

Chapter 1

The Marine Ecosystem as a Source of Antibiotics



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1 Introduction

In spite of the remarkable impact on health that the antimicrobials have achieved in the 1960s and 1970s, 40 years later infectious diseases remain the second-leading cause of death worldwide [1].

Nowadays, one of the most important health problems is the increase, emergence, and spread of antimicrobial resistance among the different microorganisms (bacteria, fungi, virus, and parasites). In the case of bacteria, resistance to antibiotics is increasing in both community and hospital settings in association with an increase in mortality and morbidity. As shown in Fig. 1.1, the discovery of new antibiotics with new mechanisms of action slowed in the year 1968 after the discovery of cephalosporins [2]. After that, most of the antibiotics developed belonged to the existing classes and were considered as “new generations.”

Unfortunately, the development of an antibiotic has, sooner or later, been followed by the emergence of bacterial strains resistant to these antibiotics. Figure 1.1 shows several examples of this [3]:

- In the 1940s penicillin was introduced into the clinical setting. Yet, in the mid-1940s, the first *Staphylococcus aureus* strains producing penicillinases resistant to penicillin were identified.
- In the 1950s, aminoglycoside, chloramphenicol, tetracycline, and macrolides were developed, with multiresistant strains of *S. aureus* emerging within the same decade.

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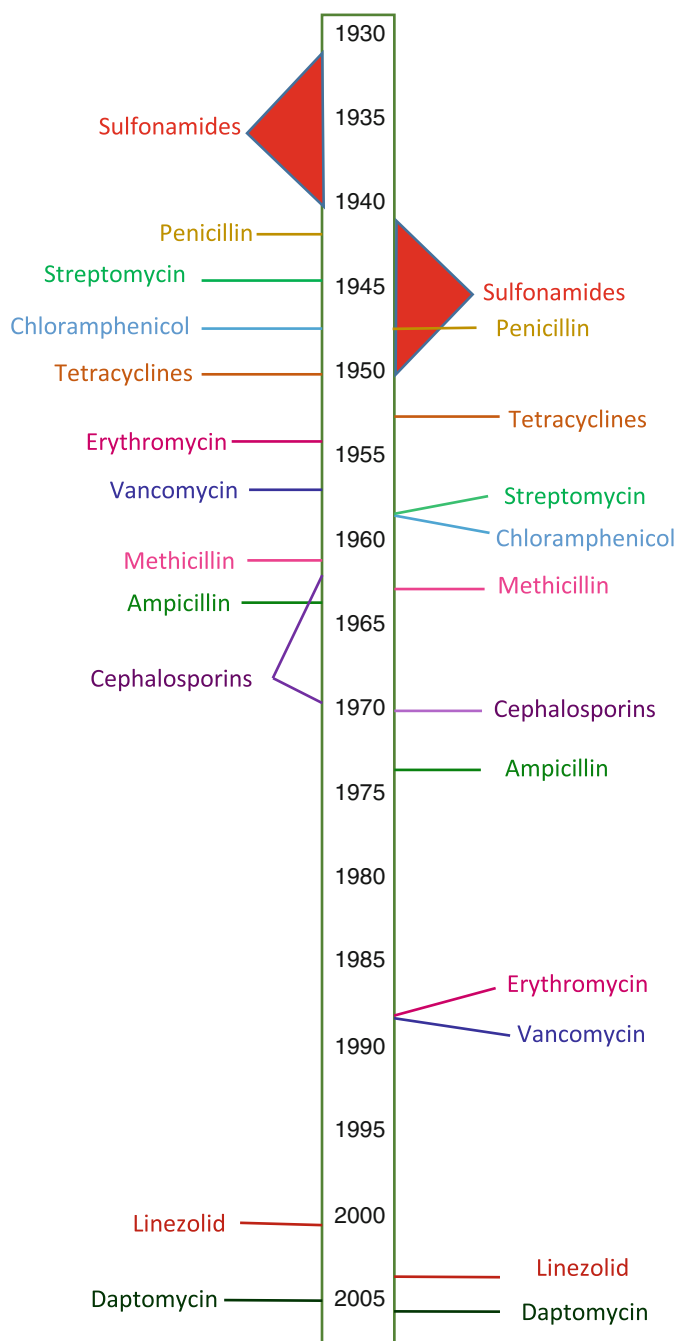
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Antibiotic discover**Antibiotic resistance emerged****Fig. 1.1** Emergence of resistance to the antibiotics

- In 1960, methicillin was synthesized, and only 1 year later, the first methicillin-resistant *S. aureus* strain (MRSA) appeared, constituting an important health problem today.
- In 1980, the third generation of cepheems was developed, followed by the emergence of the first extended-spectrum beta-lactamases (ESBL) Gram-negative-producing strains only 3 years later.

Antimicrobial resistance has an impact not only on health, but it also has an important economical impact. Thus, it has been estimated that the annual cost due to antimicrobial-resistant *S. aureus* infections is about \$4.6 billion taking in account both hospital and nosocomial infections [4].

The high impact of antimicrobial resistance on healthcare and the economy has led to an urgent need to develop novel compounds with new mechanisms of action to treat infectious processes. However, the demand for new antibiotics is not in parallel with the development of new antimicrobial agents. Thus, since 2000 only three new classes of antibiotics have been marketed for human use, one of which was only for topical use.

At present, the following classes of new antibiotics have been approved and/or are under development: monocyclic beta-lactams, pleuromutilins, quinolones, carbapenems, polymyxins, cephalosporins, cephalosporin+beta-lactamase inhibitors, ketolides, glycopeptides, tetracyclines, beta-lactamase inhibitors, aminoglycosides, and oxazolidinones [5].

The question is whether the need for new antibiotics is really urgent or not, and what problems do their development entail? There are three main problems to address [6]:

1. Regulations for drug approval by each government.
2. Economic factors affecting the price, demand, and availability of a product or market forces. The cost associated with the development of a new drug is estimated to be \$400–\$800 million, with the market for antimicrobials being estimated at between \$26 and 45 billion per year [2].
3. Problems encountered by scientists working on this subject. In this regard, scientists are encouraged to find [1]:
 - New molecules active against multiple bacterial species and active against multiple types of infections
 - New molecules that do not generate resistance
 - New compounds acting in targets found thanks to genomics

In addition, antimicrobial agents are less economically attractive than other drug classes for several reasons [1]:

- Their use in short-course therapies in a given patient.
- The development of new agents generates high competitiveness among the pharmaceutical companies.
- There is a preference for broad-spectrum antimicrobial agents that are very rare to find.

In spite of all these reasons, the development of new antimicrobial agents has several advantages in the pharmaceutical industry: the time needed for the clinical development of these agents is shorter compared with other pharmaceutical products aimed at other pathologies, and antibiotics have the highest approved success rates due to the variety of “in vitro” assays and the current animal model tests [2].

In this sense, nature is an important source of molecules demonstrating different activities. The most studied natural products are those produced by bacteria and fungi as demonstrated by 9 of the 12 antibiotic classes derived from a natural product. Seventy-four of the 88 antibacterials marketed from 1981 to 2005 present structures taken from nature [7]. Nonetheless, at present, synthetic products are the most commonly used in medicine.

Several approaches have been undertaken or are currently under development in the search for new molecules from natural organisms. The platform most commonly used is the screening of microorganisms from soil by growth in different culture media followed by bioactivity assays and the identification of the compound responsible for this activity (Waksman platform). Other approaches involve the study of microorganisms isolated from extreme environments such as in the Antarctica to test combinations between known antibiotics and newly found molecules and/or to search for antivirulence compounds.

In all these approaches, the screening of enormous natural extract libraries has led to several problems which have been solved with advances in science. It is important to know the structure of the new active compound. Thus, a process called dereplication has been developed, involving the purification and characterization of metabolite(s) of interest from among a large mixture of metabolites present in the whole extract [6]. The isolation of active compounds requires multiple steps of extraction and HPLC, followed by structure identification using magnetic resonance (MR) and mass spectral (MS) protocols.

Another possible problem is the amount of active molecule available. These metabolites are usually found in small quantities, and changes in metabolism can lead to the loss of the capacity of these organisms to produce these metabolites. In addition, it must be taken into account that these metabolites are often toxic for humans or must first be chemically modified to improve their activity as an antibiotic.

However, we must also [8]:

- Explore other ecological niches such as marine environments.
- Explore peptides and compounds produced by animals and plants.
- Mimick the natural lipopeptides of bacteria and fungi.

As mentioned previously, the most important platform for the screening of new molecules is soil as part of the terrestrial environment. However, the ability of marine habitats to produce antibiotic metabolites remains largely unexplored due to the enormous diversity of potentially attainable species.

To date the following metabolites from marine environments have been found [7]:

- **Alkaloids** including 8-hydroxymanzamine (active against *Mycobacterium tuberculosis*), marinopyrrole 1 (active against MRSA), and cribrastatin 6 (active against *Streptococcus pneumoniae*).
- **Polyketides** constructed by polyketide synthases, such as abyssomicin C (active against MRSA), pestalone (active against MRSA), and ariakemicin A (active against *S. aureus*).
- **Terpenes** including axisonitrile 3 (active against *M. tuberculosis*), haliconadin C (active against *M. luteus*), and bromosphaerone (active against *S. aureus*).
- **Ribosomal peptides** which are antimicrobial peptides synthesized by ribosomes and include arenicin-1 (active against Gram-negative and Gram-positive microorganisms), halocidin (active against *Pseudomonas aeruginosa*), hadistin (active against Gram-negative microorganisms), and clavanin A (active against *Escherichia coli* and *Listeria monocytogenes*).
- **Non-ribosomal peptides** constructed by large multifunctional protein complexes named non-ribosomal peptide synthetases. Among these peptides we can find bogorol 1 and emericellamide A (both active against MRSA) and thiocoraline (active against *S. aureus* and *Bacillus subtilis*).

We have to consider that these molecules could have new targets such as virulence, type III secretion system, quorum sensing, bacterial metabolism, cell division, biosynthesis of aminoacyl-tRNAs, two-component bacterial systems, native proton force, and efflux pumps [8] as well as the use of phages. Indeed, the marine environment could be an important source of quorum-sensing inhibitors due to the multiple associations between the eukaryotic and prokaryote organisms that live in this habitat.

In the last years, Europe is funding projects related to the search of bioactive compounds. Thus, several projects have been developed in the search for new molecules with antibiotic activity. **BAMMBO** (sustainable production of Biologically Active Molecules of Marine Based Origin-FP7-265896) deals with innovative solutions to overcome existing bottlenecks associated with culturing marine organisms for sustainable production of HAVPs for the pharmaceutical, cosmetic, and industrial sectors. **MICRO B3** (Biodiversity, Bioinformatics, Biotechnology) involves a bioinformatic platform with experts in plankton ecosystems and oceanography for developing techniques for the analysis of molecular, genetic, and environmental data from oceanic expeditions. **MACUMBA** (Marine Microorganisms: Cultivation Methods for Improving their Biotechnological Applications-FP7-311975) exploits diverse marine microorganisms for the production of HAVPs.

Currently, the **NOMORFILM** project (H2020-Grant Agreement 634588) is searching for new antimicrobial molecules from microalgae. Microalgae are a source of secondary metabolites useful as new bioactive compounds. The activity of these compounds against bacterial pathogens and biofilm formation has yet to be determined. Biofilm formation is especially important in infections and tissue inflammation related to implants and catheters which may lead to implant rejection and subsequent removal and replacement with a new device, with an increase in antibiotic consumption, together with a health costs of about 50,000–90,000 € per infectious episode. Considering these complications, the search for new antimicrobial agents

that are effective against bacteria in their two media, planktonic and biofilm stage, is a priority in clinical practice. For this reason, the global objective of the NOMORFILM project is to search for antibiofilm compounds isolated from microalgae that will be useful in the treatment of this kind of infections and can be incorporated into the manufacturing of medical prosthetic devices.

Briefly, the oceans and seas are environments that offer us an enormous source of bioactive compounds. Indeed, their inhabitants have survived depredators in these habitats for millions of years. Some of these interesting marine organisms are described in this chapter.

2 Marine Cyanobacteria and Bacteria

Antibacterial agents of marine origin have been widely studied in the last decade, with microalgae and cyanobacteria constituting two of the most promising sources of novel bioactive molecules.

2.1 *Cyanobacteria*

Cyanobacteria belong to the Eubacteria kingdom and the division Cyanophyta. They are the oldest fossils identified, dating from 3.5 billion years ago. Cyanobacteria are aquatic and perform photosynthesis like plants, but they are prokaryotic organisms [9]. They constitute a rich nutrient source, producing chlorophyll, amino acids, minerals, and carotenoids, among others.

In the last decades, novel bioactive compounds have been isolated from cyanobacteria. In this regard, these fossils are considered one of the most promising groups of organisms thanks to the diversity of the secondary metabolites that they secrete. Among these compounds different proportions of toxins, lipopeptides, amino acids, fatty acids, macrolides, and amides are the most frequently found depending on the marine environment they inhabit [9].

Some highly valued metabolites of cyanobacteria showing antibacterial activity are cryptophycin and lipopeptides. Lipopeptides isolated from cyanobacteria have been extensively studied, showing antitumor, cytotoxic, antiviral, antibacterial, antimalarial, and antimycotic activity.

2.2 *Bacteria*

Nature has been a source of medicinal agents for centuries, and an impressive number of modern drugs have been isolated from microorganisms, many based on their use in traditional medicine [10].

