197	SCH_3	SCH_3		H	CH_3	CH_3
198	SCH_3	SCH_3		H	CH_3	CH_3
199	SCH_3	SCH_3		$CH(CH_3)_2$	H	H
201		CH_2OH	SCH_3	SCH_3	CH_3	H
224	H	SCH_3			H	H
239		SCH_3	SCH_3	CH_2OH	H	CH_3
240		SCH_3	SCH_3	CH_2OH	H	H



No.	R_1	R_2	R_3	R_4	n
200		H	CH_3	CH_3	2
202	H	CH_2OH	H	CH_3	4



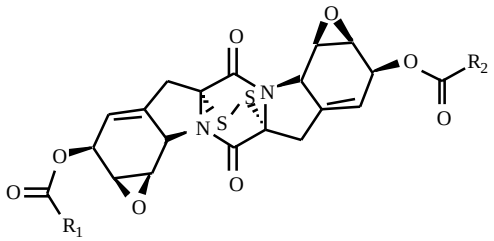
No.	R_1,R_2	A	R_3,R_4	B	R_5	R_6
203	∏	*S*	∏	*S*	=O	=O
204	∏	*R*	∏	*S*	=O	=O
205	OH, H	*R*	∏	*S*	=O	=O
206	H, H	*?*	H, H	*?*	=O	=O
207	H, H	*S*	H, H	*S*	(*S*)-OH	(*S*)-OH
208	H, H	*S*	H, H	*R*	=O	=O
209	OMe, H	*S*	H, OMe	*R*	(*S*)-OH	=O
210	SH, H	*S*	H, SH	*R*	=O	=O

Stereum hirsutum (epicorazine C) [159,160]. Epicorazine C (**205**) inhibited the growth of Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MIC 57 nmol/ml) and vancomycin-resistant *Enterococcus faecalis* (MIC 29 nmol/ml) as well as *Candida albicans* but not Gram-negative bacteria such as *Escherichia* or *Pseudomonas.* Epicorazines A-C also exhibited antiproliferative effects against several mouse fibroblast and cancer cell lines. They were highly cytotoxic against HeLa cells, where epicoracine B was most potent amongst the congeners.

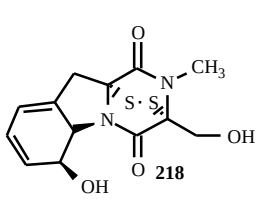
Four new cytotoxic disulfides, rostratins A-D (**207**-**210**), were isolated from the whole broth of the fungus *Exserohilum rostratum* [161], a fungal strain found associated with a marine cyanobacterial mat. Rostratins A-D showed diverse *in vitro* cytotoxicities against HCT-116 human colon carcinoma with IC_{50} values of 19.8, 4.5, 1.56, and 33.7 nmol/ml, respectively.

Two potently cytotoxic ETPs, ambewelamides A (**211**) and B (**212**), have been isolated from the lichen *Usnea* sp. collected in Sri Lanka [162]. Ambewelamide A exhibited potent *in vitro* cytotoxicity (murine leukemia P388, IC_{50} 16.2 pmol/ml) and it showed significant *in vivo* antineoplastic activity. Several scabrosin esters (**213**-**217**) from the lichen *Xanthoparmelia scabrosa* were found to exhibit potent cytotoxic activity against murine P815 mastocytomia cells and human breast MCF7 carcinoma cells, with IC_{50} values of 0.5 μM and 1 nM, respectively [163,164].

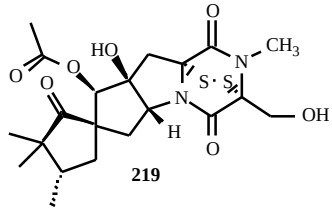
Gliotoxin (**218**) is one of the better-known members of the epipolythiodioxopiperazine class of fungal metabolites. Gliotoxin, displaying typical bridged disulphide, was described in 1943 [165] and its structure elucidated in 1966 [166]. A number of *Penicillium*, *Trichoderma virens*, and *Aspergillus* species [167-169], as well as *Gliocladium* [170], *Thermoascus* [171], and *Candida* [172] produce gliotoxin. Gliotoxin has antibacterial and antiviral properties [173] and shows selective toxicity to cells of the hematopoietic system [174,175].



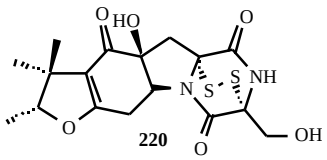
No.	R_1	R_2
211	CH_3	$CH_2CH_2CH_3$
212	CH_3	$CH_2(CH_2)_2CH_3$
213	CH_3	CH_3
214	CH_3	$CH_2CH_2CH_3$
215	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$
216	CH_3	$CH_2(CH_2)_2CH_3$
217	$CH_2CH_2CH_3$	$CH_2(CH_2)_2CH_3$



218



219



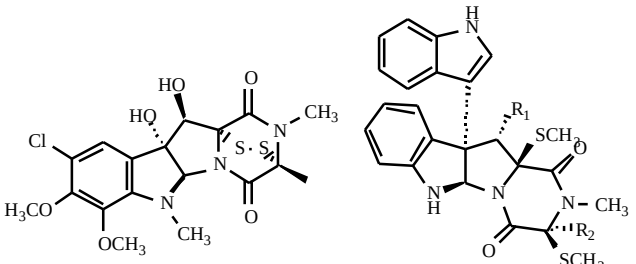
220

Sirodesmin (**219**) has phytotoxic activities suggesting its role in the blackleg disease of *Brassica napus* (canola, oilseed rape). It was isolated from *Leptosphaeria maculans* and *Sirodesmium diversum* [176,177].

Phomalirazine (**220**), which was produced by *Phoma lingam* (perfect stage *Leptosphaeria maculans)*, is active at 10^{-5} M in a cotyledon assay [178].

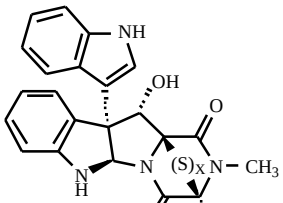
Ingestion of spores of the saprophytic fungus *Pithomyces chartarum* by grazing livestock causes the disease commonly known as facial eczema in sheep, cattle, deer, and goats. This mycotoxicosis is responsible for significant reductions in growth, reproductive performance, and wool and milk production. The spores contain the potent mycotoxin sporidesmin A **(221)** which causes extensive liver damage, particularly to the biliary system, and the resulting impaired liver function often causes a secondary photosensitization of exposed areas of skin around the eyes, ears, and muzzle [178].

New dioxopiperazine metabolites named gliocladins A-B (**222, 223**) and gliopiperazine (**224**) have been isolated from a strain of *Gliocladium* sp. [179].



221

222 R_1 = OH, R_2 = H
223 R_1 = R_2 = H
226 R_1 = OH, R_2 = CH_2OH



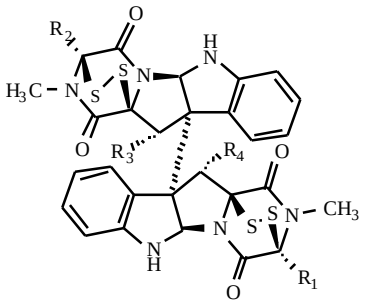
225 x = 3
227 x = 2

Three novel thiodiketopiperazine derivatives, T988 A, B, and C (**225-227**), were isolated from a *Tilachlidium* sp. All the compounds showed potent cytotoxicity against cultured P388 leukemia cells with ID_{50} values of 0.25, 2.18, and 0.56 μM, respectively [180]. The minor components, Sch 52900 (**228**) and Sch 52901 (**229**), were isolated from a fermentation broth of the fungus *Gliocladium* sp. [181], and were found to be novel compounds closely related to the major component, verticillin A (**230**), previously identified as an antitumor compound. All three compounds prevented serum-stimulated transcription of the human *c-fos* promoter, using a *fos/lac Z* reporter gene assay, with IC_{50}, values of 1.5, 18 and 0.5 μM, respectively. Northern analysis revealed that exposure of cells to compound **227** causes inhibition of both phorbol ester-induced *c-fos* induction and serum-induced JE induction in the absence of inhibiting RNA synthesis, as measured by [^{3}H] uridine incorporation. These results suggest that this class of compounds exerts antitumor activity by blocking a signal transduction pathway that is common to and necessary for the induction of at least a subset of immediate early genes involved in cell proliferation.

