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Development of antibiotics and the future of marine microorganisms to stem the tide of antibiotic resistance

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Abstract

Antibiotics remain essential tools in the control of infectious diseases. With the emergence of new diseases, resistant forms of diseases such as tuberculosis and malaria, as well as the emergence of multidrug-resistant bacteria, it has become essential to develop novel antibiotics. Development of the existing antibiotics involved three strategies, including discovery of new target sites, modification of existing antibiotic structures, and the identification of new resources for novel antibiotics. Marine microorganisms have clearly become an essential new resource in the discovery of new antibiotic leads.

Keywords

Antibiotics; *Bacillus subtilis*; *Escherichia coli*; marine fungi; marine microorganisms; resistance; *Staphylococcus aureus*

Introduction

An antibiotic is a chemical substance produced by microorganisms, which has the capacity to inhibit the growth and even to destroy bacteria and other microorganisms in low concentration [1]. This definition distinguishes between chemicals produced by microorganisms and antimicrobial compounds synthesized in the laboratory. To date the term antibiotic is used to describe both of these groups of antimicrobial agents.

The history of antibiotics began with Alexander Fleming's discovery of penicillin in 1929 and since then thousands of antibiotics have been isolated and characterized. Many antibiotics are used commercially or are useful in medicine for activities other than their antibiotic activity. Antibiotics are used as enzyme inhibitors, antitumor, immunosuppressive, hypocholesterolemic and antiparasitic agents, in addition to their applications as antibiotics.

Antibiotics have remained unrivaled in their control of infectious bacteria and fungi for the last 60 years. Ongoing research focuses on the discovery of new or novel antibiotics and further modifications to existing antibiotics are still in progress. There are several important reasons why the discovery and development of antibiotics with novel structural classes are

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particularly important, including the development of resistant bacteria and other pathogens. Not all antibiotics have activity against all microorganisms. In addition, every antibiotic which is released for clinical application has a limited shelf-life. This means that antibiotics will no longer be active against bacteria at some point in time [2•]. Some microorganisms are naturally resistant to antibiotics and antibiotic resistance can be an inherent property of a microorganism, or can be acquired. Microorganisms may have an inherent resistance to an antibiotic because, for example, the microorganism may lack the target an antibiotic inhibits, be impermeable to antibiotics, be able to alter antibiotics to an inactive form or may modify the target of the antibiotics, or be able to pump out an antibiotic entering the cell [3•].

Bacteria have a high capacity to mutate and exchange genetic material with other microorganisms and, as a result, the development of resistant bacteria has become a serious concern. The development of resistance to existing antibiotic classes by pathogenic bacteria is in direct correlation with the level of utilization of the antibiotics to treat them, and now different types of antibiotics are required to treat the same bacterial infection. For example, treatment for an infection by *Staphylococcus aureus* was changed from penicillin to methicillin, and now vancomycin is required to treat this infection.

Antibiotic resistance can be achieved by horizontal transfer of resistant genes (carried by plasmids or transposons), by recombination of foreign DNA into a chromosome, or by mutation in different chromosomal loci. Mutations in different loci produce a change in MIC values and stable maintenance of heterogeneous antibiotic resistance expression classes in bacterial populations [4]. Resistance to antifolate drugs by the malaria parasite is the result of point mutations in the substrate binding site of target enzymes. There are seven identified point mutations occurring in the dihydrofolate reductase genes which are associated with reduced drug binding capacity in resistant strains of *Plasmodium falciparum* [5]. *Mycobacterium tuberculosis* resistance to rifampin arises due to mutation in the β-subunit of RNA polymerase encoded by the gene *rpoB* [6]. Resistance to macrolide antibiotics involves modification of the ribosome, specifically methylation of an adenine residue in domain V of the 23S rRNA [7].

There are now many examples of antibiotic-resistant bacteria that are of concern such as methicillin-resistant *S aureus* (MRSA), vancomycin-resistant enterococci (VRE), multidrug resistance in *M tuberculosis* and multidrug-resistant Gram-negative bacteria. Other examples of resistance include penicillin resistance in *Neisseria gonorrhoeae*, pneumococcal infections that are resistant to macrolide therapy and drug-resistant meningococci and *Haemophilus influenzae* [8].

The toxicity of current antibiotics limits their application, thus providing another reason why the development of novel antibiotics is of importance. Many existing antibiotics have toxicity at or near to their therapeutic dose. Amphotericin B is used in the treatment of serious disseminated dimorphic fungal infection and infections caused by *Blastomyces, Candida, Cryptococcus* and *Histoplasma* spp. Unfortunately, amphothericin B causes nephrotoxicity, reduction of renal blood flow, nausea, vomiting and anorexia. Griseofulvin causes hepatotoxicity and gastrointestinal distress but is used for the treatment of certain dermatophyte infections caused by *Epidermophyton, Microsporum* and *Tricophyton* spp [9].

The development of infectious conditions has risen significantly as a result of increasingly widespread use of therapies that depress the immune system. The rise has also been due to the frequent and often indiscriminate use of broad-spectrum antibacterial agents and the common use of indwelling intravenous devices combined with the advent of chronic immunosuppressive viral infections such as AIDS. These factors have intensified the need to search for new, safer and more efficacious antibiotics [10]. A changing patient population, including immunocompromised cases, an elderly population and patients under aggressive chemotherapy, has generated increasing sensitivity to infectious diseases and spurred a resurgent interest in the search for new antibiotics [11].

Finally, increasing concerns that terrorists will use infectious diseases as weapons of mass destruction has had an impact on our need for new antibiotics, and as a result, novel antibiotics are needed to combat infections agents, eg, *Bacillus anthracis*, *Clostridium botulinum* and *Yersinia pestis* [12].

The emergence of new infectious diseases, the re-emergence of resistant forms of well known diseases such as tuberculosis and malaria, and the emergence of antibiotic-resistant bacteria have made the development of new antibiotics essential. Different approaches and strategies can be utilized in the search for novel antibiotics and include the discovery of new target sites, structural modification of currently available antibiotics, and sourcing of antibiotic producers. The first and second strategies have been discussed in a review by Schmidt [13•]. This review will emphasize the exploration of new sources for the production of novel antibiotics, with a focus on marine-derived microorganisms as a final frontier for the discovery and development of novel antibiotics.

There are many natural resources that can be explored as sources of antibiotics, such as rare actinomycetes [14], endophytes [15–17], myxobacteria [18] and marine microorganisms [19–21]. Many antibiotic researchers believe that a vast number of new antibiotics will be discovered if other groups of microorganisms are examined.