The main source of obtaining these antagonistic molecules corresponds to soil bacteria belonging to the order *Actinomycetales*, which have historically shown great benefits in the discovery of therapeutic agents such as streptomycin, another aminoglycosides, macrolides, and tetracyclines. Similarly, secondary metabolites of various chemical structures and biological activities have been extracted from some species of *Bacillus* genus; some examples of these antibiotics used in medical treatments are bacitracin, gramicidin S, polymyxin, and tyrothricin [11], which demonstrate their effectiveness in treatment of infections caused by Gram-positive and even Gram-negative anaerobes [12].

Indeed, nearly 50,000 natural compounds have been discovered, being a good source of new families of antibiotics, antitumoral and antiviral agents [13, 14].

Globally, there are about $3 \times 10^8 \text{ km}^3$ of ocean sediment saturated with $8 \times 10^7 \text{ km}^3$ of porewater inhabited by an estimated 3×10^{29} microbial cells [15]. The genetic and biochemical diversity of these marine microbes is immense, with these microbes having developed complicated biochemical and physiological systems with which they can adapt to the extreme habitats and unfavorable conditions of marine environment. Compared with terrestrial organisms, the secondary metabolites produced by marine organisms have more novel and unique structures owing to their much more complex living circumstances and greater diversity of species and bioactivity, making them a likely rich source of novel effective drugs [16, 17] (Table 1.1).

Several strains were isolated from marine environment with antimicrobial activity against wide variety of pathogenic bacteria (Table 1.2). The marine bacteria live in a biologically competitive environment for space and nutrients, under unique conditions of salinity, pressure, temperature, light, oxygen, and pH. They have therefore developed mechanisms of defense against competitors and predators for their own survival, synthesizing secondary metabolites of great value in pharmaceutical and biotechnological applications [19, 20].

Several molecules that have been isolated from marine microorganisms mainly correspond to secondary metabolites that are chemically divided into **peptides, saponins, terpenoids, alkaloids, nucleosides polycyclic ethers, sterols, amino acids**, etc. (Table 1.3; Fig. 1.2) [18]. However, a critical point in the search for

Table 1.1 Important compounds isolated from marine algae

| Secondary metabolite class | Mechanism of action | Examples |
|----------------------------|---|---------------------|
| Phlorotannins | Inhibition of oxidative phosphorylation | Phloroglucinol |
| | Binding to bacterial proteins | Phlorofucofuroeckol |
| Polysaccharides | Increasing permeability of bacterial cell | Laminarin |
| | | Fucoidan |
| Peptides | Binding bacterial cytoplasmic membranes | Lectins |
| Terpenes | Inhibiting bacterial growth | Xanthophylls |
| | | Fucoxanthin |
| Lactones | Inhibiting bacterial quorum-sensing | Furanones |

Table 1.2 List of antibacterial activity of marine bacteria against some pathogenic organisms (adapted from [18])

| Marine bacteria with antimicrobial activity | Test strain |
|---|---|
| <i>Pseudomonas putida</i> | <i>Bacillus subtilis</i> , <i>Vibrio parahaemolyticus</i> , <i>Escherichia coli</i> , <i>Serratia marcescens</i> , <i>Aeromonas hydrophila</i> , <i>Rothia</i> sp., <i>Staphylococcus aureus</i> , MRSA ^a |
| <i>Actinomycetes</i> | <i>S. aureus</i> , <i>Bacillus subtilis</i> , <i>E. coli</i> , <i>Saccharomyces cerevisiae</i> , <i>Candida albicans</i> , <i>Aspergillus niger</i> , <i>Pseudomonas aeruginosa</i> |
| <i>Pseudomonas Aeruginosa</i> | <i>Aeromonas punctata</i> , <i>Kokoris marina</i> , <i>Rothia</i> Sp., <i>Vibrio</i> sp., <i>S. aureus</i> , <i>Staphylococcus epidermidis</i> , <i>E. coli</i> , MRSA ^a , <i>Proteus vulgaris</i> , <i>Bacillus thuringiensis</i> , <i>B. subtilis</i> , <i>Enterococcus faecalis</i> |
| <i>Pseudoalteromonas</i> sp. | <i>S. aureus</i> , MRSA ^a |
| <i>Pseudomonas</i> sp. | <i>Klebsiella pneumoniae</i> , <i>S. aureus</i> , <i>Shigella flexneri</i> , <i>P. aeruginosa</i> , <i>B. subtilis</i> , MRSA ^a /ORSA ^b |
| <i>Bacillus</i> sp. | <i>Kokoris marina</i> , <i>Rothia</i> sp., <i>Aeromonas punctata</i> , <i>Rothia</i> sp., <i>Vibrio</i> sp., <i>S. aureus</i> |
| <i>Brevibacterium frigiditolerans</i> | <i>Rothia</i> sp., <i>Vibrio</i> sp., <i>S. aureus</i> |

^aMethicillin resistant *S. aureus*^bOxacillin-resistant *S. aureus***Table 1.3** Natural compounds and peptides with antimicrobial activity isolate from marine bacteria

| Metabolite | Isolated from | Chemical scaffold | Activity against | References |
|---------------------------|---|-------------------|--|------------|
| 7-methylcoumarin | <i>Streptomyces</i> spp. | Phenolic | Gram-positive bacteria (<i>Staphylococcus aureus</i>) | [21] |
| Macrolactin (D, S, S) | <i>Bacillus marinus</i> | Macrolide | <i>S. aureus</i> and two species of fungi (<i>Pyricularia oryzae</i> and <i>Alternaria solani</i>) | [22] |
| Macrolactin (T, B O) | | | <i>E. coli</i> , <i>S. aureus</i> , <i>B. subtilis</i> and several soil bacteria | |
| Tropodithietic acid (TDA) | Rosobacter clade (<i>R. gallaeciensis</i> , <i>Pheobacter</i> sp., <i>Silicibacter</i> sp. and <i>Ruegeria mobilis</i>) | | Pathogens in aquaculture as <i>Vibrio anguillarum</i> , <i>V. coralliilyticus</i> and <i>V. shiloi</i> | [23, 24] |
| Tauramamide | <i>Brevibacillus laterosporus</i> | Lipopeptide | <i>Enterococcus</i> spp. | [25] |
| TP1161 | <i>Nocardiopsis</i> spp. | Thiopeptide | Vancomycin resistant <i>Enterococcus faecalis</i> and <i>E. faecium</i> | [26] |

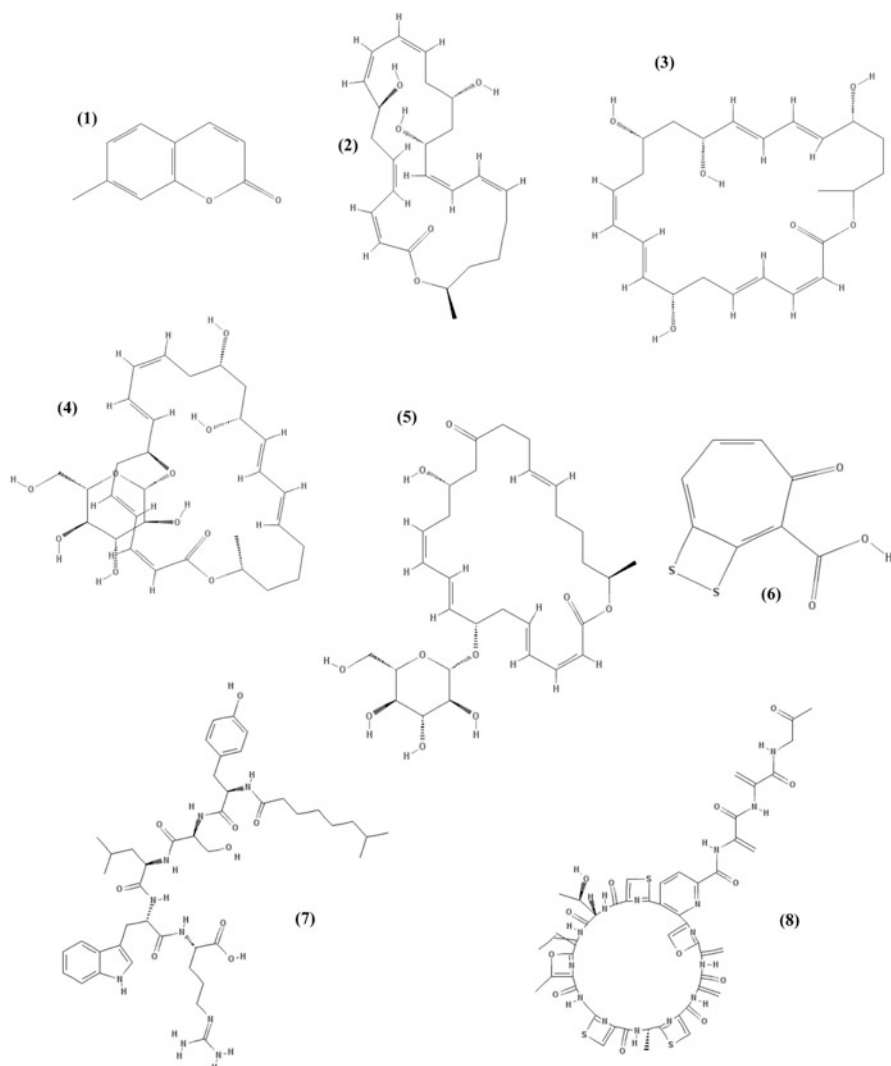


Fig. 1.2 Structures of secondary metabolites with antimicrobial activity isolate from marine bacteria. (1) 7-Methylcoumarin, (2) macrolactin A, (3) macrolactin T, (4) macrolactin B, (5) macrolactin O, (6) tropodithietic acid, (7) tauramamide, and (8) TP1161

new molecules is the extraction and identification of these metabolites to test against clinically relevant bacteria and to find the active compound to be followed by clinical trials.

Two different procedures are followed for the separation of the bioactive fractions of different groups. In the first step, the fractions of low or medium polarity contain organic lipophilic compounds that can generally be separated by standard normal or

reverse phase column chromatography and high-performance liquid chromatography (HPLC) to obtain the individual components. Another method is based on high-polarity fractions that contain water-soluble organic compounds [18]. These chromatography techniques are necessary to fractionate and concentrate the pure active molecule.

Some phenolic compounds from marine bacteria have also been described, such as **4,4',6-tribromo-2,2'-biphenol** (CMMED 290) which was isolated from an extract of the marine bacteria *Pseudoalteromonas* spp. and displays significant antimicrobial activity against MRSA [27]. Another *Pseudoalteromonas* species, the marine bacterium *Pseudoalteromonas phenolica* O-BC3 0 T [28], produces **2,2',3-tribromo-biphenyl-4,4'-dicarboxylic acid**. This compound has antimicrobial activity against MRSA with minimum inhibitory concentrations (MIC) between 1 and 4 µg/ml. In addition, this bacterium shows high activity against *B. subtilis*, *Enterococcus serolicida*, and some fungi species. On the other hand, El-Gendy et al. [21] have isolated three phenolic compounds, **7-methylcoumarin (1)** and the flavonoids **rhamnazin** and **cirsimaritin**, from the marine bacteria *Streptomyces* spp., but only **7-methylcoumarin** showed a potent antibacterial activity, principally against Gram-positive bacteria. The compounds isolated were reported to be antimicrobial products. Other antimicrobial phenolic compounds of marine origin include **ammonificins A** and **B**, which are chroman derivatives from the marine hydrothermal vent bacterium *Thermovibrio ammonificans* [29]. A new 24-member macrolide, **macrolactin T**, and a new polyene δ -lactone, **macrolactin U**, together with **macrolactins A (2), B, D, O, and S** were isolated from the culture broth of the bacterium *Bacillus marinus*, which was isolated from *Suaeda salsa* collected on the coastline of the Bohai Sea of China. Macrolactins are a large group of macrolide antibiotics, e.g., macrolactins T (**3**), B (**4**), and O (**5**) show inhibitory activity against *S. aureus* and two species of fungi, *Pyricularia oryzae* and *Alternaria solani* [22]. In addition, it has increasingly been reported that macrolactins, along with the microbes producing it, are being used to control soil-borne pathogen diseases in agricultural production [30].

A **bromophenyl** compound has been isolated from the marine bacteria resistant to a methicillin *Pseudoalteromonas haloplanktis* INH strain. Cetina et al. [31] detected seven bioactive compound producer strains and observed the presence of a likely association between pigments and toxicity in several marine heterotrophic bacteria with pigmentation. However, a study of pigment synthesis in *P. tunicate* determined that the pigment had no antimicrobial activity against the target bacteria. Lu et al. [32] found that **diketopiperazine** and **macrolide** are the two most important secondary bioactive metabolites from marine microorganisms.

Strains affiliated with *Roseobacter* clade as *Roseobacter gallaeciensis*, *Phaeobacter* sp., *Silicibacter* sp., and *Ruegeria mobilis* were isolated from the German Wadden Sea. These microorganisms have a “biphasic swim-or-stick life-style” that enables their symbiosis with phytoplankton. Several reports showed organisms have been of particular interest due to their ability to form the antibacterial compound named tropodithietic acid (TDA) (**6**) [23, 33, 34]. The new antibiotic showed strong inhibiting properties against marine bacteria and microalgae, being

considered a potent probiotic against the most important pathogens in aquaculture as *Vibrio anguillarum*, *V. coralliilyticus*, and *V. shiloi* [24, 35].