Three new antibiotics, verticillins A (**230**), B (**231**), and C have been isolated from *Verticillium* sp. Verticillin C is thought to be an epitrithio-analogue of verticillin B [182]. Antimicrobial activity, as usually, has been found against Gram-positive bacteria and mycobacteria but not against Gram-negative bacteria and fungi. The cytotoxicity effects (ED_{50}) of verticillins A and B against HeLa cells were both 0.3 nmol/ml.

Three new sulfur compounds, verticillins D-F (**232-234**), have been isolated from solid-substrate fermentation cultures of the sclerotial mycoparasite *Gliocladium catenulatum* [181,183]. All three verticillins did show antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* at 130 nmol/disk in standard Petri-plate assays.

Two new cytotoxic dioxopiperazine dimers, 11,11'-dideoxyverticillin A (**235**) and 11'-deoxyverticillin A (**236**), and the previously described verticillin A (**230**), have been isolated from the mycelium of a marine-derived fungus of the genus *Penicillium* [184]. Compounds **235** and **236** exhibit a potent *in vitro* cytotoxicity against HCT-116 human colon carcinoma (IC_{50} = 45 pmol/ml).



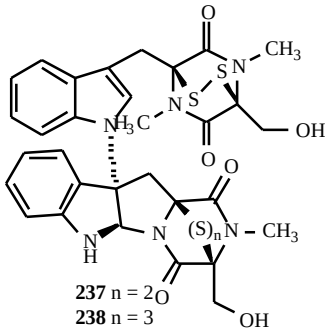
No.	R_1	R_2	R_3	R_4
228	CH_3	$CH(OH)CH_3$	OH	OH
229	CH_3	CH_2CH_3	OH	OH
230	CH_3	CH_3	OH	OH
231	CH_2OH	CH_3	OH	OH
232	$CH(OH)CH_3$	$CH(OH)CH_3$	OH	OH
233	$COCH_3$	$COCH_3$	OH	OH
234	$COCH_3$	$CH(OH)CH_3$	OH	OH
235	CH_3	CH_3	H	H
236	CH_3	CH_3	H	OH

The ascomycetes *Chaetomium seminudum* or *C. globosum* [185] have afforded a known epipolythiodioxopiperazine, chetomin (chaetomin) (**237**), together with three new chetomin-related metabolites named chetoseminudins A (**238**), B (**239**), and C (**240**) [186]. All four compounds exerted immunosuppressive activities against Con A-

induced (T-cells) and LPS-induced (B-cells) proliferations of mouse splenic lymphocytes (see Table **6**). Compounds **237** and **238**, one with epidithio- and the other with epitrithiomoiety, displayed comparatively high immunosuppressive activity while **239** and **240**, which possessed dethiodimethylthiodioxopiperazine moieties, displayed very low activity. This seems to be similar to the result already obtained with other fungal ETPs, *e.g.* sporidesmin and gliotoxin [187].

Table 6. Immunosuppressive Effects of Chetomin (237), Chetoseminudins A (238), B (239), and C (240), on Con A-induced and LPS-induced Proliferations of Mouse Splenic Lymphocytes

No.	IC_{50} (nmol/ml)	
	Con A-induced	LPS-induced
237	0.24	0.13
238	0.24	0.17
239	63	Not tested
240	>137	Not tested



237 n = 2
238 n = 3

One of the largest classes of cytotoxic fungal metabolites containing 24 members are leptosins. The producing fungus, *Leptosphaeria* sp. was isolated from the marine alga *Sargassum tortile*. The leptosins can be divided into six subclasses or groups based on structural similarity and biological activity with leptosins A (**241**), B (**242**), C (**243**), [188] G (**244**), G1 (**245**), G2 (**246**), and H (**247**) [189] differing only in the number of sulfur atoms contained within the polythio-bridge. Leptosins K (**248**), K1 (**249**), and K2 (**250**) [190] form another group that differs in the stereochemistry of one polythio-bridge and the presence of two valines. The third group is comprised of leptosins I (**251**) and J (**252**) [191], which contain an additional ether linkage between the two diketopiperazine monomers. The fourth group, leptosins M (**253**), M1 (**254**), N (**255**), and N1 (**256**) [192] lack one polythio-bridge. The fifth group includes leptosins D (**257**), E (**258**), and F (**259**) [188], which are diketopiperazine monomers with an indole appendage. The sixth group, leptosins O-S (**260-264**), lacks both oligothio-bridges; moreover, leptosin S does not contain sulfur [193].

The potency of the leptosins against a P388 leukemia cell line ranged in the order of nanograms to micrograms per ml (Table **7**). The activities show that the most detrimental effect on the activity arises from the additional ether linkage contained in I (**251**) and J (**252**). Leptosins M (**253**) and M1 (**254**) are the second least potent analogs, which must be due to the lack of a second polythio bridge in conjunction with an additional methoxyl. Comparison of M and M1 to N and N1 demonstrates that the stereochemistry of C11 and C11´ is also important for the overall activity. Leptosins A and C showed a significant antitumor activity in mice bearing Sarcoma-180 ascites. Mean survival time of the treated animal (*T*) was compared to that of the control animal (*C*) expressed as percentage (*T/C* %) and yielded *T/C* values of 260 and 293 for **241** and **243**, respectively. Studies [194] on leptosin M showed that it inhibited (30-70% inhibition) two kinases, PTK and CaMKIII at 13 nmol/ml but did not inhibit PKA, PKC or EGFR at 130 nmol/ml [192]. Additionally, leptosin M inhibited topoisomerase II (IC_{50} = 59.1 μM) but did not inhibit topoisomerase I.

Secoemestrin C (**265**) was isolated from the culture medium of the early state of *Emericella foveolata* [195]. Secoemestrin C may be the key intermediate in the biosynthesis of some ETPs. A new epidithiodioxopiperazine derivative, emethallicin A (**266**), was isolated from the mycelial extract of the heterothallic fungus, *Emericella heterothallica* (*Aspergillus heterothallicus*). Emethallicin A showed a potent inhibitory effect on histamine release from mast cells [196]. Emethallicins B (**267**) and C (**268**), novel ETPs, possessing strong inhibitory activity on the histamine release from mast cells, were isolated from the mycelial extract of *E. heterothallica* [197].

The organic extract of the fermentation broth of an unidentified fungus, *Arachniotus aureus*, and/or *Aspergillus terreus* was found [198,199] to contain three epidermal growth factor receptor antagonists, *i.e.* SCH 64874 (**269**), SCH 64875 (**270**), SCH 64877 (**271**). All three compounds showed inhibition in the epidermal growth factor receptor assay with IC_{50} of 1.4, 1.4, and 1.6 nmol/ml, respectively. Aranotin (**272**) was isolated from the same source [199].

Six new compounds, MPC1001 (**273**) and MPC1001B-F (**274-278**), were isolated from the fungus *Cladorrhinum* sp. [200]. MPC1001 and the analogs exerted potent antiproliferative activities against a human tumor cell line and DU145 human prostate cancer cell line. The decrease of the antiproliferative activities in **277**, and further analogs indicated that the macrocyclic ring and polysulfide structures were critical for the antitumor activity. Novel antifungal macrocyclic EPTs, emestrin A (**279**) [201,202] and emestrin B (**280**) [203], have been isolated from mycelial extracts of *Ernericella striata*, *E. quadrilineata*, *E. nidulans* var. *acristata*, and *E. parvathecia*.

Vertihemiptellides A (**281**) and B (**282**), unique diketopiperazine dimers, were isolated from the insect pathogenic fungus *Verticillium hemipterigenum* [204]. Both compounds exhibited growth inhibitory activity against *Mycobacterium tuberculosis* but also showed moderate cytotoxic activities, see Table **8**. They were inactive against the malarial parasite *Plasmodium falciparum* (at a concentration of 33 nmol/ml) and the fungus *Candida albicans* (at 81 nmol/ml).