Marine microorganisms as resources of antibiotics

The oceans are a resource of biodiversity that greatly exceeds that of terrestrial environments. The oceans cover 71% of the earth’s surface and 80% of life on the planet is found under the ocean surface. In addition, the marine environment provides 95% of habitat space on the planet and the oceans offer tremendous diversity with two-thirds of phyla exclusively or dominantly marine organisms [22]. The oceans have been a valuable resource for a unique and large group of structurally diverse natural products that are mainly accumulated in invertebrates, such as sponges, tunicates, bryozoans and mollusks [23–25]. Different ecological areas have been investigated as potential sources of microbial diversity. Therefore, the biological diversity will be reflected by chemical diversity of metabolites [26].

The marine environment also provides a tremendous biodiversity of microorganisms. Microorganisms play an important role in all of the major element cycles in the ocean and are involved in many ecological phenomena. Marine microorganisms have developed unique

metabolites and physiological capabilities that not only ensure survival in extreme habitats, but also offer the potential for production of metabolites not observed in terrestrial microorganisms [27]. The isolation of antimicrobial metabolites from bacteria collected from sponges suggested that these bacteria may play a role in a defense mechanism of these invertebrates [28,29].

Terrestrial microorganisms have proved to be rich sources for bioactive antibiotics and the exploration of terrestrial microorganisms and, in particular, the *Actinomycetes* gave rise to the discovery of many new species and novel bioactive compounds. The expectation to identify novel, diverse and bioactive metabolites from marine microorganisms is based on the following reasons:

- **i.** The diversity of microorganisms is certain to yield diversity in the production of secondary metabolites.
- **ii.** The production and diversity of microbial metabolites can be regulated by modification of culture conditions.
- **iii.** Most bioactive compounds are produced by a specific strain rather than a taxonomically defined species of microorganisms. Therefore, new bioactive compounds can be obtained by the cultivation of isolated strains without prior taxonomic classification.
- **iv.** Only a small quantity of plant or animal tissue is required to cultivate marine microorganisms, thus protecting macroorganisms from over sampling.
- **v.** A preservation of natural habitat is possible since once microbes are cultured and preserved, recollection is not required.
- **vi.** Culturable marine microorganisms will provide an adequate supply of bioactive compounds required for scientific and economic development.
- **vii.** Microorganisms are more readily manipulated than their macro counterparts [30•,31].

The most significant obstacles which have hindered the development of drugs from marine natural products are the supply issues. The majority of promising marine compounds have complex structures, rich in centers of asymmetry, limiting the large-scale preparation of antibiotics by chemical synthesis [32•,33•]. In addition, the yield from harvesting and isolating from macroorganisms may be quite low because the macroorganism is rare or the active compound is a minor component. Since it is impossible to collect unlimited quantities of marine animal or plant material, the isolation and cultivation of symbiotic microorganisms are of great importance for a sufficient and constant supply of bioactive compounds. Symbiotic marine microorganisms which can be cultivated will facilitate future development of marine natural product leads if the compound can be produced by an associated microorganism [34•].

The biology of the bacteria-sponge relationship has elicited considerable interest. The responsibility of bacteria for defense strategies of sponges and the production of chemical

products by the sponge-associated bacteria are important observations for the exploitation of natural substances [35,36••,37]. Several metabolites have been identified from bacterial cultures isolated from sponges. For example, diketopiperazine, previously reported as a metabolite of the sponge *Tedania ignis*, is produced by the *Micrococcus* sp isolated from *T ignis* [38].

Marine microorganisms can be isolated from seawater, sediment, animate and inanimate surfaces and the internal space of invertebrate animals. The surface of marine organisms is more nutrient rich than sea water and sediment, and numerous bacterial strains can be observed on the surface of marine plants and animals [39,40••,41•].

Antibiotics from marine bacteria

Bacteria in the marine environment are diverse, and more than 40% of sponge weight may contain bacteria. Marine bacteria are considered as an emerging source of novel bioactive metabolites with respect to their existence, diversity and function in the marine environment [42••,43]. Here, we report promising antibiotics derived from marine bacteria.

Massetolides

Bacterial isolate MK90e85 was obtained from the marine leafy red alga and produced massetolides A (Figure 1) to D, while a tube worm-derived strain (MK91CC8) produced massetolides E to H. Both isolates belong to the Pseudomonas genus based on fatty acid analysis. Massetolide A exhibited promising antimicrobial activity against M tuberculosis and Mycobacterium avium intracellulare with MIC values of 5 to 10 μg/ml and 2.5 to 5 μg/ml, respectively. No activity was demonstrated against human pathogenic bacteria, including Escherichia coli and S aureus. A single intraperitoneal injection of 10 mg/kg of massetolide A was non-toxic to mice [44].

Haliangicin

Haliangicin (Figure 1) is a β-methoxyacrylate antibiotic, which was isolated from the culture broth of a marine myxobacterium Haliangium luteum. Haliangicin demonstrated potent activities against filamentous fungi such as Aspergillus niger AJ 117374, Aspergillus fumigatus AJ 117190, Botrytis cinerea AJ 117140, Fusarium sp AJ 117167 and Mucor hiemalis AJ 117374 with MIC values of 3.1 to 12.5 μg/ml. The compound also showed potency against oomycete fungi Phythium ultimum IFO 32210 and Saprolegnia parasitica IFO 8978, which were insensitive to amphotericin B and nystatin [45].

Macrolactins

Macrolactins (Figure 2) were reported as secondary metabolites from an unidentified bacteria isolated from a deep sea sediment sample from California, USA [46], *Bacillus* sp isolated from a macroalga *Schizymenia dubyi* [47] and *Bacillus* sp SC 026 identified from a sediment sample around Sichang Island, Thailand [48]. Gustafson *et al* reported that macrolactin A inhibited herpes simplex type 1 virus strain LL (IC_{50} = 5.0 μg/ml) as well as type II virus strain G (IC_{50} = 5.0 μg/ml) and HIV (IC_{50} = 10 μg/ml) [46]. The MIC values of macrolactin A against *Bacillus subtilis* and *S aureus* were 20 and 5 μg/disk, respectively. Nagao *et al* reported that macrolactin A, G, H, I, L and M were more effective against *S*

aureus (MIC = 10 ppm) than *B subtilis* (MIC = 60 ppm). Macrolactin J was the most active with MIC values of 5 and 30 μg/ml against *S aureus* and *B subtilis*, respectively, while macrolactins F and K were considered inactive. This lack of antibacterial activity was due to the presence of ketone carbonyl at C(15) and suggested that the hydroxyl group at C(15) was necessary for antibacterial activity.