Several studies have described the isolation of antimicrobial peptides from marine bacteria. Thus, two new cyclic lipopeptides, **maribasins A** and **B**, have been isolated from the fermentation broth of the marine microorganism *Bacillus marinus* B-9987 [36]. These compounds exhibit broad-spectrum activity against phytopathogenic fungi. Another *Bacillus* species, *B. amyloliquefaciens* SH-B10, isolated from deep-sea sediments, produces two antifungal **lipopeptides** purified by bioactivity-guided fractionation. Both compounds show significant inhibitory activities against five plant fungal pathogens in a paper-agar disk diffusion assay [37]. A new lipopeptide named **tauramamide (7)** was isolated from *Brevibacillus laterosporus* PNG276 obtained from Papua New Guinea. This peptide shows a potent and relatively selective inhibition of pathogenic *Enterococcus* spp. [25]. Other antimicrobial peptides from the marine bacterium *Nocardiopsis* spp. **TP-1161(8)** are **thiopeptides** and **depsipeptides**. Structure elucidation revealed that these compounds are new thiopeptide antibiotics with an unusual aminoacetone moiety. The “in vitro” antibacterial activity of these thiopeptides against a panel of bacterial strains showed a MICs values ranging from 0.25 to 4 µg/ml against Gram-positive strains and showed a strong activity against vancomycin-resistant bacterial strains represented by *Enterococcus faecalis* and *E. faecium* 569, with a MIC of 1 µg/ml [26]. **Unnarmicine A** and **C** are new antibacterial depsipeptides synthesized by the marine bacterium *Photobacterium* MBIC06485. Both compounds selectively inhibit the growth of two strains belonging to the genus *Pseudovibrio*, one of the most prevalent genera in marine environments [38]. Other unusual peptides are the **ariakemicins A** and **B**. These peptides are linear hybrid polyketide-non-ribosomal peptides isolated from a marine gliding bacterium of the genus *Rapidithrix* spp. [39]. The ariakemicins are composed of threonine, two Ω-amino-(Ω-3)-methyl carboxylic acids with diene or triene units, and δ-isovanilloylbutyric acid. These antibiotics selectively inhibit the growth of Gram-positive bacteria, specifically antistaphylococcal activity.

3 Marine Fungi

The fungi kingdom is a large eukaryotic group including yeast and molds. Fungi are widespread in marine water as they are major decomposers of woody and herbaceous substrata. For this reason, marine fungi may be established in mangroves, algae, plants, plankton, sands, and many other ecological niches. Marine fungi may be classified into two groups:

- Obligate marine fungi: All fungi that complete their life cycle in the sea or in an estuarine habitat [40].
- Facultative marine fungi: These are fungi that can grow in marine environments, but one part of their cycle is developed in a terrestrial ambience or freshwater [41].

Marine fungi include about 10,000 species. Nevertheless, this number may differ from the real number since many ubiquitous species have been included in marine fungi [42]. The predominant species among marine fungi are *Aspergillus*, *Penicillium*, and *Alternaria* [43].

Several molecules with antibacterial activity have been isolated from marine fungi, with 195 strains isolated from beach, estuarine, and mangrove habitats [44]. The species isolated from mangroves have shown the strongest antibacterial activity against *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *E. coli*. Further activities from other fungi species are shown in Table 1.4 and Fig. 1.3.

Chevalone E is an example of an active compound obtained when the fungi *Aspergillus similanensis* is associated with other marine organism such as sponges. This metabolite does not present activity against *E. coli*, *S. aureus*, and *E. faecalis* but does show synergism with the antibiotic oxacillin against MRSA [45].

Deep-sea conditions (over 1000 m below the surface) are extreme, being characterized by complete darkness, low temperature, or high pressure, among other extreme factors. Nevertheless, marine fungi such as *Aspergillus candidus* grows in these conditions. This specie secretes the compound **terphenyl** that is active against *S. aureus*, *B. subtilis*, and *Vibrio* spp. showing antibacterial activity with 83.9–100% of inhibition [46].

Other compounds from deep-sea habitats include the prenylxanthenes **emerixanthenes A–D (1–4)**, isolated from *Emericella spand*, which show activity against *E. coli*, *K. pneumoniae*, *S. aureus*, *E. faecalis*, *Acinetobacter baumannii*, and *Aeromonas hydrophila*. The diameters of inhibition are between 1 and 3 mm. Compound D also shows mild antifungal activity against pathogens such as *Fusarium* spp., *Penicillium* spp., *Aspergillus niger*, *Rhizoctonia solani*, *Fusariumoxy sporium* f. sp. *niveum*, and *Fusariumoxy sporium* f. sp. *cucumeris*, with diameters of inhibition between 3 and 4 mm [47].

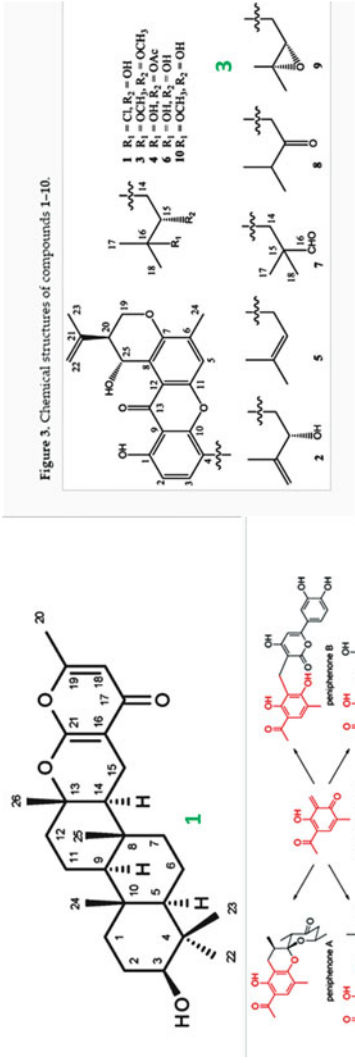
A few compounds show antibiofilm activity as in the case of **flavipesins A** from *Aspergillus flavipes*. The activity of this compound is comparable to that of penicillin in *S. aureus* biofilms. Flavipensis A can penetrate the biofilm matrix and decrease the number of living cells inside the mature biofilm from 390.6 to 97.7 µg/mL [48].

Antituberculosis agents have also been obtained from marine fungi. An example of these agents is **peniphenones A–D** from the mangrove fungus *Penicillium dipodomycicola* HN4-3A. The antituberculosis activity of these compounds against *Mycobacterium tuberculosis* is reflected by their LD50 values of between 0.16 and 1.37 µM [49].

Zopfiella marina is a facultative marine ascomycete that synthesizes two antimicrobial compounds, **zopfiellamide A (5)** and **zopfiellamide B**. Zopfiellamide A has greater antibacterial activity than zopfiellamide B. Nevertheless, the antibacterial activity of compound A shows MICs of 2–10 µg/ml against *Acinetobacter calcoaceticus* and moderate inhibitory effects against Gram-positive microorganisms such as *Arthrobacter citreus*, *Bacillus brevis*, *B. subtilis*, *B. licheniformis*, *Corynebacterium insidiosum*, *Micrococcus luteus*, *Mycobacterium phlei*, and *Streptomyces* spp. [50].

Table 1.4 Natural compounds with antimicrobial activity isolate from marine fungi

| Metabolite | Isolated from | Chemical scaffold | Activity against | References |
|-------------------|-----------------------------------|----------------------|--|------------|
| Chevalone E | <i>Aspergillus similanensis</i> | Aromatic hydrocarbon | <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> and <i>Enterococcus faecalis</i> | [45] |
| Terphenyl | <i>Aspergillus candidus</i> | | <i>S. aureus</i> , <i>Bacillus subtilis</i> and <i>Vibrio</i> spp. | [46] |
| Emenxanthones A–C | <i>Emericella spand</i> | | <i>E. coli</i> , <i>Klebsiella pneumoniae</i> , <i>S. aureus</i> , <i>E. faecalis</i> , <i>Acinetobacter baumannii</i> , and <i>Aeromonas hydrophila</i> | [47] |
| Emenxanthones D | <i>Emericella spand</i> | | <i>Fusarium</i> spp., <i>Penicillium</i> spp., <i>Aspergillus niger</i> , <i>Rhizoctonia solani</i> , <i>Fusariumoxysporium</i> f. Sp. <i>Niveum</i> and <i>Fusariumoxysporium</i> f. Sp. <i>cucumeris</i> | |
| Flavipesins A | <i>Aspergillus flavipes</i> | | <i>S. aureus</i> | [48] |
| Peniphenones A–D | <i>Penicillium dipodomycicola</i> | | <i>Mycobacterium tuberculosis</i> | [49] |
| Zopfiellamide A | <i>Zopfiella marina</i> | Alkaloid | <i>Acinetobacter calcoaceticus</i> , <i>Arthrobacter citreus</i> , <i>Bacillus brevis</i> , <i>B. subtilis</i> , <i>Bacillus licheniformis</i> , <i>Corynebacterium insidiosum</i> , <i>Micrococcus luteus</i> , <i>Mycobacterium phlei</i> , and <i>Streptomyces</i> spp. | [50] |



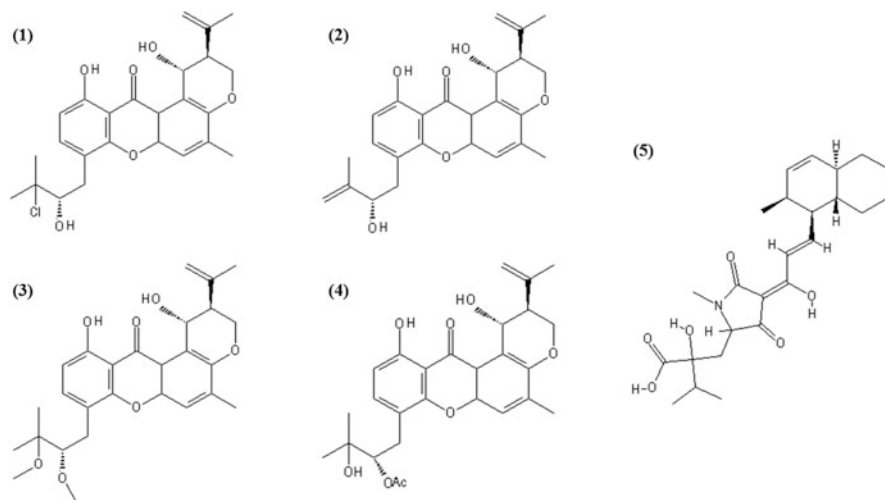


Fig. 1.3 Chemical structures of emerixanthones A (1), B (2), C (3), D (4), and zopfiellamide A (5). Emerixanthones A–C showed moderate antibacterial activity and emerixanthone D only antifungal activity. Zopfiellamide A showed a great antibacterial activity

Overall, there seems to be some evidence to indicate that new compounds from fungi that live in the sea or in estuarine or mangrove habitats are a promising source of new marine compounds. Nonetheless, it should not be forgotten that more than 10,000 species with promising metabolites remain in the oceans.

4 Sponge

Sponges (phylum Porifera) are sessile aquatic organisms, filter feeders, and the oldest multicellular animals. There are more than 8700 species including marine and nonmarine species according to the World Porifera Database [51]. They are located in all the seas and at all marine depths, adapting multiple forms and playing an important role in biogeochemical cycling [52]. In addition, sponges contribute to carbon flow from pelagic to benthic environments due to their feeding activity.

The body structure of a sponge consists in a set of specialized cells but is not organized into tissues or organs. There are two different layers: pinacoderm, the external layer formed by pinacocyte cells, and a gelatinous matrix called mesohyl, the inner layer or connective tissue. Sponges possess an aquiferous system with external pores (ostia) that allows the entry of food and oxygen. Once the fluid is filtered, waste is discarded by the oscula, the exit porus. All this water movement is facilitated thanks to choanocytes, flagellated collar cells which generate constant water flow through the sponge. The skeleton is made up of two types of skeletal

elements: the spicules, formed by siliceous or calcareous elements, and protein fibers called spongins.

The phylum Porifera is divided into four different groups:

- Calcarea or Calcispongiae sponges formed by calcium carbonate with simple one-, three- or four-rayed spicules. They are of limited size and live in shallow waters and coral reefs.
- Hexactinellida, glass sponges or Hyalospongiae made of siliceous spicules with six-rayed spicules arranged in three planes at right angles to each other. They live in deep water and areas of difficult access.
- Homoscleromorpha, the last to be added, is differentiated in a new clade separated from the Demospongiae and is composed of two families, Oscarellidae and Plakinidae [53].
- Demospongiae which may be supported by either siliceous mineral spicules (never six-rayed) or spongin protein fibers, although some sponges might have none or both. This class is the largest group with more than 95% (7300 species) of known species and includes large populations in freshwater and bath sponges.

Marine sponges are sessile organisms, and their defense mechanism against bacteria, eukaryotic organisms, or viruses is based on the production of a diverse range of secondary metabolite products, allowing efficient chemical protection. Most of these compounds are the result of either the sponge or the microorganisms that live on it [54]. The relevance of marine sponges and their importance in the discovery of new compounds has been demonstrated in the literature. Scientists are actively seeking potential molecules, and only in the last 7 years, more than 620 novel molecular structures have been identified [55]. Even though most of those compounds have shown biological activity in the laboratory, their pharmacological use and synthesis might be expensive due to their chemical complexity.

Sponges are the richest marine phylum with biologically active secondary metabolites [56]. These secondary metabolites have a vast applicability against different diseases such as cancer, malaria, and tuberculosis, among others [57]. The range of action and chemical structure of the sponges already described as antimicrobial agents differ, with the most common being the **alkaloids**, **terpenoids**, and **peptides**.