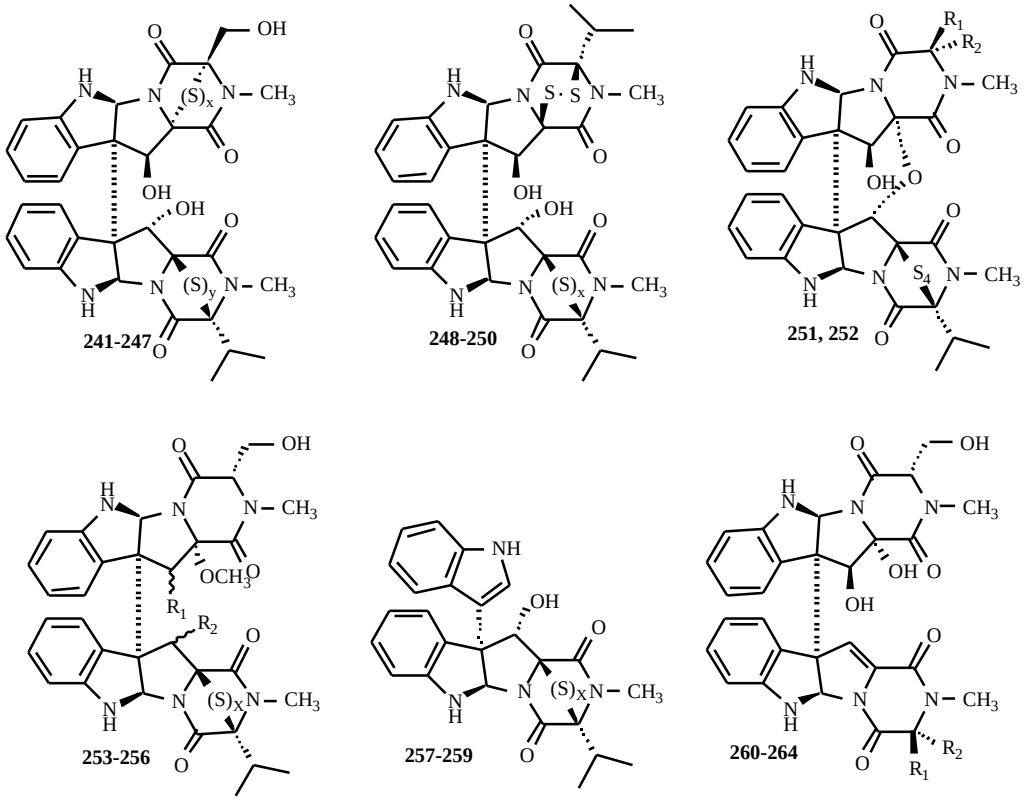
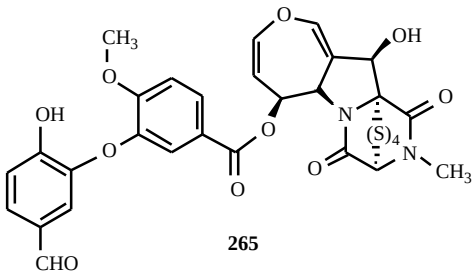


Table 7. Cytotoxicity of Leptosins Against a P388 Leukemia Cell Line

Leptosin	x	y	R_1	R_2	ED_{50} nmol/ml
A (**241**)	4	2	-	-	2.30×10^{-3}
B (**242**)	3	2	-	-	3.11×10^{-3}
C (**243**)	2	2	-	-	2.36×10^{-3}
D (**257**)	2	-	-	-	10.3×10^{-2}
E (**258**)	3	-	-	-	5.7×10^{-2}
F (**259**)	4	-	-	-	7.2×10^{-2}
G (**244**)	4	3	-	-	5.7×10^{-3}
G1 (**245**)	3	3	-	-	5.7×10^{-3}
G2 (**246**)	2	3	-	-	5.6×10^{-3}
H (**247**)	2	4	-	-	3.7×10^{-3}
I (**251**)	-	-	CH_2OH	H	1.52
J (**252**)	-	-	H	CH_2OH	1.69
K (**248**)	2	-	-	-	4.9×10^{-3}
K1 (**249**)	3	-	-	-	3.1×10^{-3}
K2 (**250**)	4	-	-	-	2.7×10^{-3}
M (**253**)	4	-	β-OH	α-OH	1.42
M1 (**254**)	2	-	β-OH	α-OH	2.84

(Table 7) contd…

Leptosin	x	y	R_1	R_2	ED_{50} nmol/ml
N (**255**)	4	-	α-OH	β-OH	0.34
N1 (**256**)	3	-	α-OH	β-OH	0.34
O (**260**)	S_2CH_3	$CH(CH_3)_2$	-	-	1.6
P (**261**)	$CH(CH_3)_2$	S_2CH_3	-	-	0.14
Q (**262**)	SCH_3	$CH(CH_3)_2$	-	-	22.4
R (**263**)	$CH(CH_3)_2$	SCH_3	-	-	23.0
S (**264**)	NH_2	$CH(CH_3)_2$	-	-	16.0



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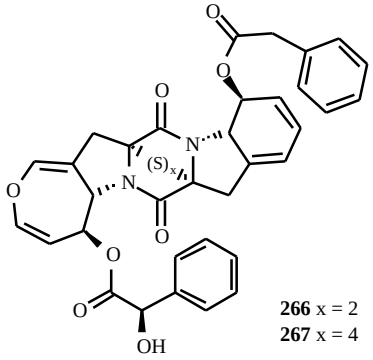
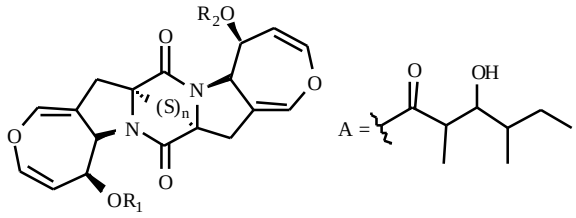
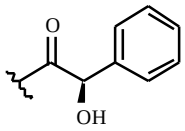
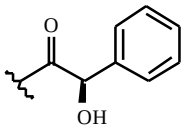


266 x = 2
267 x = 4



No.	R_1	R_2	n
268			2
269	A	A	2
270	A	A	3
271	A	A	4
272	H		2

SIX AND MORE MEMBERED OLIGOSULFIDES

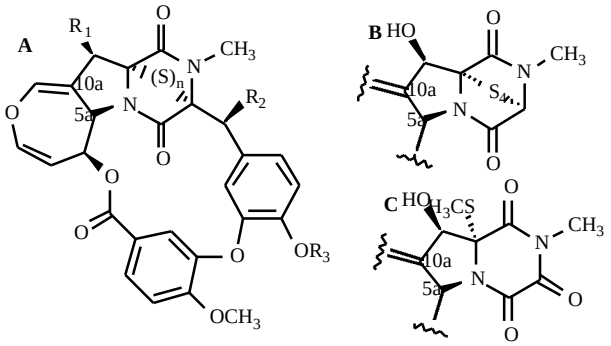
Saturated six and more membered di- and trisulfides are fairly rare. The cruciferous phytoalexins, sinalbin A (**283**) and B (**284**), are produced by white mustard (*Sinapis alba*) [205] after treatment with biotic and abiotic elicitors. Sinalbin B appears to be both a phytoalexin and a biosynthetic precursor of sinalbin A. Finally, antifungal bioassays established that metabolites **283** and **284** were active against *P. lingam*. While **284** ($5x10^{-4}$ M) caused complete inhibition of spore germination (ED_{50} $2x10^{-4}$ M at 48 h) for the duration of the assay (7 days), **283** showed moderate activity (ED_{50} $7x10^{-4}$ M at 48 h) at a similar concentration ($5x10^{-4}$ M), causing about 30% germination inhibition relative to controls, after 48 h.

Two novel sulfur derivatives (**285, 286**), one of which is a glycoside, containing substituted 1,2,3-trithiocane cycles, were isolated from *Perophora viridis* which was collected off the Atlantic coast of North Carolina [206]. The compounds gave positive results in the brine shrimp toxicity assay and the sea urchin test. Both compounds show a modest activity against different microorganisms (see Table **9**). They are active against *Staphylococcus aureus* and *Bacillus subtilis* but inactive against the Gram-negative bacterium *Escherichia coli*.

The seaweed *Dictyopteris plagiogramma* contains a seven-membered cyclic disulfide **287** [207]. 5-Methylthio-1,2,3-trithian (**288**) was isolated from green seaweed *Chara globularis* [208]. Cyclic polysulfides such as 1,2,3,5,6-pentathiecan (**289**), 1,2,4,5-tetrathian (**290**), and 1,2,3,4, 5,6,7-heptathiocan (**291**) were isolated from cultural liquids produced by cells of the genus *Thermococcus* [209]. The study of biological activity of these compounds showed that pentathiepan **289** exhibits the highest fungicidal activity (MIC 41.5 nmol/ml to *Microsporum canis*). It was established that alkyl substituted polysulfides of type **291** are unstable and decay, yielding cyclic polysulfides containing one and two sulfur atoms.

The antibiotic activity of the red alga *Chondria californica* is due to a mixture of cyclic polysulfides and their oxidation products [212]. Different thiolanes and thiepanes, and a 12-membered heterocycle (**292**) containing eight sulfur atoms, were identified in this alga.