Bacillus laterosporus antibiotics

Bacterium PNG-276 tentatively identified as *Bacillus laterosporus* produced the cyclic decapeptide antibiotics loloatin A to D (SeaTek Marine Biotechnology Inc; Figure 3) [49], the cationic peptide bogorol A (Figure 3) [50], basiliskamide A and B (Figure 3), and tupuseleiamides A and B [51]. Loloatin A to D inhibited MRSA, vancomycin-resistant *Enterococcus faecalis*, vancomycin-resistant *Enterococcus faecium*, penicillin-resistant *Streptococcus pneumoniae*, as well as *Candida albicans* with MIC values from 0.5 to 8 μg/ml. Bogorol A displayed potent activity against both MRSA (MIC = 2 μg/ml) and VRE (MIC = 10 μg/ml) but exhibited no activity against *C albicans* and drug-resistant *P aeruginosa*. Basiliskamide A and B showed activity against *C albicans* with MIC values of 1.0 and 3.1 μg/ml, respectively. In addition, the MIC values of basiliskamide A and B against *A fumigatus* were 2.5 and 5.0 μg/ml, respectively. *In vitro* cytotoxicity studies comparing basiliskamide A with amphotericin B revealed that basiliskamide A produced minimal toxicity at 100 μg/ml and no cytopathic effect, while amphotericin B produced a cytopathic effect at 12.5 μg/ml.

Tetrocarcins

Arisostatin A and B (Figure 4) are new members of the tetroarcin class of antibiotics and were isolated from the cultured broth of the actinomycetes strain TP-AO316, which was identified as a *Micromonospora* sp strain isolated from a sea water sample collected in Toyama Bay, Japan. Arisostatins exhibited antibacterial activity against Gram-positive bacteria but no activity against Gram-negative bacteria and yeast [52]. The MIC values are shown in Table 1.

Lomaiviticins

The antitumor antibiotics lomaiviticins A and B (Figure 5) were isolated from halophilic strain LL-371366 and identified as the new species *Micromonospora lomaivitiensis*. The strain was isolated from the inner core of the ascidian *Polysyncraton lithostrotum*. In a plate assay, the antibiotics demonstrated potent activity against *S aureus* and *E faecium* with MIC values of 6 to 25 ng/spot [53].

Pelagiomicins

The new marine bacterium *Pelagiobacter variabilis* was isolated from macroalga *Pocockiella variegata* collected at Palau. This bacterium is halophilic, Gram-negative and produced a series of compounds called pelagiomicin A (Figure 6) to C. Pelagiomicin A was active against Gram-positive and -negative bacteria (Table 2) but was inactive against yeast. The pelagiomicins are the third example containing griseoluteic acid. The other two were

griseoluteins and senacarcin produced by terrestrial *Streptomyces* sp, and this raises interesting questions regarding the propagation of secondary metabolite genes [54].

Thiocoraline

A new depsipeptide, thiocoraline (PharmaMar SA; Figure 7), was isolated from marine *Micromonospora* strain L-13-ACM2-092 identified as *Micromonospora marina*. The strain was obtained from a soft coral collected in the Indian Ocean. Thiocoraline showed potent antibiotic activity against Gram-positive bacteria *S aureus, B subtilis* and *Micrococcus luteus* with MIC values of 0.05, 0.05 and 0.03 μg/ml, respectively, while demonstrating low activity against Gram-negative bacteria. This antibiotic inhibits RNA synthesis more specifically than DNA synthesis [55].

Parimycin

Marine actinomycete strain B8652, found in the sediment of the Laguna de Terminos at the Gulf of Mexico, produced the antibiotic parimycin (Figure 8). The antibiotic showed moderate activity against *E coli*, *B subtilis* and *S aureus* at concentrations of > 20 μg [56].

IB-00208

A polycyclic xanthone IB-00208 (Figure 9) was isolated from strain BL-42-PO13-046 identified as *Actinomadura*. This compound showed good antibiotic activity against Gram-positive bacteria, but poor activity against Gram-negative bacteria. The MIC values against *B subtilis* ATCC 6051, *S aureus* ATCC 6538P and *M luteus* ATCC 9341 were 1.4, 1.4 and 0.09 nM, respectively [57,58].

YM-266183 and YM-266184

Bacillus cereus QN 03323 was isolated from a marine sponge *Halicondria japonica*, collected at Hoshisuna Beach, Irimoto Island, Japan and produced novel thiopeptide antibiotics YM-266183 (Figure 10; Yamanouchi Pharmaceutical Co Ltd) and YM-266184 (Figure 10; Yamanouchi Pharmaceutical Co Ltd) along with thiocillin I and II. Both compounds were active against Gram-positive bacteria, including MRSA (MIC = 0.78 and 0.39 μg/ml, respectively), VRE (MIC = 0.025 μg/ml) and methicillin-resistant *Staphylococcus epidermidis* (MRSE) (MIC = 1.56 and 0.2 μg/ml, respectively), but inactive against Gram-negative bacteria, fungi and yeast. The activities against MRSA, MRSE and VRE were comparable to vancomycin [59,60].

Chalcomycins

Marine *Streptomyces* sp B7064 was isolated from a mangrove sediment near Pohoiki, Hawaii and was reported to produce new types of macrolide antibiotics chalcomycin A and B (Figure 11). The compounds showed activity against *B subtilis*, *E coli* and *S aureus* with MIC values of 6.25, 750 and 0.39 μg/ml, respectively [61].

Antibiotics from marine fungi

Terrestrial fungi have proven a rich source of pharmaceutically interesting secondary metabolites, and it has been postulated that marine fungi will also become an important

source of secondary metabolites. Higher marine fungi are distributed in littoral and deep water. Their geographic distribution is largely a function of temperature and salinity requirements. These fungi are morphologically different and have different growth requirement from terrestrial fungi. The diversity, biological activity and secondary metabolite production of fungi associated with marine sponges were investigated in order to assess the potential of these fungi for the production of novel biologically active compounds [62,63].

Zopfiellamides

Two novel antimicrobial compounds zopfiellamide A and B (Figure 12) were isolated from a submerged culture *Zopfiella latipes*. The strain was isolated from a sediment sample from the Indian Ocean near New Delhi. Zopfiellamide A was reported to be active against *Arthrobacter citreus, Bacillus brevis, B subtilis, B licheniformis, Corynebacterium insidiosum, M luteus, Mycobacterium phlei* and *Streptomyces* sp with MIC values between 2 and 10 μg/ml; zopfiellamide B was 5-fold less active [64].

Pestalone

Pestalone (Figure 13) was obtained by mixed fermentation of the fungus *Pestalotia* sp and an unidentified antibiotic-resistant marine bacteria. The fungus was isolated from the surface of the brown alga *Rosenvingea* sp collected at the Bahamas Island. Interestingly, pestalone was not produced when the fungus and bacteria were cultured separately. This fact suggests that the antibiotic is produced in response to antagonism. It is possible that the pathogens responsible for the biosynthesis of certain compounds are regulated by factors elicited by one microbe and detected by another. The compound showed activity against MRSA (MIC = 37 ng/ml) and VRE (MIC = 78 ng/ml) [65].