Axinellamines (Fig. 1.4) **A** (**1**) and **B** (**2**) are alkaloids with a complex polycyclic skeleton isolated from the sponge *Axinella* sp. Their biological activity was first described in 1999. They exhibit activity against several Gram-positive and Gram-negative bacteria. The antibacterial activity of compound **A** shows MICs of 0.5–16 µg/ml and compound **B** of 1–32 µg/ml. Moreover, axinellamines cause membrane destabilization in *E. coli* [58]. Axinellamines **A** and **B** have recently been chemically synthesized using a new asymmetric synthesis method [59].

Alkaloids such as **oroidin** (isolated from *Agelas oroides*) have been described activity against *S. aureus* and *E. faecalis* [60]. **Sceptrin**, **dibromosceptrin**, and **bromoageliferin** (from *Agelas conifera*) show antimicrobial activity against *S. aureus* and *A. baumannii* (Table 1.5). This evidence has encouraged scientists

Fig. 1.4 Structure of axinellamines. Axinellamines showed activity against several Gram-positive and Gram-negative bacteria

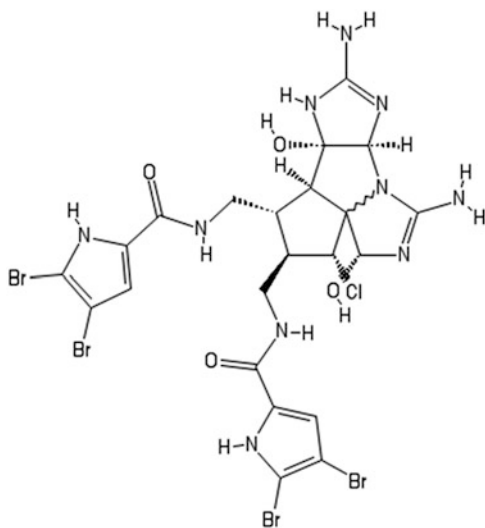


Table 1.5 Natural compounds with antimicrobial activity isolate from sponge

| Metabolite | Isolated from | Chemical scaffold | Activity against | References |
|---|------------------------|-------------------|---|------------|
| Axinellamines A and B | <i>Axinella</i> sp | Alkaloid | <i>Escherichia coli</i> , <i>Pseudomona aeruginosa</i> , MRSA ^a , <i>Staphylococcus epidermidis</i> , <i>Enterococcus faecalis</i> , <i>Corynebacterium efficiens</i> , <i>Yersinia pestis</i> | [58] |
| Oroidin | <i>Agelas oroides</i> | Alkaloid | <i>S. aureus</i> and <i>E. faecalis</i> | [60] |
| Sceptrin, Dibromosceptrin and Bromoageliferin | <i>Agelas conifera</i> | Alkaloid | <i>S. aureus</i> and <i>Acinetobacter baumannii</i> | [61] |

^aMethicillin resistant *Staphylococcus aureus*

to continue with their research on these alkaloids and to perform assays against Gram-negative and Gram-positive bacteria [61].

In the search for new metabolites with antibacterial properties, sponges are promising organisms, and continued investigation of these organisms will hopefully lead to the discovery of powerful new biomolecules to improve the fight against multidrug resistance.

5 Cnidaria

The phylum Cnidaria includes more than 10,000 species which are widespread throughout the seas, with only a few species having been found in freshwater [62]. These organisms are usually found in shallow warm waters. They have two embryonic cell layers, ectoderm and mesoderm (diploblastic organisms), that form the epidermidis and gut cavity, respectively, in adult organisms. Cnidarians are organisms with radial symmetry although they can also exhibit directional asymmetry or bilateral symmetry. These organisms play an important role in inorganic carbon precipitation in reef-building corals [63]. This phylum has been divided into five classes: Anthozoa (including corals), Cubozoa (cube jellyfishes), Hydrozoa (the most variable class), Scyphozoa (true jellyfishes), and Staurozoa (the most recently characterized class) [64].

Cnidarians are an interesting group because of their venomous properties. Their chemical products have been reported to cause mainly local damage, but some species, such as Australian species, produce several cardiac or neurological problems. Some proteins are considered to be responsible for the hemolytic effects of these organisms due to alterations in cell permeability resulting in ion transport, pore formation, oxidative stress, or osmotic lysis [65].

Cnidarians lack an external structure to protect them from other organisms. Thus, secondary metabolites, which can be used against bacteria, have been developed from the need for self-protection. Among these excreted products are **sesquiterpenes, diterpenoids, steroids, terpenes, and peptides** that present antimicrobial activity.

The chemistry of the cnidarians is dominated by metabolites derived from terpene biosynthesis. **Terpenes** are a large diverse class of organic compounds biosynthetically derived from isoprene units. Some authors have described terpenes and terpenoids as the same compound. Nevertheless, terpenoids can be considered as modified terpenes. Terpenes may be derived from terpenoids, diterpenoids, sesquiterpenoids, and cembranoids.

Bipinnapterolide B is a terpenoid that has been isolated from the octocoral *Pseudopterogorgia bipinnata*. The activity of bipinnapterolide B has been tested “in vitro” against *Mycobacterium tuberculosis* showing inhibition in 66% of the samples at a concentration of 128 mg/mL [66].

Xeniolide I is a diterpenoid that has been isolated from the cnidarian *Xenia novaebritanniae*. It has shown antibacterial activity in *E. coli* ATCC and *B. subtilis* at a concentration of 1.25 mg/mL [67]. Other diterpenes isolated from *P. elisabethae* and their posterior derivatives have shown activity against the resistant *M. tuberculosis* H37Rv strain [68]. Another four diterpenoids (**elisabethin E, elisabethin F, pseudopterodin P, and pseudopterodin Q**) have been described, but only P and Q exhibit selective activity against three Gram-positive bacteria (*Streptococcus pyogenes*, *S. aureus*, and *E. faecalis* [69].

One diterpene, a compound composed of two terpene units, has been related to antimycobacterial activity. This compound is the **homopseudopteroxazole** that induces 80% of *M. tuberculosis* inhibition with a MIC value of 12.5 µg/mL [70]. The diterpene **pseudopterosins U**, isolated from *P. elisabethae*, exhibits activity against *S. aureus* and *E. faecalis* with an IC₅₀ of 2.97 µM and 3.19 µM, respectively [71].

Three new phenolic bisabolane-type **sesquiterpenoids** from *Dichotella gemmacea* have been isolated and show moderate antimicrobial activity against *S. aureus* and MRSA [72].

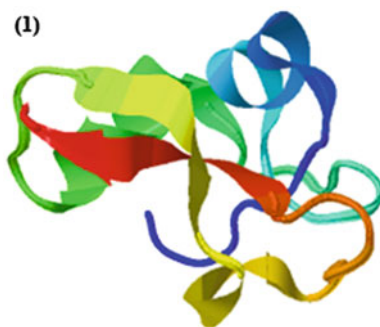
Soft corals also yield a variety of **steroids**. **Litosterol** and **nephasterols B and C** have been isolated from Red Sea *Nephthea* spp. Litosterol and nephasterols C inhibit the growth of *M. tuberculosis* with MICs of 3.13 and 12.5 mg/mL, respectively [73].

The cembranoid **sarcophytolide** was isolated from soft corals of the genus *Sarcophyton*. This cembranoid shows activity against *S. aureus* with a MIC of 125 mg/mL [74].

Finally, cnidarians are able to synthesize peptides. Thus, **aurelin**, a new peptide with 40 residues purified from the mesoglea of the jellyfish *Aurelia aurita* (order Semaestomeae), is considered a promising peptide. Its activity consists in its structural similarity with the defensins and K⁺ channel blockers of sea anemones. This compound shows activity against Gram-positive (*L. monocytogenes*) and Gram-negative (*E. coli*) bacteria [75].

The cnidarian *Hydra magnipapillata* produces a large number of peptides (**hydramacin-1**, **periculin-1**, **arminin**, and **kazal-2**), which show activity against a wide range of Gram-positive and Gram-negative bacteria. **Hydramacin-1** (Fig. 1.5) has demonstrated the greatest activity, with concentrations less than 1 µM and killing 99.9% of bacteria against Gram-negative species such as *E. coli*, *K. pneumoniae*, *K. oxytoca*, *S. typhimurium*, *Citrobacter freundii*, *Enterobacter cloacae*, and *Yersinia enterocolitica*. On the other hand, the highest activity against Gram-positive pathogens was observed in *Staphylococcus haemolyticus* with a concentration of 1.8 µM. Its structure is quite similar to the scorpion oxin-like superfamily [76].

Fig. 1.5 Hydramacin-1 peptide with the greatest antibacterial activity isolated from *Hydra magnipapillata*



The peptide **periculin-1** has shown antimicrobial activity against *Bacillus megaterium* at concentrations ranging between 0.2 and 0.4 μM [77].

Arminin is another peptide synthesized by cnidarians and consists of a strongly positively charged C-terminal region and a highly negatively charged N-terminal region. This peptide has shown antimicrobial activity against *E. coli*, *Bacillus megaterium*, and *S. aureus* at concentrations of less than 0.5 μM . In addition, MRSA is inhibited at concentrations of 0.4–0.8 μM and a LD 90 of 0.2 μM . Arminins tested under physiological conditions did not show adverse effects to human erythrocytes. All of these properties make arminin an interesting antibacterial agent [78].

Finally, the peptide **kazal-2**, a serine protease inhibitor, has shown antimicrobial activity against *S. aureus* at concentrations of 0.7–0.8 μM [79]. A summary of the activity and derived species of the aforementioned compounds is illustrated in Table 1.6.

6 Bryozoa

The Bryozoa or Ectoprocta phylum includes 5869 species which are found to be widespread in marine water, brackish-water, and freshwater [80]. They live in sessile colonies, formed by several individual units called zooids, and are colonial filter feeders. The colonies provide an external skeleton or zoecium that may be formed by chitin and gelatin and might include calcium.

Ectoprocta are one of the most abundant and diverse members of Antarctic benthos. Approximately 55% of all bryozoan species are found below a depth of 40 m, and approximately 27% of these species live in very deep water (>700 m) [81]. To date, the ecology of the Antarctica remains poorly studied because of few groups that have investigated the Antarctic benthos [82].

In recent years, there has been increasing interest in secondary metabolites of the Bryozoa species.

The majority of bryozoan metabolites isolated to date are **alkaloids**, **ceramides**, and **sterols**, being alkaloids the group with the most active compounds discovered (Table 1.7).

Some examples of alkaloids from Bryozoa include the **amathaspiramides** (isolated from *Amathia wilsoni*), **euthyroideones** (from *Euthyroides episcopalis*), and **pterocellins** (from *Pterocella vesiculosa*). Amathaspiramide A has shown modest antibacterial activity against *B. subtilis* but has not been tested against marine bacteria which may be a more effective indicator of its activity [83]. A new alkaloid, **5-bromo-8-methoxy-1-methyl-carboline**, was isolated from the bryozoan *Pterocella vesiculosa*. This new alkaloid has demonstrated antimicrobial activity against *B. subtilis* (MIC = 2–4 $\mu\text{g/mL}$) and *C. albicans* and *Trichophyton mentagrophytes* (MIC of 4–5 $\mu\text{g/mL}$) [84].

Other important metabolites with antibacterial activity extracted from bryozoan are terpenes. Thus, **eusynstyelamide F** was isolated from *Tegella* cf. *spitzbergensis*

Table 1.6 Natural compounds and peptides with antimicrobial activity isolate from Cnidaria

| Metabolite | Isolated from | Chemical scaffold | Activity against | References |
|---|--------------------------------------|-------------------|--|------------|
| Bipinnapterolide B | <i>Pseudopterogorgia bipinnata</i> | Terpenoid | <i>Mycobacterium tuberculosis</i> | [66] |
| Xeniolide I | <i>Xenia novaebritanniae</i> | Diterpenoid | <i>Escherichia coli</i> and <i>Bacillus subtilis</i> | [67] |
| 21-((1 <i>H</i> -imidazol-5-yl) methyl)-pseudopteroxazole | <i>Pseudopterogorgia elisabethae</i> | Diterpenoid | <i>M. tuberculosis</i> | [68] |
| Elisabethin P, Q | <i>Pseudopterogorgia elisabethae</i> | Diterpenoid | <i>Streptococcus pyogenes</i> , <i>Staphylococcus aureus</i> and <i>Enterococcus faecalis</i> | [69] |
| Homopseudopteroxazole | <i>Pseudopterogorgia elisabethae</i> | Diterpenoid | <i>M. tuberculosis</i> | [70] |
| Pseudopterostins U | <i>Pseudopterogorgia elisabethae</i> | Steroids | <i>S. aureus</i> and <i>E. faecalis</i> | [71] |
| Litosterol and Nephasterols B–C | <i>Nephthea</i> spp. | | <i>M. tuberculosis</i> | |
| Sarcophytolide | Genus <i>Sarcophyton</i> | Cembranoid | <i>S. aureus</i> | [74] |
| Aurelin | <i>Aurelia aurita</i> | Peptide | <i>Listeria monocytogenes</i> and <i>E. coli</i> | [75] |
| Hydramacin-1 | <i>Hydra magnipapillata</i> | Peptide | <i>E. coli</i> , <i>Klebsiella pneumoniae</i> , <i>K. oxytoca</i> , <i>Salmonella typhimurium</i> , <i>Citrobacter freundii</i> , <i>Enterobacter cloacae</i> , <i>Yersinia enterocolitica</i> and <i>Staphylococcus hemolyticus</i> | [76] |
| Periculin-1 | <i>Hydra magnipapillata</i> | Peptide | <i>Bacillus megaterium</i> | [77] |
| Arminin | <i>Hydra magnipapillata</i> | Peptide | <i>E. coli</i> , <i>B. megaterium</i> and <i>S. aureus</i> ^a | [78] |
| Kazal-2 | <i>Hydra magnipapillata</i> | Peptide | <i>S. aureus</i> | [79] |