The first naturally occurring pentathiepin, varacin A (**293**), was isolated from the marine ascidian *Lissoclinum*



No.	type	n	R_1	R_2	R_3	DU145 human prostate cancer cell line (IC_{50}, nM)
273	A	2	OH	OH	CH_3	9.3
274	A	2	H	OH	CH_3	39
275	A	2	OH	H	CH_3	12
276	A	3	OH	OH	CH_3	16
277	B	-	-	-	CH_3	85
278	C	-	-	-	CH_3	-
279	A	2	OH	OH	H	-
280	A	3	OH	OH	H	-

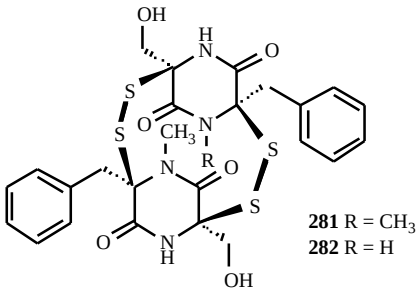
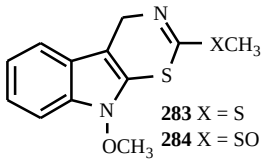


Table 8. Antimycobacterial and Cytotoxic Activities of Compounds 281 and 282

No.				cytotoxicity (IC_{50}, nmol/ml)	
	anti-TB (MIC, nmol/ml)	KB[a]	BC[b]	NCI-H187[c]	Vero[d]
281	20.1	>32	13.4	7.1	7.9
282	20.6	>33	27.7	5.8	16.0

[a] Human epidermoid carcinoma in the mouth.
[b] Human breast cancer cells.
[c] Human small cell lung cancer.
[d] African green monkey kidney fibroblast.

vareau in 1991. A few other closely related pentathiepins were isolated in the following few years, *e.g.* lissoclinotoxin A (**294**) from *Lissoclinum* sp., lissoclinotoxin B (**295**) and isolissoclinotoxin A (**296**) from *L. perforatum*, *N,N*-dimethyl-5-(methylthio)varacin (**297**) from *L. japonicum*, 5-(methylthio)varacin (**298**) as an inseparable 2:3 mixture with the corresponding trithioles from a different *Lissoclinum* species, and 3,4-desmethylvaracin (**299**) from a *Eudistoma* sp. [211-213].

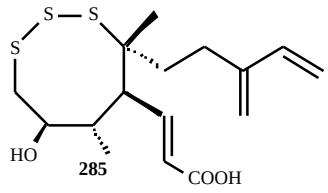


Varacin A (**293**) exhibits potent antifungal activity against *Candida albicans* (IC_{50} 0.15 nmol/ml) and cytotoxicity toward the human colon cancer HCT 116 with an IC_{50}

Table 9. Bioactivities of Sulfur Compounds 285 and 286

Test organism	285	286
Staphylococcus aureus[a]	19.5	24.1
Bacillus subtilis[a]	9.8	18.2
Escherichia coli[a]	0	0
Saccharomyces cerevisiae[a]	0	0
Artemia salina[b]	39.6	11.4
Paracentrotus lividus[b]	62.3	0.3

[a] inhibition zone in mm; [b] MIC (nmol/ml)



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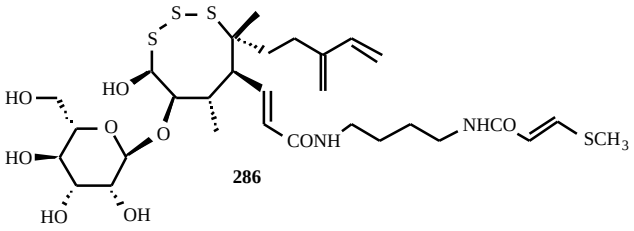
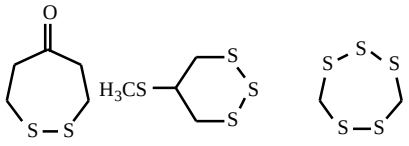
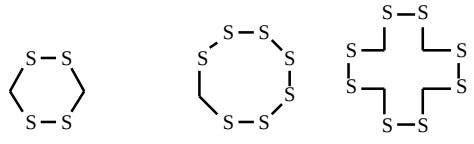


286

of 0.15 fmol/ml. It also exhibited a 1.5 fold differential toxicity toward the CHO cell line EM9 (chlorodeoxyuridine sensitive) versus BR1 (BCNU resistant), providing preliminary evidence that varacin damages DNA [214].

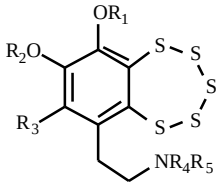


287 **288** **289**



290 **291** **292**

Lissoclinotoxin A (**294**) has a fungicidal and antimicrobial activity against various Gram-positive (MIC for *Staphylococcus aureus, Streptococcus faecalis* and *Citrobacter* sp. 0.25-1.8 nmol/ml) and Gram-negative bacteria (MIC for *E. coli, Salmonella sp. Proteus sp.* and *Pseudomonas aeruginosa* 0.25-1.8 nmol/ml), and against the yeasts *Candida albicans* (123 nmol/ml) and *Trichophyton mentagrophytes* (62 nmol/ml) [211]. Lissoclinotoxins A and B suppress ichtyopathogenic genera *Aeromonas salmonicida* and *Vibrio anguillarum*. Lissoclinotoxin A has a rather high activity against *Plasmodium falciparum* resistant to antimalarial agents such as mefloquine or chloroquine. The ability of lissoclinotoxin A to inhibit the growth of cells of leukaemia L 1210 in a concentration of 3 nmol/ml is remarkable. Lissoclinotoxin A shows cytotoxic properties, suppressing cell fission in the sea urchin test [212].

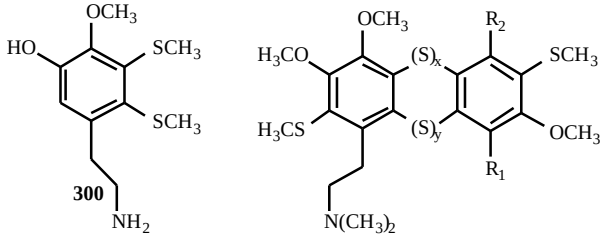


No.	R_1	R_2	R_3	R_4	R_5
293	CH_3	CH_3	H	H	H
294	CH_3	H	H	H	H
295	CH_3	H	$-(CH_2)_2-$		H
296	H	CH_3	H	H	H
297	CH_3	CH_3	SCH_3	CH_3	CH_3
298	CH_3	CH_3	SCH_3	H	H
299	H	H	H	H	H

New alkaloids, lissoclinotoxin C (**300**) and the dimeric lissoclinotoxin D (**301**), were isolated along with lissoclinotoxin A from *Lissoclinum* sp. collected from the Great Barrier Reef, Australia. Lissoclinotoxin D (**301**) exhibits antifungal activity against *Candida albicans* [215]. Bioassay-guided fractionation of the MeOH extract from a Philippine didemnid ascidian *Lissoclinum* resulted in the isolation of two new dimeric alkaloids, lissoclinotoxins E (**302**) and F (**303**). Both lissoclinotoxins displayed IC_{50} values at 4.0 and 2.5 nmol/ml, respectively, on human breast carcinoma cell line MDA-MB-468 [216].

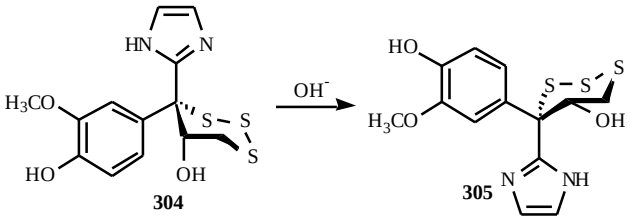
The ascidian *Aplidium pliciferum* contains trisulfide **304**, possessing antimicrobial, antineoplastic, and cytotoxic activity [217]. In neutral or slightly basic solutions, the sulfur cycle is transformed to yield the more stable diastereoisomer **305** with a changed chiral carbon atom configuration. Both

stereoisomers possess a high biological activity: cytotoxic activity of the isomers **304** and **305** against leukemia P388 cells can be characterized by IC_{50} of 38 and 35 nmol/ml, respectively.



300

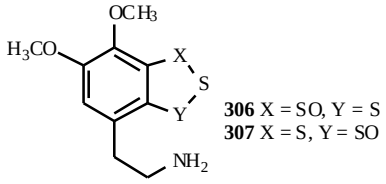
No.	x	y	R_1	R_2
301	2	2	OCH_3	$N(CH_3)_2$
302	1	1	OCH_3	$N(CH_3)_2$
303	2	1	$N(CH_3)_2$	OCH_3



304 **305**

Varacins A-C (**293, 306, 307**) have been isolated from the Far Eastern ascidian *Polycitor* sp.; all exhibit potent antifungal and antimicrobial activities *in vitro* against *Candida albicans* and *Bacillus subtilis* [218].