Halorosellinic acid

A culture broth of marine fungus *Halorosellina oceanica* BCC 5149 collected in Thailand was the source of an ophiobolane sesterpene halorosellinic acid (Figure 14). The compound exhibited moderate antimalarial activity against *Plasmodium falciparum* with an IC_{50} value of 13 μg/ml but weak antimicrobial activity [66].

Trichodermamides

Two new modified dipeptides trichodermamides A and B (Figure 15) were isolated from marine-derived fungi CNL 910 and CNK 266, identified as *Trichoderma virens*. The fungus CNL 910 was isolated from the well-known ascidian *Didemnum molle* collected in Papua New Guinea, while the fungus CNK 266 was isolated from the surface of the green alga of the genus *Halimeda*. Trichodermamide B exhibited moderate antimicrobial activity against MRSA, VRE and amphotericin-resistant *C albicans* with MIC values of 15 μg/ml for all three strains, while trichodermamide A was completely inactive suggesting that the chlorine atom at C(5) is essential for activity [67].

Varixanthone

The fungus *Emericella variecolor* isolated from a sponge collected in Venezuelan waters of the Caribbean produced varixanthone (Figure 16) with activity against *E coli*, *Proteus* sp, *B subtilis* and *S aureus* (MIC = 12.5 μg/ml in all cases) [68].

YM-202204

Marine fungus *Phoma* sp Q60596 was isolated from the sponge *Halicondria japonica* collected at Hoshisuna beach, Okinawa, Japan. The fungus produced the antifungal antibiotic YM-202204 (Figure 17; Yamanouchi Pharmaceutical Co Ltd) which showed activity against *C albicans* ATCC 10231, *Cryptococcus neoformans* TIMM0362, *A fumigatus* TIMM1776 and *Saccharomyces cerevisae* YFC805 with IC_{80} values of 6.25, 1.56, 12.5 and 1.56 μg/ml, respectively. The compound has activity as an inhibitor of glycosyl-phosphatidyl inositol (GPI)-anchoring. Since GPI-anchoring protein is essential for the growth of yeast and fungi, this may be a unique target for antifungal chemotherapy [69].

Future prospects

Novel antibiotics from marine microorganisms are attracting attention because of the growing demand for antibiotics to combat infectious diseases. Many unusual antibiotic classes with different mechanisms of action from existing antibiotics have been discovered. These require further development of fermentation processes since their yields from natural sources are quite low. Fenical recommended 100-liter fermentation of marine bacteria to yield sufficient quantities of the target compound for analysis [70•].

Bioprocess intensification focusing on optimizing fermentation of marine microorganisms will become an important strategy for improving the yields [71••]. For industrial purposes, marine microorganisms should have properties related to current industrial microorganisms, such as the production of the metabolite of interest, availability in pure culture, genetic stability and culture on a large-scale, stability over long periods, rapid growth and production of the desired product quickly, ability to grow in inexpensive liquid media, ease of separation between media and biomass, and finally the microorganisms should be amenable to genetic manipulation.

Many technologies that have been successfully applied to terrestrial microorganisms can be applied to marine microorganisms in order to achieve overproduction of the desired product. Random mutagenesis and selection techniques are common for strain improvement [72].

Recent advances in molecular biology, knowledge of biosynthetic pathways and biosynthetic gene clusters offer many possibilities in strain improvement, such as increasing gene dosage by introduction of additional copies of genes encoding the pathway, increasing gene expression by introduction of regulatory genes in high copy numbers and gene replacement technologies [73–76]. Further understanding of antibiotic biosynthesis pathways will increasingly permit reprogramming of antibiotic biosynthesis, resulting in more desirable molecular structures, simplified production patterns and enhanced product yield [77].

Conclusion

Marine microorganisms provide a wide range of diversity in chemical structure. The activity of compounds derived from marine microorganisms is comparable with existing antibiotics and can address the problem of resistance and reemergence in infectious diseases. In addition to the molecular novelty and bioactivity, marine microbes present a secure and renewable supply of targeted metabolites for scientific enquiry and commercial development.

Rapid developments in molecular biology, genetic engineering and fermentation technologies provides the capacity to solve the problem of renewable supply associated with marine natural products as well as uncultivable and unexplored marine microorganisms. Considerable effort will be required before the potential of the marine microorganism is fully realized.

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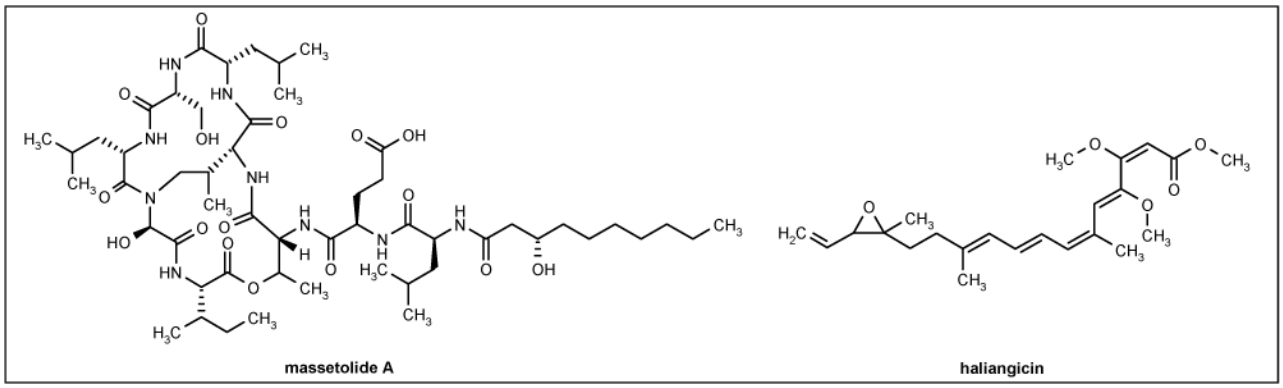


Figure 1.
The structures of massetolide A and haliangicin.