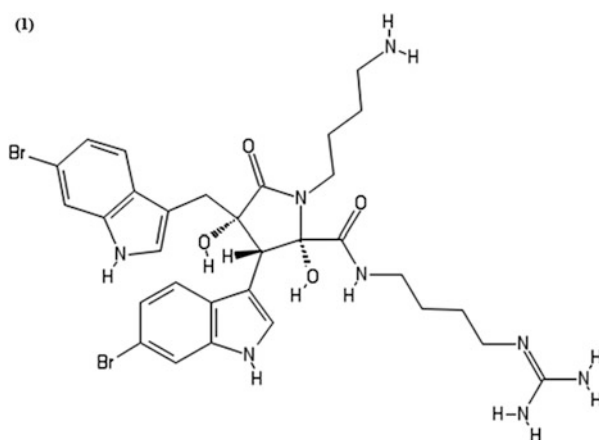
^aMethicillin resistant *Staphylococcus aureus*

Table 1.7 Natural compounds with antimicrobial activity isolate from Bryozoa

| Metabolite | Isolated from | Chemical scaffold | Activity | References |
|--------------------------------------|--|-------------------|--|------------|
| Amathaspiramide A | <i>Amathia wilsoni</i> | Alkaloid | <i>Bacillus subtilis</i> | [83] |
| 5-Bromo-8-methoxy-1-methyl-carboline | <i>Pterocella vesiculosa</i> | Alkaloid | <i>B. subtilis</i> , <i>Candida albicans</i> and <i>Trichophyton mentagrophytes</i> | [84] |
| Eusynstyelamide F | <i>Tegella</i> cf. <i>spitzbergensis</i> | Alkaloid? | MRSA ^a , <i>Escherichia coli</i> , <i>Pseudomona aeruginosa</i> and <i>Corynebacterium glutamicum</i> | [85] |

^aMethicillin resistant *Staphylococcus aureus*

Fig. 1.6 Structure of eusynstyelamide F, isolated from *Tegella* cf. *spitzbergensis*, exhibited stronger antibacterial activity



together with three other compounds (**eusynstyelamides D and E** and **ent-eusynstyelamide B**) (Fig. 1.6). However, eusynstyelamide F showed higher activity compared to the others. Their range of action was tested in *S. aureus*, *E. coli*, *P. aeruginosa*, *Corynebacterium glutamicum*, and MRSA, with eusynstyelamide F being more active against *S. aureus* and *C. glutamicum* with a MIC of 6.25 µg/mL and 12.5 µg/mL, respectively [85].

Nowadays, only a few compounds are in experimental study phases compared to other phylum such as sponges. Some reasons for this may be the problems associated with working with these compounds. To the naked eye, phylum Bryozoa is commonly confused with hydroids and other species. Moreover, they are located in places of difficult accessibility since they live in deep areas, in which there is a lack of biomass available for extraction.

7 Mollusca

The phylum Mollusca is one of the most attractive invertebrate phyla, and they are widely distributed worldwide, having many representatives in the marine and estuarine ecosystems including slugs, whelks, clams, mussels, oysters, scallops, squids, and octopods [86–88]. Molluscs are a highly diverse group in size, anatomical structure, behavior, and habitat. Around 10,000 extant species and another 70,000 fossil molluscs have been described. However, many species have not yet been identified, making these the largest marine phylum with about 23% of all named marine organisms. Representatives of this phylum live in an enormous range of habitats including marine, freshwater, and terrestrial environments [89].

Taxonomically, there are three well-known types of molluscs, including clams and mussels (Bivalvia, 20,000 species), snails and slugs (Gastropoda, 70,000 species), and squids and octopuses (Cephalopoda, 900 species). In addition, there are four other classes: Chitons (Polyplacophora, 1000 species), tusk shells (Scaphopoda, 500 species), *Neopilina* and its relatives (Monoplacophora, 25 species), and the vermiform and primitive Aplacophora (200 species). It is of note that the members of these seven classes are phenotypically very different but remarkably similar in terms of their organizational model [86, 88, 90].

The many relationships between humans and molluscs mainly involve them as sources of food, money, jewels, and art, but molluscs play an important role in science as model organisms in the study of neurobiology and evolutionary biology and as a source of bioactive metabolites [90].

Among marine invertebrates, many classes of bioactive compounds from different species of molluscs have exhibited antitumor, antileukemic, antibacterial, and antiviral activity [87, 91]. Another important feature of some species of molluscs, including gastropod and bivalves, is that they are widely used as biomarkers of environmental pollution by infection and heavy metal exposure [92, 93].

Concerning the antibacterial activity related to this phylum, several molecules have been studied as shown in the Table 1.8 and Fig. 1.7. Thus, **hexadecylglycerol**, which is isolated from the digestive gland and skin of *Archidoris montereyensis* (nudibranch), has shown “in vitro” antibacterial activity against *S. aureus* and *B. subtilis* [94, 95]. The same compound obtained from *Perna viridis* (bivalve) extracts showed the highest activity against *E. coli* K1, *A. baumannii*, and *P. aeruginosa* [96]. Furthermore, there have been many studies on antimicrobial compounds from gastropods, using different samples including hemolymph, egg masses, or whole body extracts [87, 89].

Other molecules, such as **diemensin A–B**, **kelletin I–II (1, 2)**, and **chromodorolide A (3)**, have been isolated from extracts of the gastropod species *Siphonaria diemenensis*, *Kelletia kelletii*, and *Chromodoris* sp., respectively. All these molecules inhibit the growth of Gram-positive and Gram-negative bacteria as well as the cell division of sea urchin eggs and the growth of L1 leukemia [94, 96–98].

Interesting compounds active against human pathogens have been isolated as a result of the relationship between a coral and *Trochus tentorium* [99]. Crude whole

Table 1.8 Natural compounds with antimicrobial activity isolate from Mollusca

| Metabolite | Isolate from | Chemical scaffold | Activity against | References |
|---------------------------|--|-------------------|--|-------------|
| Hexadecylglycerol | <i>Archidoris montereyensis</i> | | <i>Staphylococcus aureus</i> and <i>Bacillus subtilis</i> | [94–96] |
| | <i>Perna viridis</i> | | <i>Escherichia coli</i> , <i>Acinetobacter baumannii</i> and <i>Pseudomonas aeruginosa</i> | |
| Diemensin A–B | <i>Siphonaria diemensis</i> | | Gram-positive and Gram-negative bacteria | [94, 96–98] |
| Kelletinin I–II | <i>Kelletia kelletii</i> | | Gram-positive and Gram-negative bacteria | |
| Chromodorolide A | <i>Chromocloris</i> sp. | | Gram-positive and Gram-negative bacteria | [94, 96–98] |
| Crude whole body extracts | <i>Trochus tentorium</i> | | <i>S. pneumoniae</i> and <i>K. pneumoniae</i> , and lower activity against <i>E. coli</i> , <i>S. pneumoniae</i> , <i>S. aureus</i> and <i>V. cholerae</i> | [99] |
| Polysaccharides | <i>Sepia aculeata</i> and <i>Sepia brevi</i> | | <i>B. subtilis</i> , <i>E. coli</i> , <i>K. pneumoniae</i> , <i>V. cholerae</i> , <i>V. parahaemolyticus</i> , <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>Salmonella</i> spp. and four fungal species (<i>Candida</i> sp., <i>Rhizopus</i> sp., <i>Aspergillus flavus</i> , and <i>A. fumigatus</i>) | [100, 101] |

body extracts using solvents such as acetone, ethyl acetate, dichloromethane, and methanol presented high activity against *S. pneumoniae* and *K. pneumoniae* and lower activity against *E. coli*, *S. pneumoniae*, *S. aureus*, and *V. cholerae*.

The marine mollusc *Melo melo* is a potential source of bioactive antibacterial and antifungal compounds. Kanagasabapathy et al. [102] observed that methanol extract of mucus, nerve tissue, body tissue, and kidney showed antimicrobial activity against *K. pneumoniae*. Protein studies such as thin-layer chromatography (TLC) and SDS-PAGE were used to determine the presence of peptides or amide groups. Thus, four proteins of 14, 17, 22, and 45 kDa were found to provide antimicrobial activity. Similar results were reported by Periyasamy et al. [87] who found many proteins between 2 and 110 kDa in weight from *B. spirata* muscle presenting antibacterial activity against pathogenic microbial forms. However, some novel and uncharacterized mechanisms of action that might ultimately benefit from the ongoing global search for clinically useful antimicrobial agents need to be explored to explain the antimicrobial activity of *B. spirata*.

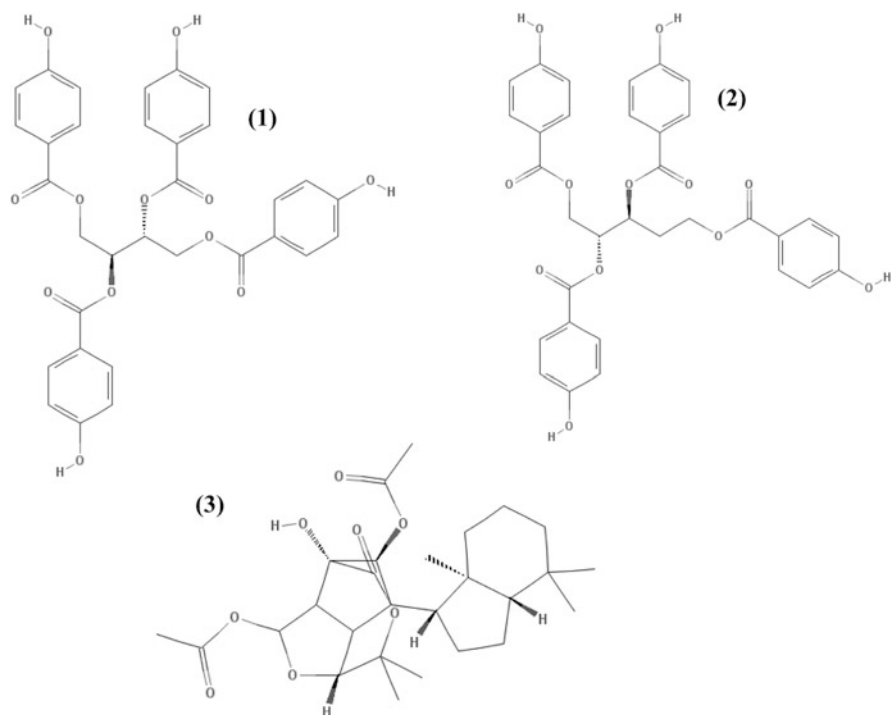


Fig. 1.7 Structures of secondary metabolites with antimicrobial activity (1) kellethinin I, (2) kellethinin II, and (3) chromodorolide A isolate from *Siphonaria diemenensis*, *Kelletia kelletii*, and *Chromodoris* sp., respectively. (Gastropod, Mollusca)

Four novel antimicrobial peptides from *Rapana venosa* have been detected. This gastropod of Asiatic origin is a successful invasive organism, which represents a serious threat to the malacological resources of marine waters worldwide. However, it has also demonstrated to be an interesting source of antimicrobial peptides. **Proline-rich peptides**, with molecular masses of between 3 and 95 kDa, have been isolated from hemolymph samples, showing strong antimicrobial activity against *S. aureus* and *K. pneumoniae* [103, 104].

Cephalopods are another interesting group to study. This group has the highest number of extinct species (~4000). For this reason, the living species are considered to be true survivors [90, 105]. These species have developed interesting defense mechanisms including high melanin production that can be distributed by ejected water as jet propulsion. The ejected cloud of melanin is usually mixed, upon expulsion, with mucus forming a thick cloud and resulting in visual and chemosensory impairment of the predator, like a smoke screen, avoiding their predation [88, 106]. In addition, cephalopods are important as a food source as well as animal models in scientific investigations, being a storehouse of many biologically important substances [107, 108].

Most studies on cephalopods are focused on the potential use of **polysaccharides** as potent antibacterial molecules. Shanmugam et al. [101] investigated the antibacterial and antifungal activity of extracts from the cuttle of two cephalopod species, *Sepia aculeata* and *Sepia brevimana*, against eight species of bacteria (*B. subtilis*, *E. coli*, *K. pneumoniae*, *V. cholerae*, *V. parahaemolyticus*, *S. aureus*, *P. aeruginosa*, *Salmonella* spp.) and four fungal species (*Candida* sp., *Rhizopus* sp., *Aspergillus flavus*, and *A. fumigatus*). They found that their potent activity is due to the presence of polysaccharides, and that this activity is directly related to the concentration of the extracts. In addition, similar results were found using methanolic extracts and fractionated polysaccharides from other cephalopod species such as *Loligo duvauceli* [100]. This same research group reported that **glycosaminoglycans** (GAGs) or **mucopolysaccharides** from a small cephalopod species named *Euprymna berryi* showed very good activity against five pathogenic bacteria and four fungal strains including *Shigella* spp. and *E. coli*. In addition, the mucopolysaccharides showed potent antifungal activity against *C. albicans* and *A. fumigatus* [109].