All natural benzopentathiepins isolated so far have an aminoethyl substituent. The positively charged amino substituent may confer DNA affinity on the pentathiepin antibiotics. 3,4-Desmethylvaracin (**299**) and *N,N*-dimethyl-5-(methylthio)varacin **297** selectively inhibit protein kinase C with IC_{50} of ca. 2.4 μmol/ml.



306 X = SO, Y = S
307 X = S, Y = SO

DISULFIDES

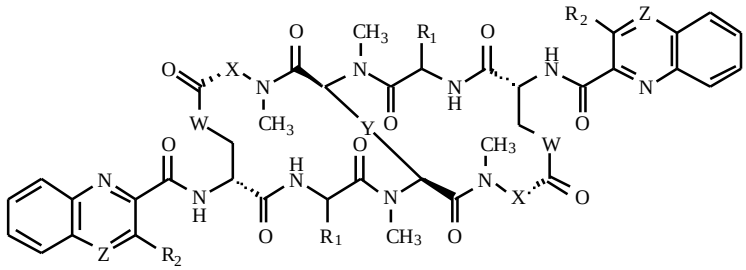
Thiocoraline (**308**) [219,220] is a potent antitumor antibiotic isolated from *Micromonospora* sp. found in the Mozambique Strait [221,222]. Thiocoraline does not inhibit DNA topoisomerase I or II but it does inhibit DNA polymerase R at concentrations that inhibit cell cycle progression and clonogenicity. It was found to unwind double-stranded DNA. Thiocoraline shows a potent antibiotic activity against Gram-positive bacteria (~0.04 nmol/ml for *Staphylococcus aureus*, *Bacillus subtilis*, and *Micrococcus luteus*), while its activity against Gram-negative bacteria is very low (>86 nmol/ml for *Escherichia coli*, *Klebsiella pneumonie*, and *Pseudomonas aeruginosa*). This antibiotic inhibits RNA synthesis more specifically than DNA synthesis (DNA synthesis IC_{50} 0.35 nmol/ml, RNA synthesis IC_{50} 0.007 nmol/ml). Thiocoraline has a strong cytotoxic effect against P-388, A-549, and MEL-28 lines (IC_{50} ~ 0.0017 nmol/ml), this activity being five fold higher for these cell lines than for HT-29 (IC_{50} 0.009 nmol/ml).

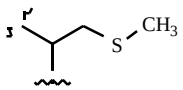
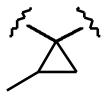
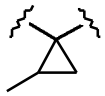
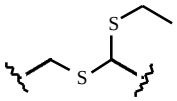
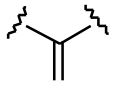
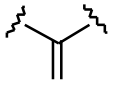
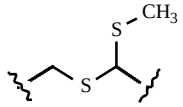
Very similar SW-163C (**309**) and E (**310**) were isolated from the culture broth of a *Streptomyces* strain [223,224]. Both compounds exhibited potent antitumor activities against various tumor cell lines *in vitro* and against murine leukemia P388 *in vivo*. They were predominantly active against Gram-positive bacteria such as *Staphylococcus aureus* including the drug resistant strains, *Bacillus subtilis, Micrococcus luteus*, and *Mycobacterium phlei*. The activity of SW-163E was higher than that of SW-163C. Both compounds were potent cytotoxic agents and strongly suppressed the growth of all tested tumor cell lines. SW-163C and E showed cytotoxicity against the cell lines with IC_{50} values of 17-140 nM and 0.2-1.6 nM, respectively. SW-163C showed a significant prolongation of survival at doses of 1 and 10 mg/kg (mice), giving TIC value of 139 and 156%, respectively. The acute toxicities (LD_{50}) of SW-163C and E were >100 mg/kg and 0.6 mg/kg, respectively, when administered intraperitoneally to mice.

A new topoisomerase II inhibitor, designated BE-22179 (**311**), was isolated from the mycelial cake of the culture broth of *Streptomyces* sp. [220,225], which resembles *Streptomyces gangtokensis*. BE-22179 inhibited topoisomerase II strongly but not topoisomerase I and showed potent antitumor activity against various tumor cell lines both *in vitro* and *in vivo*.

Echinomycin (**312**) [226,227] was originally isolated from *Streptomyces echinatus*. Based on its antitumor activity against two i.p. implanted murine tumors, the B16 melanoma and the P388 leukemia, it was brought into clinical trials by the National Cancer Institute, USA. Studies on its cytotoxic action have related its antitumor activity with its ability to bifunctionally intercalate with double stranded DNA. Toxicological studies were carried out in mice and beagle dogs using intravenous injections. The major toxic effects were found in the gastrointestinal, hepatic, and lymphoreticular systems. These trials will further characterize echinomycin´s toxic effects and its antitumor activity.

Several brominated tyrosine-derived compounds, psammaplins A (**313**), K (**323**) and L (**324**) as well as bisaprasin (**325**), were isolated from the Fijian marine sponge *Aplysinella rhax* during a bioassay guided isolation protocol. Psammaplin A was found to moderately inhibit chitinase B from *Serratia marcescens*, the mode of inhibition being noncompetitive. Psammaplin A was found to be a potent antifungal agent [228] that also possesses potent antibiotic activity against Gram-positive bacteria, especially methicillin-resistant *Staphylococcus aureus*, is cytotoxic and inhibits the human topoisomerase II enzyme. Psammaplin A was found to be particularly effective against vine downy mildew, *Plasmopora viticola*.



No.	R_1	R_2	W	X	Y	Z
308	H	OH	S		$-CH_2-S-S-CH_2-$	CH
309	CH_3	OH	O		$-CH_2-S-S-CH_2-$	CH
310	CH_3	OH	O			CH
311	H	OH	S		$-CH_2-S-S-CH_2-$	CH
312	CH_3	H	O			N

Psammaplin A 11-sulfate and bisaprasin 11-sulfate have been isolated from *Aplysinella rhax*, along with psammaplin A. Psammaplin A and his sulfate inhibited [^{3}H]1,3-dipropyl-8-cyclopentylxanthine binding to rat-brain adenosine A_1 receptors. Psammaplin A and psammaplin A 11-sulfate inhibited [^{3}H]8-cyclopentyl-1,3-dipropylxanthine binding to rat-brain adenosine A1 receptors with IC_{50} of 20 μM and 90 μM, respectively. Bisaprasin 11-sulfate did not show the inhibition up to the concentration of 2.2 mM [229, 230]. Psammaplin A displays significant cytotoxic activity against the RAW264.7 cell line. It was also found that psammaplin A could substantially inhibit SV40 DNA replication *in vitro*, in which polymerase α-primase is one of its main targets [231]. It was also shown to inhibit mammalian aminopeptidase N that plays a key role in tumor cell invasion and angiogenesis, with an IC_{50} of 18 μM in a non-competitive manner [232]. The sponge *Aplysinella rhax* collected from Guam, Palau, and Pohnpei contained aplysinellin A (**326**) and B (**327**) that exhibited moderate cytotoxicity and inhibitory activities against farnesyl protein transferase and leucine aminopeptidase [233].

Three new bromotyrosine derivatives *i.e.* psammaplin trisulfide (**328**), brom-psammaplin (**329**), and bi-psammaplin (**330**) were isolated from an association of two sponges, *Jaspis wondoensis* and *Poecillastra wondoensis*, along with the previously described psammaplin A, psammaplin D, and bisaprasin [234]. The compounds displayed significant cytotoxicity against human lung (A549), ovarian (SK-OV-3), skin (SK-MEL-2), CNS (YF498), and colon (HCT15) cancer cell lines. Psammaplin trisulfide exhibited more potent antibacterial activity than meropenem against several strains. Psammaplin A from the sponge *Psammaplysilla purpurea* inhibited strongly the development of Gram-positive bacteria - *Staphylococcus aureus* and *Trychophyton mentagrophytes*. Prepsammaplin A (**331**), and three new bromotyrosine-cysteine derivatives, psammaplins B (**314**), C (**315**), and D (**316**) were also isolated and identified from this sponge. Psammaplin D showed antimicrobial and mild tyrosine kinase inhibitory activity [235].