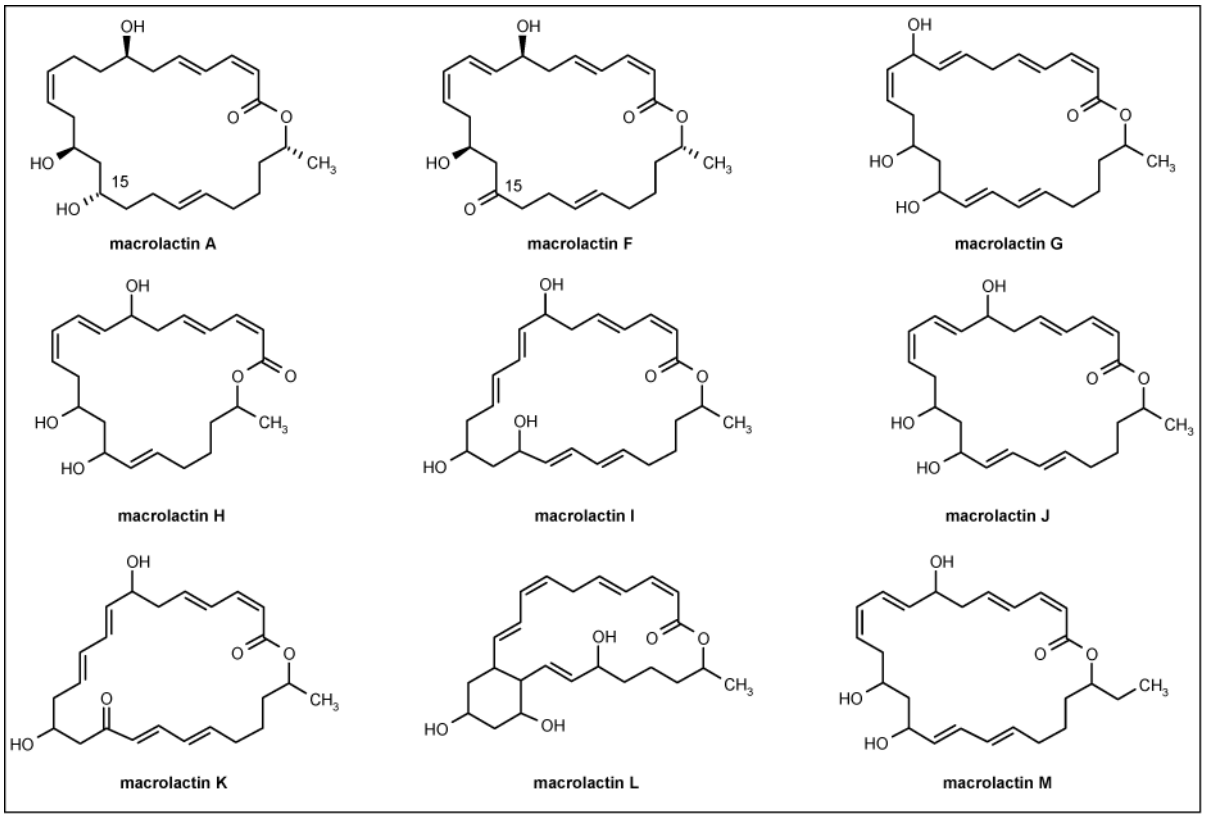
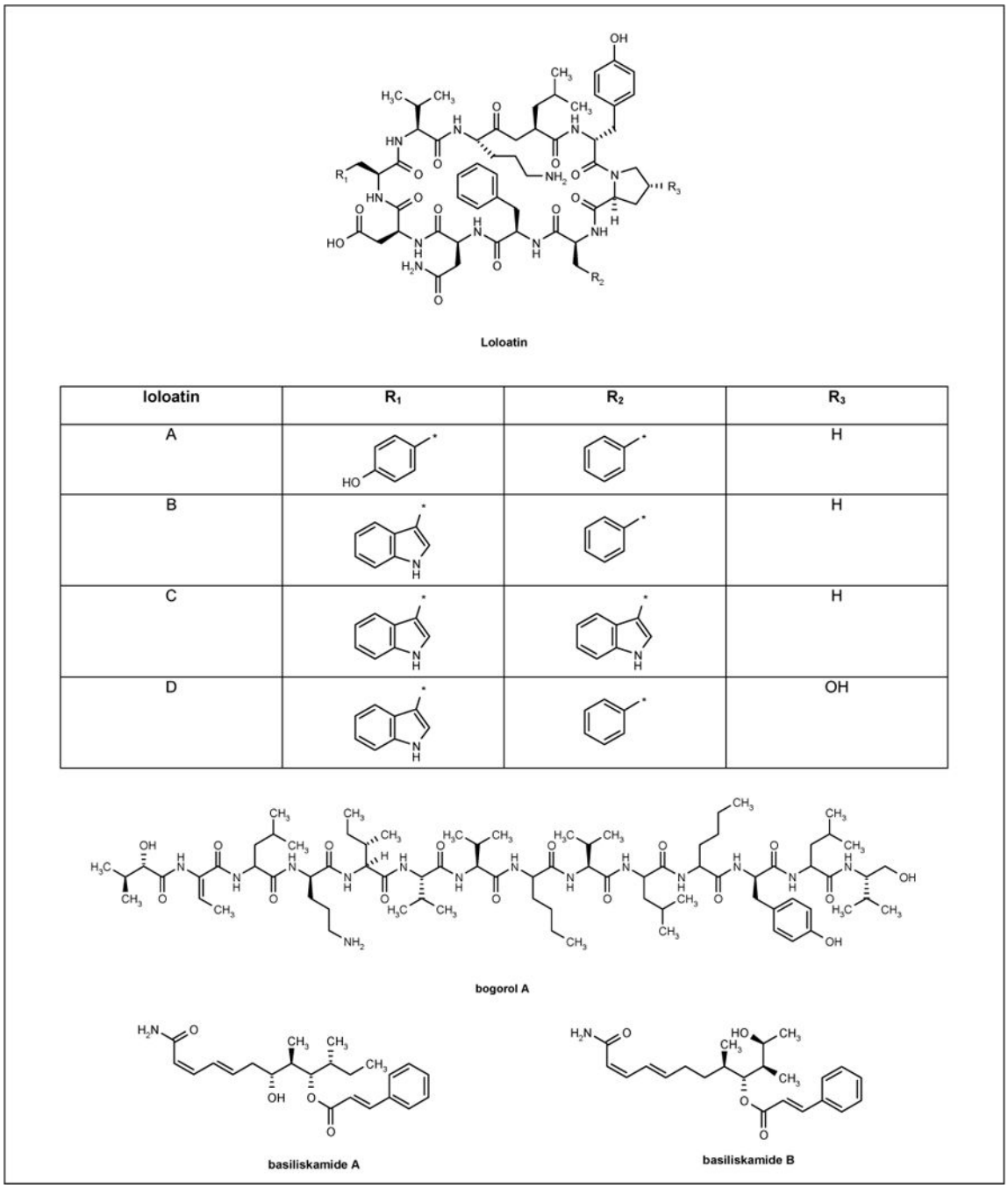


Figure 2.
The structures of macrolactins.



Ioloatin	R_1	R_2	R_3
A			H
B			H
C			H
D			OH

Figure 3.
The structure of antibiotics isolated from *Bacillus laterosporus*.