8 Annelida

The Annelida phylum is made up of **bilaterally symmetrical** animals with bodies that consist of three regions. The body is divided into parts or similar segments also called metamerisms, which are arranged in a linear series along the anteroposterior axis of the body. Annelids are of particular phylogenetic interest, because they are the first coelomates with a complete digestive tract, a closed circulatory system with hemoglobin in the plasma to carry oxygen and carbon dioxide, and their nervous system is developed and they have an excretory structure [86, 88].

The diversity of annelids comprises ringed or segmented worms including rag worms, earthworms, and leeches, with over 17,000 living species ranging in size from less than 1 mm to well over 3 m (Gippsland earthworm and *Amyntas mekongianus*, respectively) [88, 110].

Annelids have been divided into three taxonomic classes (Polychaeta, Oligochaeta, and Hirudinea). However, more recent phylogenetic investigations only consider two taxonomic classes, Polychaeta and Clitellata, with the latter being subdivided into the subclasses Oligochaeta and Hirudinea [88]. The Polychaeta class is the largest and most diverse group, with more than 12,000, mainly marine, species having been described. The Clitellata class (of about 5000 species) includes the subclass Oligochaeta, which is composed of freshwater annelids, earthworms and a variety of marine species, and the subclass Hirudinea which the best-known members are leeches. Marine species are mostly blood-sucking parasites, mostly of fish, while most freshwater species are predators [86, 111].

The annelids have successfully invaded all habitats where sufficient water is available. They are important players in the benthic communities as they are critical for the biomass of seashore, estuary, freshwater, and terrestrial soils. Moreover, they

occupy the central position in the trophic networks and are a major food source for fishes, birds, and terrestrial fauna [112]. The importance of this group of invertebrates not only lies in their great diversity and evolutionary success but also as a source of novel molecules of biological interest, highlighting the potential use of biomarkers to monitor the influence of environmental perturbation and as a potential source of new molecules with antimicrobial activity, synthesized in response to defense mechanisms to fight pathogens [112–114].

The immunity system of annelids is an attractive object of study for comparative immunologists, because, despite being primitive, they have developed both cellular and humoral innate response against pathogens [115]. This immune system is very conserved throughout the animal kingdom and is the most ancient first line of immune protection for invertebrates. Numerous components participate in innate immune response, including important humoral factors such as antimicrobial proteins and antimicrobial peptides (AMPs) as well as cells that kill invasive pathogens using phagocytic or cytotoxic systems [112, 115, 116].

In the last years, great emphasis has been given to the study of compounds with antimicrobial activity obtained from marine annelids, especially from Polychaete worms. In this sense, the most studied antimicrobial proteins are **lysozymes** [112]. The first bacteriolytic molecules were identified as **lysozyme-like** of 14 kDa from *Nereis diversicolor* produced by a type of cells named G1 and detected in the coelomic fluid about 24 h after infection with bacteria. This protein has bacteriostatic activity, cleaving the B-1-4 bonds between N-acetylglucosamine and N-acetylmuramic acid of peptidoglycan and providing access of the latter to the bacterial cell wall of Gram-positive bacteria, being mostly active against this bacterial type [112].

Other antimicrobial factors have been isolated from coelomic fluid from *Glycera dibranchiata* (Polychaeta, Glyceridae), probably as part of the organisms defense against bacterial infection. They show very good activity against Gram-negative bacteria, including *S. marcescens*, *P. aeruginosa*, and *E. coli* strains [117].

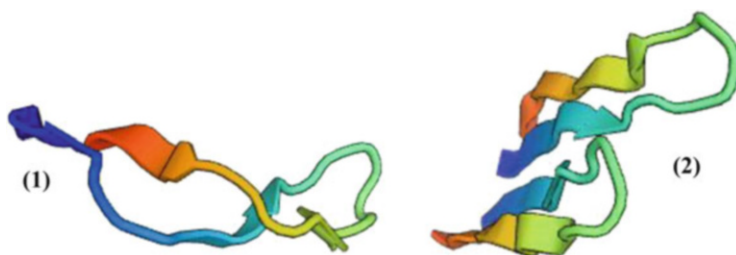
On the other hand, numerous studies on the effectors of the innate immune system have demonstrated the contribution of antimicrobial peptides (AMPs) to host defense [112, 114] (Table 1.9; Fig. 1.8). **Antimicrobial peptides** are small well-conserved molecules among strains. Based on their structural features, five major classes have been defined:

1. Linear α -helical peptides without cysteines. The prototypes of this family are the cecropins.
2. Loop-forming peptides containing a unique disulfide bond. These are mainly isolated from amphibian skin.
3. Open-ended cyclic cysteine-rich peptides, being defensins the most widespread.
4. Linear peptides containing a high proportion of one or two amino acids such as indolicidin.
5. Peptides derived from larger molecules known to exert multiple functions [112, 114, 121].

The principal source of AMPs in annelids has been found in three species of marine Polychaetes, *Arenicola marina*, *Nereis diversicolor*, and *Perinereis aibuhitensis*. **Perinerin** is the bioactive compound isolated from the clam worm

Table 1.9 Peptides with antimicrobial activity isolate from Annelida

| Metabolite | Isolated from | Chemical scaffold | Activity against | References |
|-----------------|----------------------------|-------------------|--|------------|
| Perinerin | <i>Arenicola marina</i> | Peptide | Gram positive and negative bacteria | [118] |
| Arenicins (1–2) | <i>Arenicola marina</i> | Peptide | <i>E. coli</i> | [112, 119] |
| Hedistin | <i>Nereis diversicolor</i> | Peptide | Gram-positive bacteria, gram-negative bacteria (marine bacteria as <i>Vibrio alginolyticus</i>) | [114, 120] |

**Fig. 1.8** Structures of two novel antimicrobial peptides isolate from lugworm *Arenicola marina* (marine Annelida). (1) Arenicin 1 and (2) arenicin 2

Perinereis aibuhitensis [118]. This peptide consists of 51 amino acids, including 4 cysteine residues presumably implicated in 2 disulfide bridges. Antimicrobial assays show high activity against Gram-negative and Gram-positive bacteria and fungi at physiological concentrations. Moreover, perinerin has shown rapid bactericidal activity against *Bacillus megaterium* during the exponential phase, suggesting a pore-forming activity [112, 113, 122]. It has also been reported that the coelomic fluid of *Perinereis cultrifera* presents potent antibacterial and antifungal activity [123].

Two novel AMPs of interest have been described in the lugworm *Arenicola marina* [119]. **Arenicin-1 (1)** and **arenicin-2 (2)** are amphipathic peptides formed by 21 residues, and each isoform possesses 2 cysteine residues implicated in 1 disulfide bond. Both isoforms show equal activity against fungi, as well as Gram-positive and Gram-negative bacteria [112]. Thus, the use of concentrations of about 5 μM of arenicin kills *E. coli* in 5 min, producing a rapid membrane permeabilization accompanied by peptide intercalation into the bilayer and the release of cytoplasmic material [113, 115, 119].

The most important AMP isolated from marine annelids is **hedistin**. It is purified from the rag worm *Nereis diversicolor*. Hedistin is a linear peptide of 22 amino acids that contains bromotryptophan residues. Moreover, the primary structure of hedistin includes a C-terminal amidation that could increase the net charge and, consequently, the electrostatic attraction to target membranes like the negatively charged bacterial membrane. This suggests that this C-terminal amidation of hedistin might be implicated in its bactericidal properties [113, 120, 124]. Hedistin is active against

a large spectrum of Gram-positive bacteria, but, interestingly, it is also very active against Gram-negative bacteria, especially the marine bacteria *V. alginolyticus* which is a causative agent of episodes of mass mortality of larvae of bivalves in commercial hatcheries. This could be attributable to the capacity of *Vibrio* to degrade the native cuticle collagen of *Nereis*. Vibrial collagenase helps bacteria to enter the worm body, making the mechanical defense barrier of the cuticle inefficient against *Vibrio* invasion [114]. No cytotoxicity of either hedistin forms was observed against *Nereis caelomocytes* [120].

9 Echinodermata

Echinoderms are deuterostome invertebrates with a phylogenetic position closely related to chordates and hemichordates [125]. The phylum contains about 7000 extant species, including sea lilies, feather stars, brittle stars, starfish, sea urchins, sand dollars, and sea cucumbers, as well as about 13,000 extinct species with a fossil registry from the early Cambrian period [86, 88]. These animals have a unique morphology that includes a water vascular system and a pentamerously symmetrical body shape in adult invertebrates that have an endoskeleton consisting of magnesium calcite [88, 126, 127].

Taxonomically, the phylum Echinodermata is categorized into two subphyla, the Pelmatozoa, including the class of Crinoidea (sea lilies and feather stars, 625 recent species), and the Eleutherozoa which comprises the classes of Asterozoa (starfish, 1500 recent species), Echinozoa (sea urchins, sand dollars, and sea biscuits, 950 recent species), Holothurozoa (sea cucumbers, 1150 recent species), and Ophiurozoa (brittle stars, 2000 recent species) [86, 88, 127, 128].

Echinoderms are exclusively marine organisms. They are generally benthic animals found in shallow water and occupy habitats from the intertidal zone to the deep sea. The size of the echinoderms ranges from small sea cucumbers and brittle stars of 1 cm to starfish that surpass 1 m in diameter and sea cucumbers of up to 2 m in length [86, 88].

The environment where the echinoderms live is exposed to relatively high amount of bacteria, fungi, viruses, and parasites, many of which are potentially pathogenic. The survival of these organisms relies on the production of efficient antimicrobial components to defend themselves against microbial infections and fouling [126, 129, 130]. As invertebrates in general, echinoderms have an innate immune system, but as all other invertebrates, they lack a vertebrate-type adaptive immune system [126, 131]. Principally, the immune response occurs in the coelomic fluid mediated by coelomocytes, in which a series of cells are activated for their defense (phagocytes, vibratile cells, colorless and red spherule cells). In addition, compounds like complement factors, lectins, lysozymes, and AMPs have been identified as participants in the host defense system [126, 129, 132, 133].

In this regard, there are many studies searching for new antimicrobials in a great variety of echinoderm species. For example, antimicrobial activity has been reported

Table 1.10 Natural compounds and Peptides with antimicrobial activity isolate from Echinodermata

| Metabolite | Isolated from | Chemical scaffold | Activity against | References |
|-----------------|--|-------------------|---|------------|
| Echinochrome-A | <i>Echinus esculentus</i> | Quinona pigments | Gram positive and negative bacteria | [139, 140] |
| Spinochromes D | <i>Echinus esculentus</i> | Quinona pigments | Gram positive and negative bacteria | [139, 140] |
| Strongylocins | <i>Strongylocentrotus droebachiensis</i> | Peptide | Marine fish pathogens (<i>Listonella anguillarum</i>) | [141] |
| Centrocins 1- 2 | <i>Strongylocentrotus droebachiensis</i> | Peptide | Gram positive and negative bacteria | [142, 143] |
| EeCentrocins | <i>E. esculentus</i> | Peptide | Gram-negative bacteria, gram-positive bacteria and against fungi | [130] |
| 5-cc | <i>Paracentrotus lividus</i> | Peptide | Antistaphylococcal bacteria <i>S. epidermidis</i> (inhibitor of formation young and mature biofilm) | [144, 145] |

in several species of echinoderms collected from the Gulf of California, Mexico, the Caribbean, and the coast of Norway [62, 134]. A variety of antimicrobial factors, including **steroidal glycosides**, **polyhydroxylated sterols**, **naphthoquinone pigments** (Service and Wardlaw 1984), **lysozymes** [135, 136], **complement-like substances** [137], and **antimicrobial peptides** [138] (Table 1.10; Fig. 1.9), have been isolated from sea cucumbers. Additionally, six new **cytotoxic triterpene glycosides** from *Mensamaria intercedens* lampert, displaying a broad range of antibacterial, antifungal, and cytotoxic activity, have been identified by extensive spectroscopic analysis (NMR and ESIMS) and chemical methods [146].

Various extracts from the gut and gonads of the sea urchin *Tripneustes gratilla* have shown antimicrobial activity. Compared to chloroform extracts, methanol extracts exhibit higher activity against Gram-positive and Gram-negative bacteria as well as against selected fungal species such as *Penicillium* spp. Furthermore, tissues tested in different reports showed high antibacterial activity [147] and, interestingly, did not exhibit hemolytic activity against human erythrocytes, being a very good early indicator of potentially low toxicity toward mammalian/human cells [134]. In contrast, other parts of the body of sea cucumbers such as the Cuvierian organs did not present activity [132].