Four novel bisulfide bromotyrosine derivatives, psammaplins E (**317**), F (**318**), G (**319**), and H (**320**), and two new bromotyrosine derivatives, psammaplins I (**321**) and J (**322**), were isolated from the sponge *Pseudoceratina purpurea*, along with known psammaplins A, B, C, and D and bisaprasin [236]. Psammaplins A, F, and bisaprasin, are potent histone deacetylase inhibitors and show mild cytotoxicity. Furthermore, psammaplin A, G, and bisaprasin, are potent DNA methyltransferase inhibitors (Table **10**).

Table 10. Enzyme-based Histone Deacetylase[a], Cell-based p21 Promoter Activity Data[b], Inhibition Data[c], and DNA Methyltransferase[d] for the Psammaplins and Bisaprasin

Compound	IC_{50} (nM)[a]	IC_{50} (μM)[b]	at 0.5 μM[c] (in %)	IC_{50} (nM)[d]
psammaplin A (**313**)	4.2 ± 2.4	7.5	15	18.6
psammaplin B (**314**)	48 ± 12	15.0	7	nt
psammaplin C (**315**)	nt	nt	nt	nt
psammaplin D (**316**)	44 ± 10	>15.0	5	> 30.0
psammaplin E (**317**)	327 ± 39	>15.0	2	> 30.0
psammaplin F (**318**)	2.1 ±0.4	0.7	18	> 30.0
psammaplin G (**319**)	18 ± 8	7.5	7	12.8
psammaplin H (**320**)	79 ± 22	3.8	7	> 30.0
psammaplin I (**321**)	299 ± 70	2.6	9	> 30.0
psammaplin J (**322**)	20 ± 17	3.6	8	nt
bisaprasin (**325**)	9 ± 5	0.7	9	3.4

A new alkaloid, polycarpine (**332**), has been isolated from extracts of the ascidian *Polycarpa aurata* collected in Micronesia and from *Polycarpa clavata* growing near Australia [237-239]. The dimeric disulfide **332** inhibited the enzyme inosine monophosphate dehydrogenase (IC_{50} 0.03 μM) but the inhibition could be reversed by addition of excess dithiothreitol suggesting that **332** reacts with thiol groups on the enzyme. Polycarpine differentially activated p38 kinase, JNKs, and ERKs in JB6 Cl 41 cells. The polycarpine induced apoptosis was decreased in cells expressing a dominant-negative mutant of JNK. The compound stimulated p53-dependent transcriptional activity and phosphorylation. Polycarpin dihydrochloride is cytotoxic against the human colon-tumor-cell line HCT-116.

Three disulfide metabolites were isolated from the fruiting bodies of the basidiomycete (mushroom) *Cortinarius* sp. [240]. Both **333** and **334** showed significant antimicrobial activity and cytotoxicity. Compounds **334** and **335** were probably artifacts of the isolation procedure.

A novel endothelin-converting enzyme (ECE) inhibitor, B-90063 (**336**), was isolated from the culture supernatant of the marine bacterium *Blastobacter* sp. [241]. Human and rat ECEs were inhibited more potently by B-90063, with respective IC_{50} valves of 1.0 and 3.2 μM, than were other neutral endopeptidases such as NEP (66 μM) and type-I (11 μM), and -IV collagenases (9.9 μM). B-90063 also inhibited the binding of ET-1 to rat ETA and bovine ETB receptors, though its antagonistic activities were weak. B-90063 may thus abolish the physiological actions of endothelins through the ECE inhibitory and receptor antagonistic mechanisms.

A novel derivative was obtained when 10 mM 2-aminoethyl-L-cysteine, an inhibitor of aspartokinase, was added to the culture of *Streptomyces griseus*. The compound A-500359 M-1 (**337**) inhibited bacterial translocase I with an IC_{50} of 0.058 μM. A-500359 M-1 inhibited the growth of mycobacteria as well.

Unfertilized sea urchin eggs (*Paracentrotus lividus*) contain, in addition to glutathione, a new low molecular weight compound derived from His (**338**) which was isolated and characterized as the disulphide.

Four novel benzenoids, polycarpamines A-D (**339**-**342**), with rare, sulfur-containing functional groups, have been isolated from the solitary marine ascidian *Polycarpa auzata* [244]. Polycarpamine B (**340**) exhibited significant antifungal activity *in vitro* against *Saccharomyces cerevisiae* and *Candida albicans*.

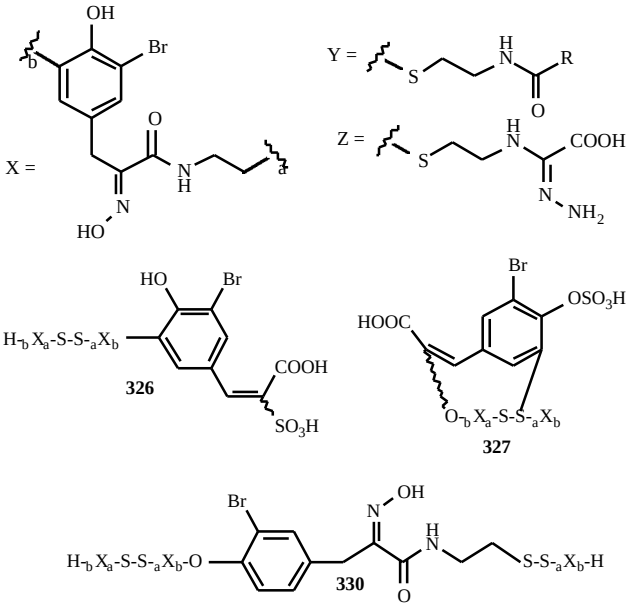
ENEDIYNE ANTIBIOTICS

The 10-membered enediyne antibiotics, which were discovered in the eighties of the last century, have long been appreciated for their novel molecular architecture, their remarkable biological activity, and their fascinating mode of action [245-254]. Enediyne antibiotics were originally derived by fermentation of microorganisms, including *Micromonospora*, *Actinomadura*, and *Streptomyces*. They have been referred to as a class of the most potent and highly active antitumor reagents yet discovered.

To date, at least twelve members of this family of antibiotics have been discovered [255], all of which fall roughly in two categories. The members of the first category of enediynes are classified as chromoprotein enediynes because they possess a 9-membered ring chromophore core structure, which also requires a specific associated protein for chromophore stabilization. The members of the second category of enediynes are classified as non-chromoprotein enediynes without additional stabilization factors.

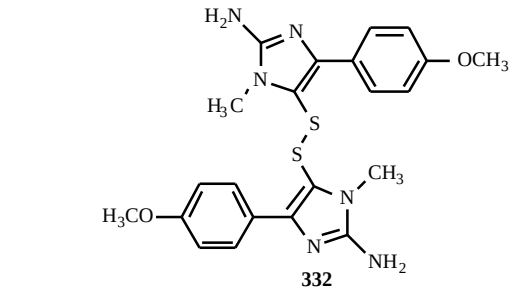
The non-chromophore enediyne subfamily is comprised of *e.g.* calicheamicins (**343**-**349**) from *Micromonospora echinospora* spp. *calichensis*, which were isolated from caliches soils in Texas, and esperamicins (**350**-**356**) from *Actinomadura verrucosospora* isolated from a soil sample collected in Argentina, both containing sulfur in their mole-

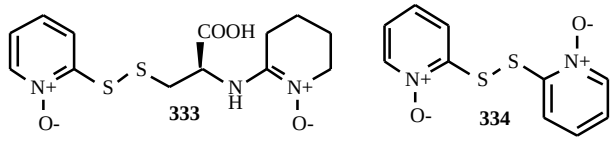
cules. Iodine- containing calicheamicins were obtained after the addition of iodide to the fermentation broth. This treatment yielded not only new representatives of this class of compounds but also resulted in an increased content of iodine-containing compounds at the expense of bromine derivatives [256,257].