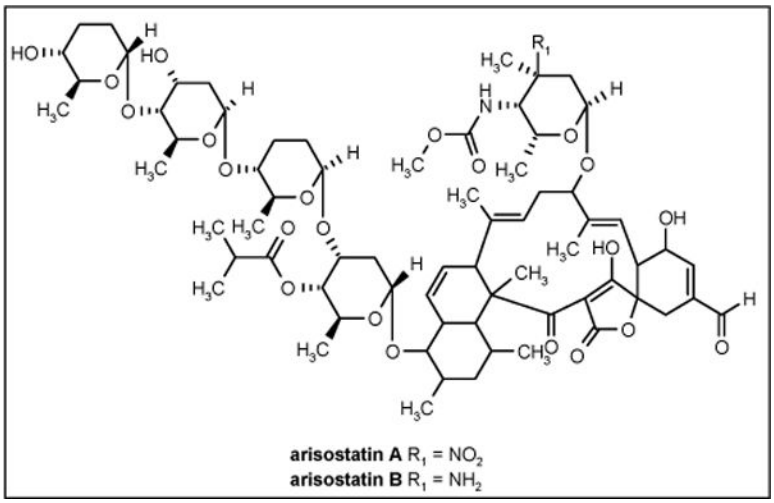


Figure 4.
The structures of arisostatins.

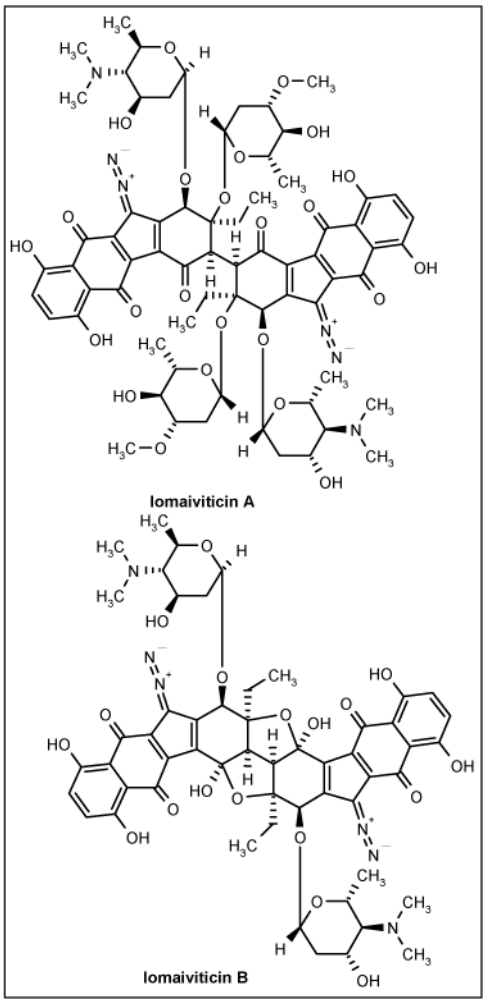


Figure 5.
The structures of lomaiviticins.

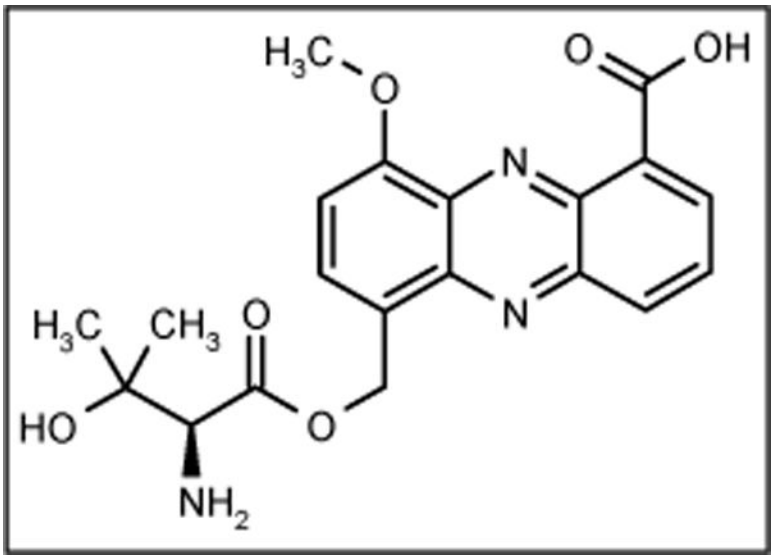


Figure 6.
The structure of pelagiomicin A.

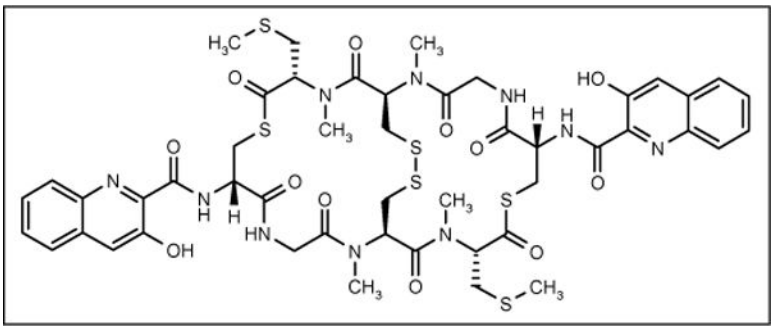


Figure 7.
The structure of thiocoraline.

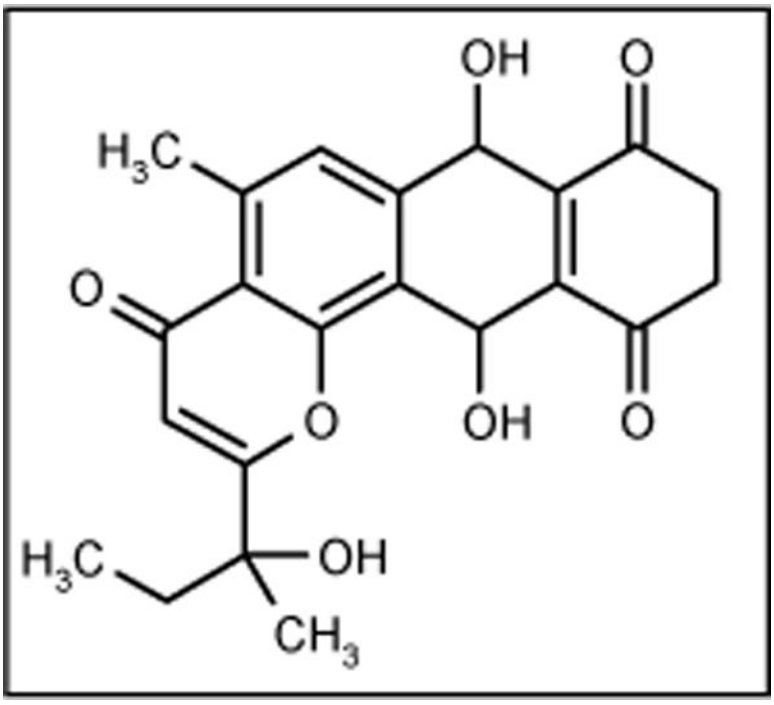


Figure 8.
The structure of parimycin.