Other important metabolites with biological activity from echinoderms are **saponins**. These compounds from sea stars and sea cucumbers have been extensively studied, but were not found to be useful as drugs because of their tendency to cause cell lysis [17]. On the other hand, **asterosaponins** are other metabolites that could play an important role in chemical defense to protect the starfish from parasites and predators. In addition, they have hemolytic, antineoplastic, cytotoxic, antitumor,

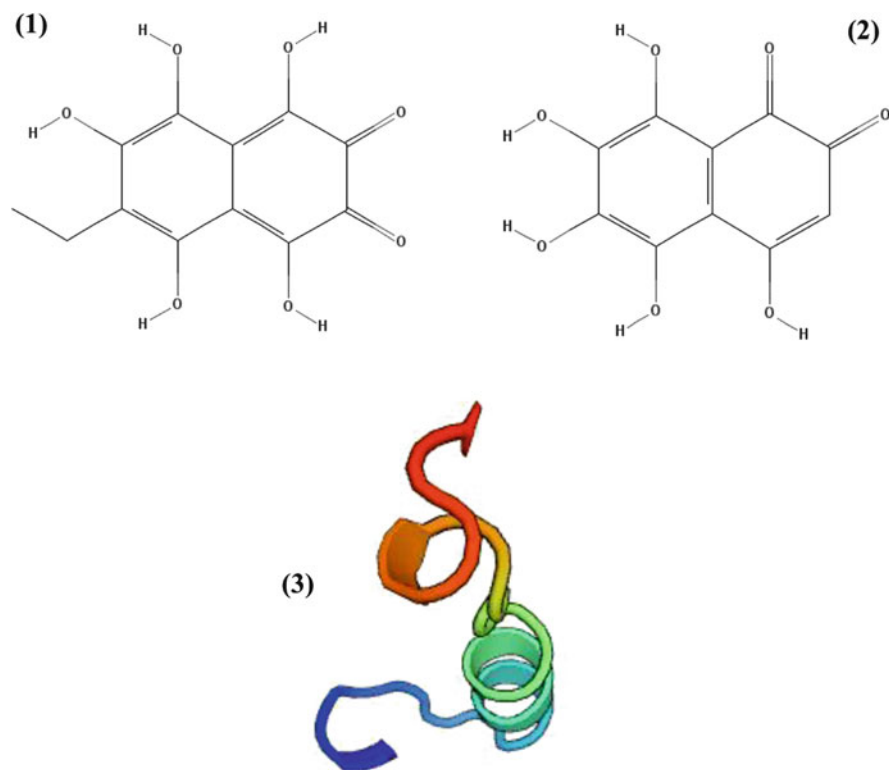


Fig. 1.9 Structures of two quinone pigments (1) echinochrome and (2) spinochromes D with antimicrobial activity and novel antimicrobial peptide (3) EeCentrocins isolate from *Echinus esculentus* (sea urchin, Echinodermata)

antiviral, antifungal, and anti-inflammatory activity. However, further studies on the activities of asterosaponins have been hampered by their poor accessibility [17, 148].

Interestingly, some **quinone pigments** from *Echinus esculentus* (sea urchin) contain antimicrobial compounds, in which **echinochrome-A (1)** and **spinochromes D (2) and E** have been identified [139]. Both compounds may play decisive roles in the regulation of lipid peroxidation and in immune defense. Specifically, echinochrome is synthesized by sea urchin pigment cells and possesses a strong bactericidal effect during embryonic and larval development [140, 149]. Another important drug isolated from sea urchin pigment cells is **histochrome** (Moscow, Russia) which presents cardiological and ophthalmological activity [150].

A large number of antimicrobial peptides (AMPs) have previously been found in the coelomic fluid of echinoderms [49, 129, 130]. The first completely sequenced AMPs in echinoderms were the **strongylocins** that are expressed in the coelomocytes of the green sea urchin *S. droebachiensis* [141]. This family includes two isoforms, the **SdStrongylocins 1b** and **2b**. Both are cysteine-rich peptides containing three disulfide bonds with MW in the 5.6–5.8 kDa range and display

strong activity against the marine fish pathogens such as *Listonella anguillarum* with a MIC of 1.3–2.5 μM [126]. Another peptide family identified from the coelomic fluid of *S. droebachiensis* is **centrocins 1 and 2** [142]. The centrocins are a family of heterodimeric AMPs ranging between 4.4 and 4.5 kDa in mass and are composed of two peptide chains: a 30-amino acid residue heavy chain (HC) and a 12-amino acid residue light chain (LC) connected by a single disulfide bond. Studies of bioactivity have shown that the cationic HC displays potent activity against both bacteria and fungi [143].

An interesting AMP of 5 kDa with antistaphylococcal biofilm properties has been isolated from *Paracentrotus lividus* [144, 145]. The **5-CC** peptide inhibits the formation of young biofilm (6-h old) from *S. epidermidis* 1457 as well as the formation of mature biofilm (24-h old) in the same clinical strain. This antibiofilm activity could be due to interference of 5-CC peptide with microbial surface proteins (adhesins, autolysins) that facilitate attachment to plastic surfaces in the first step of staphylococcal biofilm formation. However, further studies are needed to explain the mechanism of action of 5-CC in the prevention of adhesion and biofilm formation [151].

Novel peptides of 5–6 kDa were first characterized from coelomocyte extracts of the edible sea urchin, *E. esculentus*, collected from sub-Arctic waters. These AMPs are novel members of the centrocin and strongylocin families. The **EeCentrocins (3)** have a heterodimeric structure composed of a HC and a LC connected by a single disulfide bond. Studies on bioactivity show that both chains seem to be necessary for maintaining antibacterial activity. The secondary structure and the three-dimensional conformation of EeStrongylocin 2 as dictated by its three disulfide bonds remains unknown but should be explored [130].

Interesting peptides have been isolated from other species of echinoderms. For example, two peptides of about 2 kDa have been isolated from the starfish *Asterias rubens* and identified as fragments of the histone H2A molecule [130]. In addition, two other new peptides were found in *A. rubens*, corresponding to fragments of actin and filamin A. Additionally, these peptides showed that peptides can agglutinate Gram-positive and Gram-negative bacteria, exhibiting strong antibacterial activity in “in vivo” and “in vitro” conditions [152].

10 Tunicate

The Urochordata or Tunicata phylum frequently includes marine animals. Most are sessile and their body is covered by a complex exoskeletal robe. They have a highly developed perforated pharynx, but in the adult, the notochord and nerve cord usually disappear, and only the larval stages that look like microscopic tadpoles have the distinctive characteristics of chordates. Currently, about 1250 species have been described, and the earliest probable species of tunicate appears in the fossil record in the early Cambrian period [86, 88, 153, 154].

Taxonomically, members of the Tunicata phylum are divided into four classes: (1) Appendicularia, also known as larvaceans, are solitary, pelagic, and the free-swimming; (2) Thaliacea or pelagic tunicates are planktonic, solitary, or colonial and include three orders of free-swimming (Salpida, Pyrosomida, and Doliolida); (3) Sorberacea are organisms with great similarity to ascidians. They are benthic, live at great depths, have a dorsal nerve cord in the adult state, and are carnivorous; and (4) Ascidiacea or sea squirts are benthic, solitary, or colonial forms, with a cuticle made of polysaccharide [86, 88, 154, 155].

Ascidia is the group of organisms mainly studied because of the greater diversity of species that they possess and their interesting chemistry and physiology [155]. The way of life of ascidians and their bright colorful exterior make them more vulnerable to predation and more attractive to predators. Since they do not possess an escape mechanism, these organisms must rely on the production of chemical toxins for their survival [156]. Most of the ascidians and tunicates generally do not possess endobionts and have a well-developed immune system, which is activated against the presence of pathogenic species. However, a variety of studies have shown that these organisms appear to be good hosts of Cyanobacteria or even bacteria, which favor the production of toxins and molecules with biological interest as novel antimicrobials, according to a mechanism that has yet to be elucidated [155, 157].

Many antibacterial compounds have previously been isolated from tunicates or tunicate-associated marine bacteria/algae (Table 1.11 and Fig. 1.10). Thus, the **rubrolides (1)** are a family of approximately 20 polysubstituted butenolides isolated from an African ascidian *Synoicum globosum*, and they display highly sought-after biological properties including, anticancer, antidiabetic, anti-inflammatory, and antiviral activity. In addition, some of their synthetic analogs have shown potent

Table 1.11 Natural compounds and peptides with antimicrobial activity isolate from tunicate

| Metabolite | Isolated from | Chemical scaffold | Activity against | References |
|--------------------|--------------------------------|--------------------------|--|------------|
| Rubrolides A | <i>Synoicum globosum</i> | Butenolides | <i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> | [158, 159] |
| Cystodimine A–B | <i>Cystodytes dellechiaiei</i> | Pyridoacridine alkaloids | <i>Escherichia coli</i> and <i>Micrococcus luteus</i> | [160] |
| Synoxazolidinone A | <i>Synoicum pulmonaria</i> | Pyridoacridine alkaloids | <i>Corynebacterium glutamicum</i> | [161] |
| Styelin D | <i>Styela clavate</i> | Peptides | <i>Staphylococcus aureus</i> and MRSA ^a | [162] |
| Halocidin | <i>Halocynthia aurantium</i> | Peptides | MRSA ^a | [163] |
| Peptidolipins | <i>Trididemnum orbiculatum</i> | Lipopeptide | MRSA and MSSA ^b | [164] |

^aMethicillin resistant *Staphylococcus aureus*

^bMethicillin susceptible *Staphylococcus aureus*

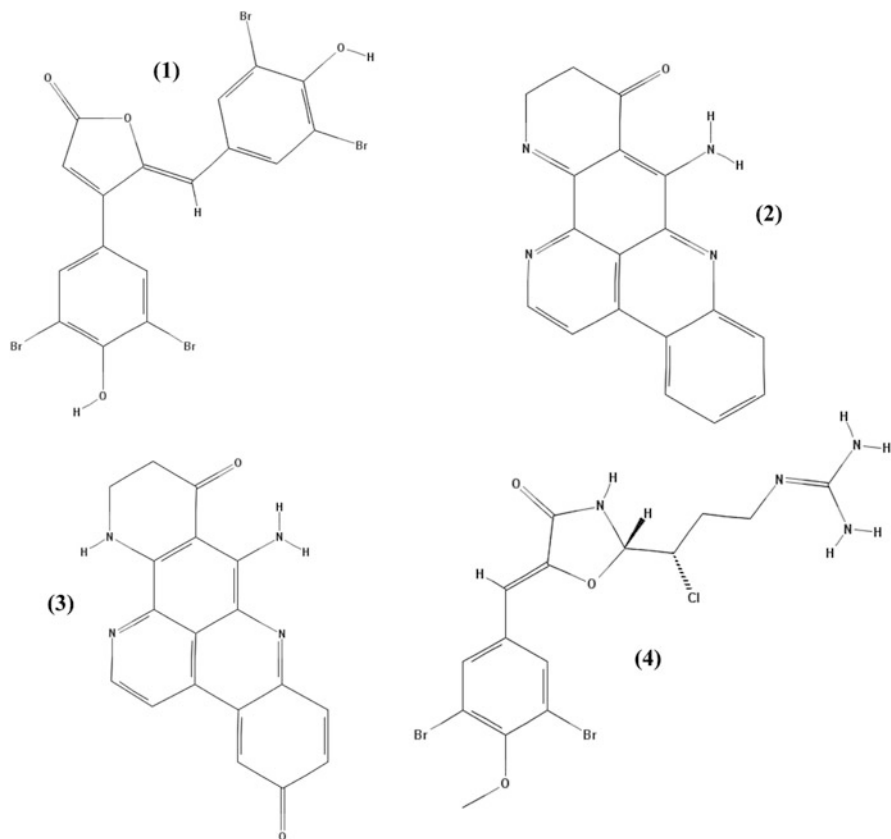


Fig. 1.10 Structures of antimicrobial compounds isolate from ascidians (tunicate). (1) Rubrolide A isolates from *Synoicum globosum*, and (2) cystodimine A, (3) cystodimine B, and (4) synoxazolidinone A isolate from *Cystodytes dellechiaiei*

antimicrobial activity against *S. aureus* and *B. subtilis* and also possess significant herbicidal and biofilm inhibitory activities (Sikorska et al. 2012; [159]).

Mediterranean and Norwegian ascidians (*Cystodytes dellechiaiei* and *Synoicum pulmonaria*, respectively) are sources of interesting alkaloids. **Pyridoacridine alkaloids** (N-deacetylshermilamine B, cystodimine A (2), and cystodimine B (3)) from *C. dellechiaiei* are active against *E. coli* and *Micrococcus luteus* [160], and **synoxazolidinone A** (4) from *S. pulmonaria* is active against *Corynebacterium glutamicum* [161].

Several antimicrobial peptides have been isolated from ascidian. The peptide **Styelin D** has been isolated from *Styela clava* and corresponds to a peptide of 32 residues that contains a 6-bromotryptophan residue in its sequence. This native peptide showed antibacterial activity against *S. aureus* and MRSA at any range of pH and salinity. However, the non-brominated version showed decreased activity at low pH and/or high salinity [162]. **Halocidin peptide** (3443 Da) was isolated from

hemocytes of *Halocynthia aurantium*. This peptide presents strong activity against a wide variety of pathogenic bacteria including MRSA [163]. Another peptide isolated from hemocytes of the tunicate *Halocynthia aurantium* is **di-cynthaurin** that contains unpaired cysteine and forms a covalent homodimer, with each monomer consisting of 30 amino acid residues with similar biological characteristics to halocidin.

The symbiotic association between the ascidian *Trididemnum orbiculatum* and the bacteria *Nocardia* spp. has been studied. The compound **peptidolipins** produced by *Nocardia* spp. shows activity against methicillin-sensitive *Staphylococcus aureus* (MSSA) and MRSA [164]. On the other hand, the relationship between cyanobacteria and ascidians has also been studied, mainly between *Synechocystis* or *Prochloron* species and the ascidian family Didemnidae [155]. Ascidians provide protection to cyanobacteria that live inside the tissues, tunic, or on the surface of the ascidians (for this reason the tunic is often transparent), and the cyanobacteria provide organic materials and cytotoxic constituents for protection to ascidians against depredator organisms. For example, the protein **didemnin B** prevents translocation by stabilizing aminoacyl-tRNA bound to the ribosomal A-site, similar to the antibiotic kirromycin. This molecule could be an interesting antibiotic to study. Furthermore, there are a substantial number of algal metabolites isolated from “free-living” algae which are similar or identical to ascidian metabolites [156, 165]. However, a better understanding of the mutual relationships of these organisms and biosynthetic studies are necessary to firmly establish whether these biologically interesting compounds are produced by the tunicate, the alga, or through a combined effort of both organisms.