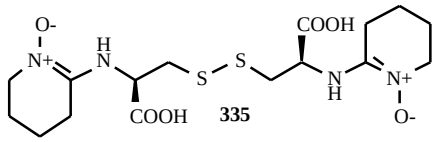


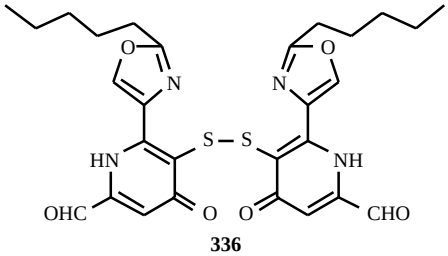
No.	Structure	R
313	H-$_b$X$_a$-S-S-$_a$X$_b$-H	-
314	H-$_b$X$_a$-SCN	-
315	H-$_b$X$_a$-SO_2NH_2	-
316	H-$_b$X$_a$-S-Y	OCH_3
317	H-$_b$X$_a$-S-Y	$CONH_2$
318	H-$_b$X$_a$-S-Y	COOH
319	H-$_b$X$_a$-S-Z	-
320	H-$_b$X$_a$-S-Y	OCH_2CH_3
321	H-$_b$X$_a$-SO_2CH_3	-
322	H-$_b$X$_a$-S-SO-$_a$X$_b$-H	-
323	H-$_b$X$_a$-S-S-$_a$X$_b$-OH	-
324	HO-$_b$X$_a$-S-S-$_a$X$_b$-OH	-
325	H-$_b$X$_a$-S-S-$_a$X$_b$-$_b$X$_a$-S-S-$_a$X$_b$-H	-
328	H-$_b$X$_a$-S-S-S-$_a$X$_b$-H	-
329	H-$_b$X$_a$-S-S-$_a$X$_b$-Br	-
331	Y-Y	OCH_3

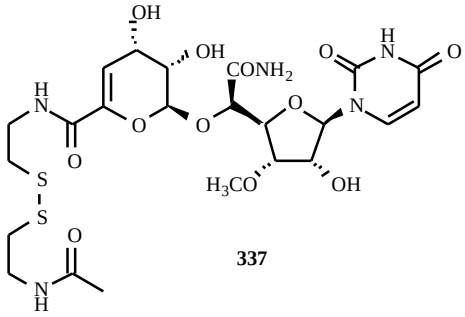
Detailed biosynthetic studies on esperamicin A were performed [258] by the incorporation of labeled compounds fed to cultures of *Actinomadura verrucosospora*. In addition to contributions on the enediyne core, several significant re-

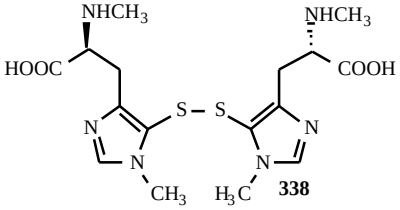


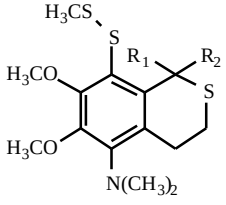












339 R_1 = H, R_2 = OCH_3
340 R_1,R_2 = O
341 R_1,R_2 = S
342 R_1 = OCH_3, R_2 = $COOCH_3$

ports have appeared on the preparation of the sugar moieties. The chemical structure of calicheamicins and esperamicins includes two parts: the enediyne bicyclic core which is responsible for DNA cleavage following the Bergman cycloaromatisation mechanism and the carbohydrate domain which plays a key role in the drug-DNA interaction (Fig. **3**). The typical half-lives of most unstrained 10-membered enediynes (for Bergman cyclization) are approximately 3-18 h at physiological temperature, making them ideal for *in vitro* and *in vivo* studies. For example, the oligosaccharide domain of calicheamicin γ_1^I is largely responsible for the selectivity and specificity of DNA cleavage, particularly towards 5'-TCCT-3' and 5'-TTTT-3' sequences (the coordination with the conformation of pyrimidine containing fragments is more favorable than with purine fragments) and has been shown to bind into the minor groove of the DNA [245].

This activity of calicheamicin has sparked considerable interest in the pharmaceutical industry culminating in the calicheamicin-antibody conjugate MyloTarg [259] to treat acute myelogenic leukemia. Additionally, similar strategies have been used in phase I trials to treat breast cancer. A massive program to examine calicheamicin conjugated to alternative delivery systems has also recently been undertaken. The biological activity and molecular architecture of calicheamicin has also prompted a search for potentially useful analogs. In addition to synthesizing calicheamicin analogs, random mutagenesis of *M. echinospora* and screening for mutant strains with improved biosynthetic potential has also been pursued. Calicheamicin γ_1 (SAc instead of SSSMe) proved to be more potent than the naturally occurring compound both in terms of cleaving DNA and killing tumor cells by initiating apoptosis [260].

The toxicity of the enediyne compounds, including calicheamicin, centers on the problem of directing the compound to cleave only the DNA of interest, such as tumor cell DNA, and not the DNA of the host. The enediyne ring structure induces DNA damage, which is frequently a double-stranded cleavage. This type of DNA damage is usually non-reparable for the cell and is most often lethal; however, many of these compounds are too toxic to be used currently in clinical studies. The enediynes also potentially have utility as anti-infective agents, if toxicity can be managed. For inhibition of DNA synthesis IC_{50} was 39 fmol/ml, inhibition of RNA synthesis 12 pmol/ml and inhibition of protein synthesis 193 fmol/ml, *in vitro* ED_{50} was 0.02 pmol/ml, *in vivo* optimal dose 1.9 μmol/kg (mice). Calicheamicin possesses antineoplastic activity 4000 times higher than that of the best currently used cancerostatic - adriamycine.

Esperamicin P (**353**) was active in a P-388 leukemia model at 0.7-2.2 nmol/kg. *In vitro* cytotoxicity tests of esperamicin P vs. cultured cell lines yielded the following IC_{50} values (nmol/ml): human lung, 0.007; A549 (VP-resistant), 0.007; murine melanoma, <0.0015; human colon, <0.0015; resistant human colon <0.0015. It has also antibacterial activity (Table **11**).

The naturally occurring enediyne cytostatics turned out to be highly toxic in animal experiments; much effort was spent on the synthesis of more selective derivatives to achieve high biological activity towards tumor cells in combination with a minimum toxicity towards normal cells [261-263].

Table 11. Antibacterial Activity of Esperamicin P (353)

Organism	MIC (nmol/ml)
Escherichia faecalis	0.0015
Streptococus aureus	0.0007
Escherichia coli	0.012
Klebsiella pneumoniae	0.74
Pseudomonas vulgaris	0.098
Pseudomonas aeruginosa	0.098

Namenamicin, (**357**) a new enediyne antitumor antibiotic from the marine thin encrusting orange ascidian *Polysyncraton lithostrotum* (order *Aplousobranchia*, family *Didemnidae*) from Namenalala Island (Fiji) [264], exhibited potent *in vitro* cytotoxicity with a mean IC_{50} of 3.5 pmol/ml and *in vivo* antitumor activity in a P388 leukemia model in mice (3 nmol/kg). Namenamicin also showed potent antimicrobial activity (Table **12**). DNA cleavage experiments indicated that namenamicin cleaved DNA with a slightly different recognition pattern than calicheamicin γ_1^I.

Table 12. Antimicrobial Activity (MIC, pmol/ml) of Calicheamycin (348) and Namenamicin (357)

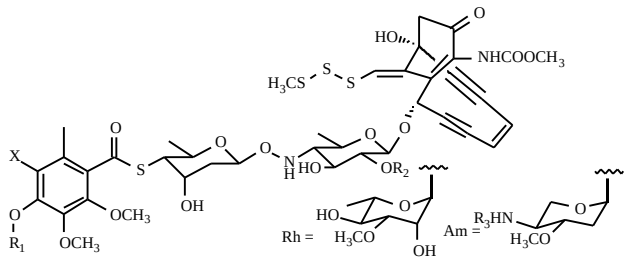
Organism	348	357
Bacillus subtilis	0.037	30.3
Staphylococcus aureus	0.00074	1.01
Enterococcus faecium	0.089	30.3
Escherichia coli	88.6	121
Klebsiella pneumoniae	185	60.5
Candida albicans	22.2	252
Ustilago maydis	0.74	4.04
Saccharomyces cerevisiae	5.91	60.5
Neurospora crassa	44.3	252

Three new derivatives of enediyne antibiotics, shishijimicins A-C (**358**-**360**), have been isolated from the ascidian *Didemnum proliferum* collected in southern Japan [265]. They encompass a novel sugar component, which is a conjugation product of a hexose and a β-carboline, attached to the calicheamicinone aglycone. Shishijimicins showed extremely potent cytotoxicity against HeLa cells with IC_{50} values of 1.8-6.9 pM. Shishijimicins [265] are highly cytotoxic, as is namenamicin (Table **13**). However, shishijimicin A was almost 10 times more active than namenamicin in the three cell lines tested. It is highly likely that shishijimicins cleave DNA as in the case of other enediyne antibiotics including namenamicin. The sequence specificity of calicheamicin γ_1^I (**344**) was ascribed to the presence of the aryl-substituted saccharides. Because β-carboline not only intercalates DNA

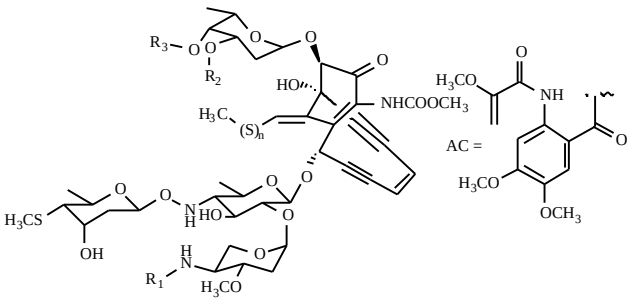
but also the β-carboline unit in shishijimicin A occupies a position similar to that of the aryl group in calicheamicin γ_1^I, it may exhibit a different pattern of DNA sequence recognition from those of other enediynes.