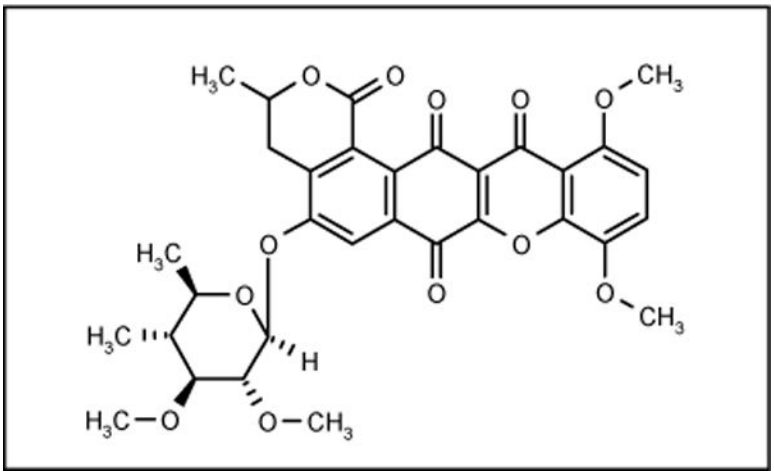


Figure 9.
The structure of IB-00208.

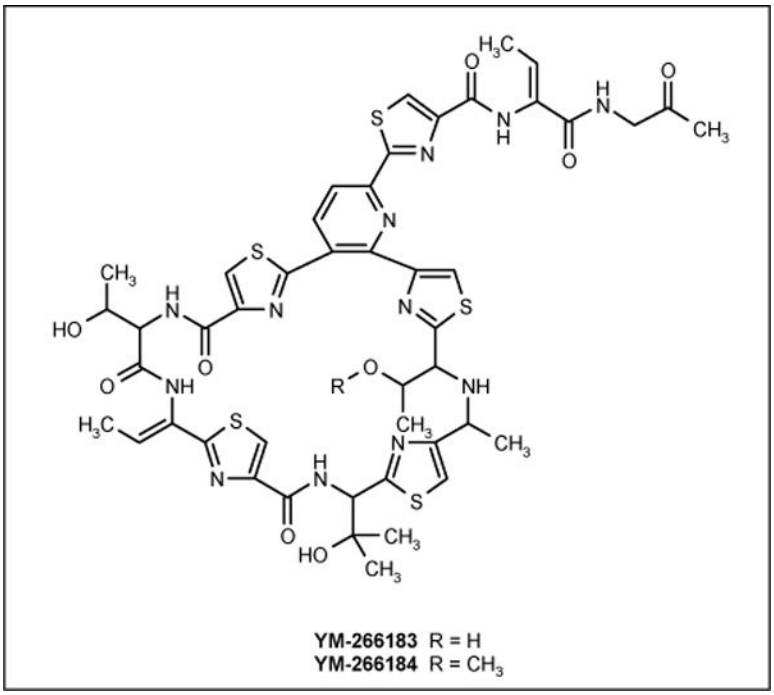


Figure 10.
The structures of YM-266183 and YM-266184.

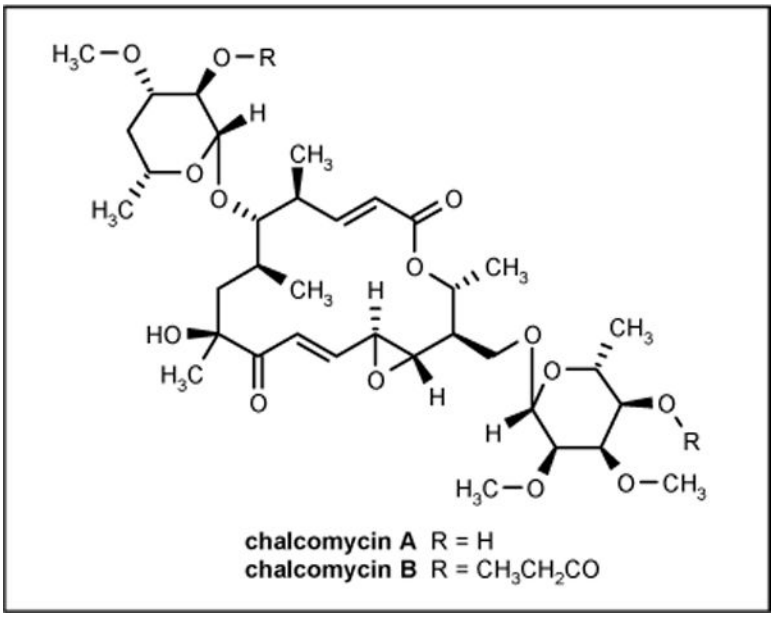


Figure 11.
The structures of chalcomycins.

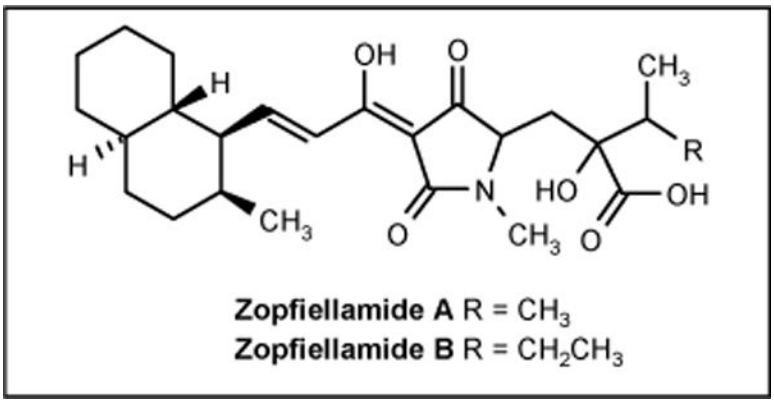


Figure 12.
The structures of zopfiellamides.

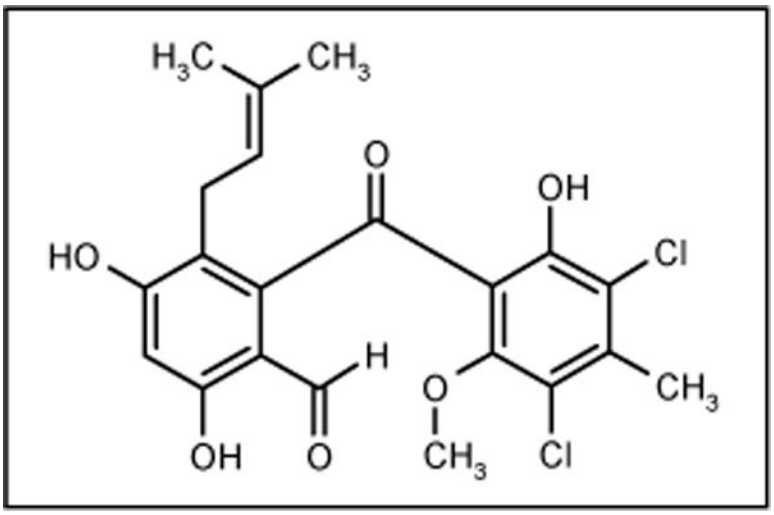


Figure 13.
The structure of pestalone.