Table 13. Cytotoxicity of Compounds 357-360 (IC_{50}, fmol/ml)

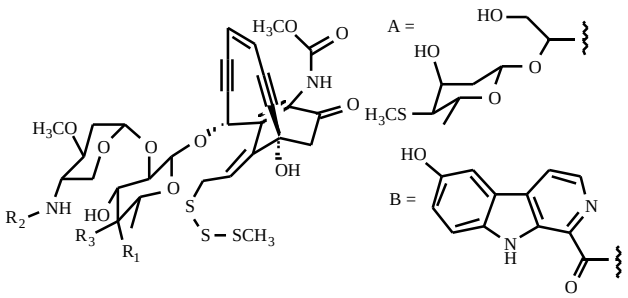
	357	**358**	**359**	**360**
3Y1	13	2.1	3.2	4.9
HeLa	34	1.9	3.4	6.4
P388	3.3	0.50	2.0	1.7



No.	Calicheamicin	X	R_1	R_2	R_3
343	γ_1^{Br}	Br	Rh	Am	i-Pr
344	γ_1^{Br}	Br	Rh	Am	Et
345	γ_2^I	I	H	Am	Et
346	γ_3^I	I	Rh	H	-
347	γ_1^{Ir}	I	Rh	Am	i-Pr
348	γ_1^I	I	Rh	Am	Et
349	γ_1^I	I	Rh	Am	Me



No.	Esperamicin	n	R_1	R_2	R_3
350	A_1	3	i-Pr	AC	H
351	A_{1b}	3	Et	AC	H
352	A_{1c}	3	Me	AC	H
353	P	4	i-Pr	AC	H
354	A_2	3	i-Pr	H	AC
355	A_{2b}	3	Et	H	AC
356	A_{2c}	3	Me	H	AC

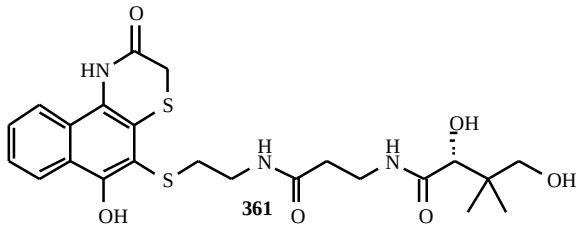


No.	R_1	R_2	R_3
357	SCH_3	i-Pr	A
358	H	i-Pr	A
359	SCH_3	Et	A
360	SCH_3	i-Pr	B

Micromonospora is a genus of bacteria of the family *Micromonosporaceae*. They are Gram-positive, spore-forming, generally aerobic, and form a branched mycelium; they occur as saprophytic forms in soil and water. Various bacterial species are sources of most varied secondary metabolites including aminoglycoside antibiotics and enediynes discussed in this review. A logical question can be asked, *viz.* why are these compounds produced at all and whether they may be useful for the producing microorganisms. This holds particularly for cancerostatics. Since soil or water microorganisms are involved, it may be assumed that the secondary metabolites produced by them help them to survive in the highly competitive environment and give them a selective advantage over other microorganisms that do not produce secondary metabolites.

OTHER COMPOUNDS

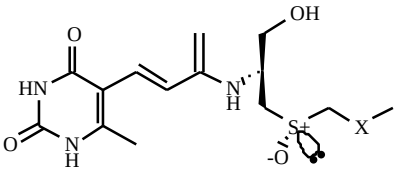
FR901537 (**361**) was produced as a new aromatase inhibitor by a bacterium *Bacillus* sp. [266,267]. It showed a potent inhibitory activity against aromatase from human placenta or rat ovary but did not inhibit the activity of 11β-hydroxylase from bovine adrenal cortex. Lineweaver-Burk plot analysis revealed that FR901537 is a competitive inhibitor. The acute toxicity of FR901537 was determined in *ddY* mice by a single intraperitoneal injection of graded doses of FR901537. The LD_{50} was over 330 mg/kg.



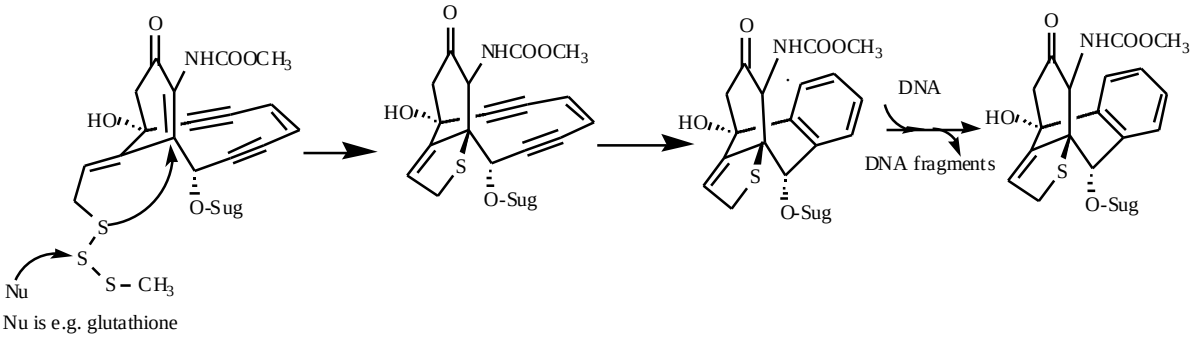
361

Streptomyces sparsogenes isolated from a soil sample collected in Hokkaido was found to produce sparoxomycins A1 (**362**) and A2 (**363**), new modulators of proliferation of mammalian cells, and sparsomycin (**364**) [268,269]. Sparoxomycins A1 and A2 reverse the morphology of temperature-sensitive mutant Rous sarcoma virus-infected NRK cells

(src(ts)-NRK cells) from the transformed phenotype to the normal phenotype at the permissive temperature. Sparsomycin (**364**) exhibits antibiotic activity against a variety of Gram-negative and Gram-positive bacteria, and it shows potent antitumor activity against KB human epidermoid carcinoma cells *in vitro*. The biological activity of sparsomycin is the result of its ability to inhibit the peptide bond-forming step of protein biosynthesis.



No.	
362	X = S^R (=O)
363	X = S^S (=O)
364	X = S

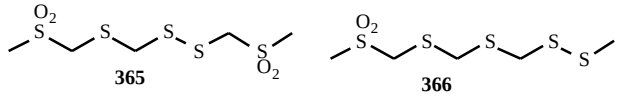


Nu is e.g. glutathione

Fig. (3). Mechanism of the DNA cleavage by calicheamicin.

Local residents use the herb *Dysoxylum richii*, growing on Fiji islands, as the additive to tea with the purpose of preventing the diseases caused by pathogenic microorganisms. Dysoxysulfone (**365**) was isolated from this plant and shown to exhibit powerful inhibitory effects on duplication of *Staphylococcus aureus* (MIC 338 nmol/ml), *Bacillus subtilis* (MIC 68 nmol/ml), and *Candida albicans* (MIC 34 nmol/ml) [270].

High antineoplastic activity is displayed by compound **366** (IC_{50} for mouse cells is 2.35 nmol/ml), isolated from the shiitake mushroom, *i.e. Lentinus edodes* [271].



365 **366**

CONCLUSION

Although only very few of the sulfur-containing compounds described in this review have already been applied commercially, some of them exhibit highly interesting antibacterial, antifungal, and anticancer activities. Some of them are much more potent than the currently used drugs but often also more toxic.

It is generally accepted that the introduction of any antibiotic, although efficient and safe, results in its widespread use and in the consequent development of resistant microorganisms. Similarly, cancer cells may also become resistant.

There are several ways how to combat the resistance, including search for new compounds and new producers, often from extreme environments, production of new derivatives of known compounds by their transformation, either chemical or biological, and design of new compounds by combinatorial biochemistry.

Natural sulfur-containing compounds could be useful as leads for new compounds. Further research will be needed to decrease their toxicity and develop them into commercially applicable drugs.

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