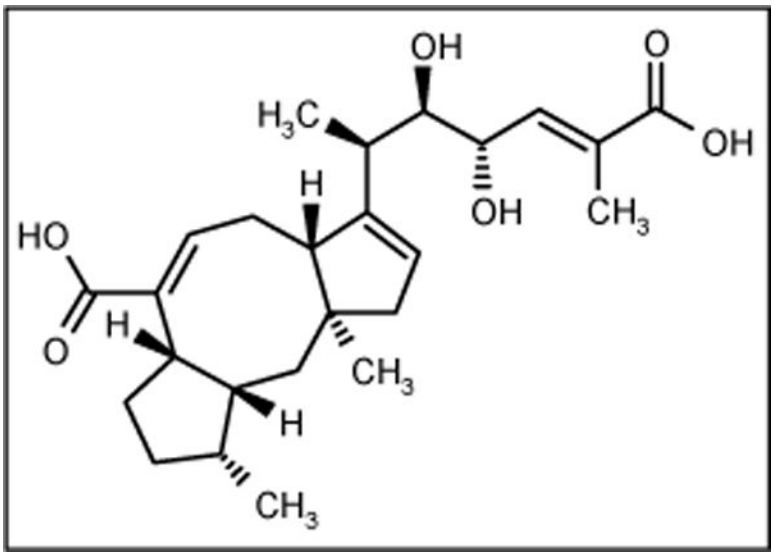


Figure 14.
The structure of halorosellinic acid.

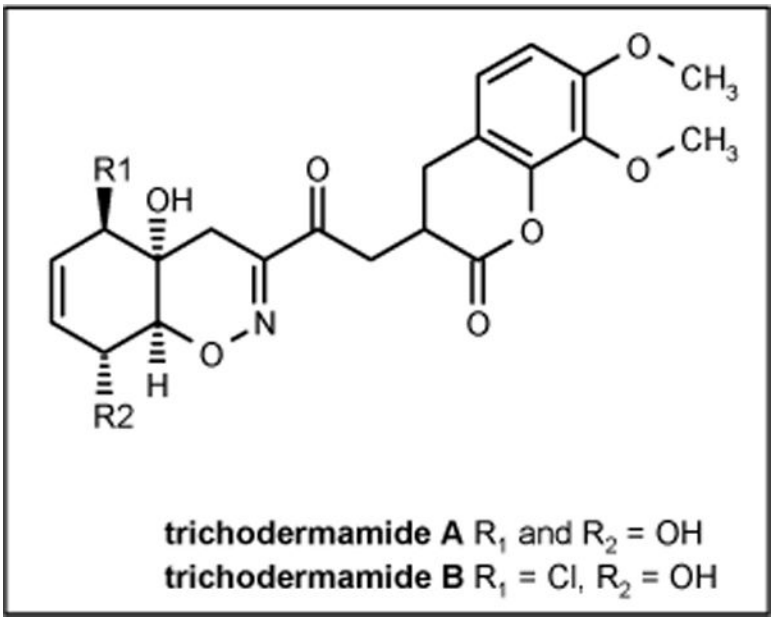


Figure 15.
The structures of trichodermamides.

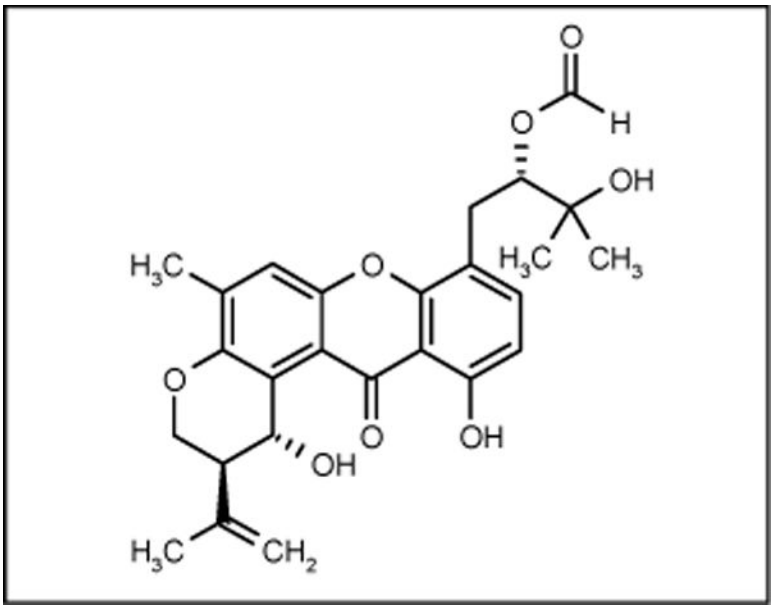


Figure 16.
The structure of varixanthone.

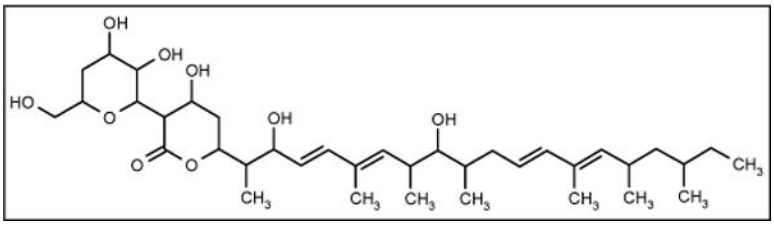


Figure 17.
The structure of YM-202204.

Table 1

The MIC values of arisostatins against Gram-positive bacteria.

	Arisostatin A (μg/ml)	Arisostatin B (μg/ml)
Staphylococcus aureus 209P JC-1	100	50
Bacillus subtilis ATCC 6633	0.39	25
Micrococcus luteus ATCC 9341	12.5	3.1

Table 2

MIC values of pelagiomicin A against Gram-positive and -negative bacteria.

Bacteria	MIC (μg/ml)
Staphylococcus aureus ATCC 6538P	2.6
Enterococcus hirae ATCC 10541	0.16
Bacillus subtilis # 10707	0.16
Klebsiella pneumoniae ATCC 10031	0.16
Escherichia coli ATCC 26	1.3
Pseudomonas aeruginosa Bin H#1	5.2
Proteus vulgaris ATCC 6897	< 0.04
Shigella sonnei ATCC 9290	1.3