11 Marine Algae

Marine algae, including microalgae, have been submitted to modern screening methods in order to identify secondary metabolites showing antibacterial activity [166]. Algae are a large group of photosynthetic and autotrophic organisms distributed extensively throughout the aquatic environment, with some terrestrial species in extremophile environments. Morphologically they can be unicellular cells (diatoms) or large multicellular algae. Their size can range from microscopic organisms to large macroscopic structures of up to several meters in length. The great power adaptation of algae species in all media (terrestrial or aquatic) and their ability to live in extremophile conditions (osmotic stress, salinity, oxygen, and high UV radiation) and constant competition with seawater bacteria make them species of interest to study their secondary metabolites.

Microalgae are microscopic and unicellular algae that have the ability to perform photosynthesis, producing about half of the atmospheric oxygen. The biodiversity of microalgae is enormous, and only 50,000 species have been described among 200,000–800,000 species estimated to exist.

Microalgae such as diatoms have developed precise systems to fight pathogenic bacteria that coexist in the marine environment. The secretion of secondary metabolites is one of these systems. Among these metabolites, fatty acids, peptides, polysaccharides, aromatic organic acids, alcohols, aldehydes, terpenes, sterols, phlorotannins, polyketides, and hydroquinones, among others, are produced by marine algae [55, 167]. About 15,000 novel compounds from microalgae have been chemically characterized, including carotenoids, toxins, fatty acids, enzymes, polymers, antioxidants, and sterols [168].

Some of the most studied compounds from marine algae with antibacterial activity are shown in Table 1.12.

Fatty acids act against bacteria inhibiting their electron transport chain and oxidative phosphorylation in cell membranes. One of the most studied fatty acids

Table 1.12 Natural compounds with antimicrobial activity isolate from Bryozoa

| Metabolite | Isolated from | Chemical scaffold | Activity against | References |
|-------------------------|---|-------------------|---|------------|
| Cyclopentaneacetic acid | Sargassum spp. | Fatty acid | <i>Staphylococcus aureus</i> and <i>Klebsiella pneumonia</i> | [166] |
| Algae extract | <i>Solieria filiformis</i> | Lectin | <i>Pseudomona aeruginosa</i> , <i>Enterobacter aerogenes</i> , <i>Serratia marcescens</i> , <i>Salmonella typhi</i> , <i>K. pneumoniae</i> and <i>Proteus</i> sp. | [169] |
| Phlorofucofuroeckol-A | <i>Eisenia bicyclis</i> | Phlorotannin | <i>S. aureus</i> ^a | [170] |
| Fucoidan | <i>Cladosiphon ocamuranus</i> , <i>Fucus evanescens</i> , and <i>F. vesiculosus</i> | Polysaccharide | <i>Helicobacter pylori</i> | [171] |
| Laminarin | <i>Ascophyllum nodosum</i> and <i>Laminaria hyperborea</i> | Polysaccharide | <i>S. aureus</i> , <i>Listeria monocytogenes</i> , <i>Escherichia coli</i> and <i>Salmonella typhimurium</i> | [172] |
| Fucoxanthin | <i>Himanthalia elongate</i> , <i>Turbinaria triquetra</i> and <i>Laurencia obtusa</i> | Terpene | <i>L.monocytogenes</i> , <i>E. coli</i> , <i>Bacillus cereus</i> , <i>Bacillus subtilis</i> , <i>K. pneumoniae</i> , <i>S. aureus</i> and <i>P. aeruginosa</i> | [173, 174] |

^aMethicillin resistant *Staphylococcus aureus*

is **cyclopentaneacetic acid** that is able to perforate cell walls causing a rupture of the chromatin and changing the cell shape and size in microorganisms including *S. aureus* and *K. pneumoniae* [166].

Peptides are another important active group. The particularity of the peptides that makes them effective against bacteria is their amphipathic conformation. This property enables them to bind to both polar and nonpolar sites of the bacteria cytoplasmic membrane, causing changes in the cellular processes [175]. Among these peptides, **lectins** are very promising as antibacterial agents. Thus, lectin isolated from the red algae *Solieria filiformis* has shown “in vitro” antibacterial activity against Gram-negative and Gram-positive microorganisms, including *P. aeruginosa*, *E. aerogenes*, *S. marcescens*, *S. typhi*, *K. pneumoniae*, and *Proteus* sp. [169].

Phlorotannins also have important antibacterial activity. The mechanisms used by phlorotannins to inhibit bacterial growth are by the inhibition of oxidative phosphorylation and by binding with bacterial proteins and cell membrane, leading to cell lysis. They have been shown to be more effective against Gram-positive microorganisms. One phlorotannin, **phlorofucofuroeckol-A** (with low molecular weight), has high antibacterial activity against MRSA. It suppresses the expression of the *mecI*, *mecR1*, and *mecA* genes, responsible for resistance to methicillin. The repression of these genes causes a suppression of penicillin-binding protein 2a production [170].

Polysaccharides are compounds formed by repeated monosaccharide units linked by glycosidic bonds. Their antibacterial activity consists of increasing the permeability of the cytoplasmic membrane, protein leakage, and binding of bacterial DNA [176]. Among these polysaccharides, **fucoidan** and laminarin are able to inhibit the growth of *S. aureus* and *E. coli* as well as the biofilm formation of *Helicobacter pylori* [171, 172].

Terpenes are compounds formed by repeated isoprene units. Among them, **xanthophyll** is produced by diatoms and has been reported to be active against bacteria. One of the most important terpenes of this group is **fucoxanthin** (Fig. 1.11) [173, 174] which inhibits the growth of *L. monocytogenes*, *E. coli*, *Bacillus cereus*, *B. subtilis*, *K. pneumoniae*, *S. aureus*, and *P. aeruginosa*.

Finally, **lactones** are cyclic esters of hydroxycarboxylic acids. The most frequently studied group of lactones are furanones. The red alga *Delisea pulchra* produces **furanones** that are delivered to the surface at concentrations able to regulate bacterial colonization. Thus, furanones have been used as inhibitors of bacterial- and macro-fouling. Furanones inhibit bacterial colonization and biofilm development through interference with a key bacterial quorum-sensing pathway, the acylated homoserine lactone regulatory system in Gram-negative bacteria. They also interfere with the alternative AI-2 signaling system in Gram-negative and Gram-positive bacteria.

Some of these secondary marine metabolites enhance the antibacterial activity of antibiotics used in clinical practice to fight against pathogenic bacteria. Thus, fucoidan, together with ampicillin or gentamicin, decreases the MIC and the minimal bactericidal concentration (MBC) of both antibiotics fourfold. Marine alginate-derived

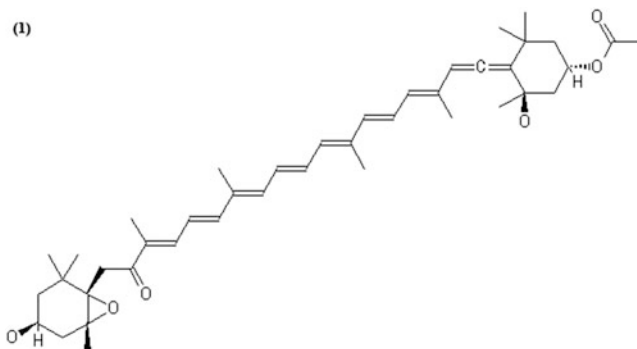


Fig. 1.11 Chemical structure of fucoxanthin, antimicrobial agent which inhibits a large range of Gram-positive and Gram-negative bacteria

oligosaccharide (ADO) together with azithromycin decreases the MIC of the azithromycin 2.8-fold, from 152.6 $\mu\text{g/ml}$ to 54.2 $\mu\text{g/ml}$ [92, 93].

12 Conclusions

Despite the enormous diversity of our oceans and seas and the different bioactivities found, very few new antibiotics obtained from marine organisms are currently on the market.

Much research remains to be done in this regard if we are to find new antibiotics from among marine microorganisms such as microalgae. The integration of microbiology with chemistry will be an important tool to advance in the successful search for new marine drugs.

References

1. Spellberg B, Powers JH, Brass EP, Miller LG, Edwards J Jr (2004) Trends in antimicrobial drug development: implications for the future. *Clin Infect Dis* 38:1279–1286
2. Powers JH (2004) Antimicrobial drug development—the past, the present, and the future. *Clin Microbiol Infect* 10(Suppl 4):23–31
3. Saga T, Yamaguchi K (2009) History of antimicrobial agents and resistant bacteria. *Jpn Med Assoc J* 52:103–108
4. Cassell GH, Mekalanos J (2001) Development of antimicrobial agents in the era of new and reemerging infectious diseases and increasing antibiotic resistance. *JAMA* 285:601–605
5. Bassetti M, Merelli M, Temperoni C, Astilean A (2013) New antibiotics for bad bugs: where are we? *Ann Clin Microbiol Antimicrob* 28:12–22
6. Wright GD (2014) Something old, something new: revisiting natural products in antibiotic drug discovery. *Can J Microbiol* 60:147–154

7. Hughes CC, Fenical W (2010) Antibacterials from the sea. *Chemistry* 16:12512–12525
8. Aminov RI (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. *Front Microbiol* 1:134
9. Singh S, Kate BN, Banerjee UC (2005) Bioactive compounds from cyanobacteria and microalgae: an overview. *Crit Rev Biotechnol* 25:73–95
10. Supriya JS, Yogesh CS (2010) Marine: the ultimate source of bioactives and drug metabolites. *Int J Res Ayurveda Pharm* 1:55–62
11. Leiva S, Yáñez M, Zaror L et al (2004) Actividad antimicrobiana de actinomycetes aislados desde ambientes acuáticos del sur de Chile. *Rev Méd Chile* 132:151–159
12. Mutaz Al-Ajlani M, Hasnain S (2010) Bacteria exhibiting antimicrobial activities; screening for antibiotics and the associated genetic studies. *Open Conf Proc J* 1:230–238
13. Abad MJ, Bedoya LM, Bermejo P (2011) Marine compounds and their antimicrobial activities. *Fortamex*:1293–1306
14. Carté BK (1996) Biomedical potential of marine natural products. *Bioscience* 46:271–286
15. Jan PA, Douglas EL (2016) Ocean sediments—an enormous but underappreciated microbial habitat. *Microbe* 427–437
16. Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR (2010) Marine natural products. *Nat Prod Rep* 27:165–237
17. Kumar Jha R, Zi-Rong X (2004) Biomedical compounds from marine organisms. *Mar Drugs* 2:123–146
18. Biswas K, Paul D, Sinha SN (2016) Marine bacteria: a potential tool for antibacterial activity. *J Appl Environ Microbiol* 4:25–29
19. Armstrong E, Yan L, Boyd KG, Wright PC, Burgess JG (2001) The symbiotic role of marine microbes on living surfaces. *Hydrobiologia* 461:37–40
20. Jeganathan P, Rajasekaran KM, Devi NKA, Karuppusamy S (2013) Antimicrobial activity and characterization of marine bacteria. *Indian J Pharm Biol Res* 1:38–44
21. El-Gendy MMA, Shaaban M, El-Bondkly AM, Shaaban KA (2008) Bioactive benzopyrone derivatives from new recombinant fusant of marine streptomyces. *Appl Biochem Biotechnol* 150:85–96
22. Lu XL, Xu QZ, Shen YH et al (2008) Macrolactin S, a novel macrolactin antibiotic from marine *Bacillus* sp. *Nat Prod Res* 22:342–347
23. Berger M, Neumann A, Schulz S, Simon M, Brinkhoff T (2011) Tropodithietic acid production in *Phaeobacter gallaeciensis* is regulated by N-acyl homoserine lactone-mediated quorum sensing. *J Bacteriol* 193:6576–6585
24. D'Alvise PW, Magdenoska O, Melchiorson J, Nielsen KF, Gram L (2013) Biofilm formation and antibiotic production in *Ruegeria mobilis* are influenced by intracellular concentrations of cyclic dimeric guanosinmonophosphate. *Environ Microbiol* 16:1252–1266
25. Desjardine K, Pereira A, Wright H, Matainaho T, Kelly M, Andersen RJ (2007) Tauramamide, a lipopeptide antibiotic produced in culture by *Brevibacillus laterosporus* isolated from a marine habitat: structure elucidation and synthesis. *J Nat Prod* 70:1850–1853
26. Engelhardt K, Degnes KF, Kemmler M, Bredholt H, Fjaervik E, Klinkenberg G, Sletta H, Ellingsen TE, Zotchev SB (2010) Production of a new thiopeptide antibiotic, TP-1161, by a marine *Nocardiosis species*. *Appl Environ Microbiol* 76:4969–4976
27. Fehér D, Barlow R, McAtee J, Hemscheidt TK (2010) Highly brominated antimicrobial metabolites from a marine *Pseudoalteromonas* sp. *J Nat Prod* 73:1963–1966
28. Isnansetyo A, Kamei Y (2009) Anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity of MC21-B, an antibacterial compound produced by the marine bacterium *Pseudoalteromonas phenolica* O-BC30T. *Int J Antimicrob Agents* 34:131–135
29. Andrianasolo EH, Haramaty L, Rosario-Passapera R, Vetriani C, Falkowski P, White E, Lutz R (2012) Ammonificans C and D, hydroxyethylamine chromene derivatives from a cultured marine hydrothermal vent bacterium, *Thermovibrio ammonificans*. *Mar Drugs* 10:2300–2311

30. Han JS, Cheng JH, Yoon TM, Song J, Rajkarnikar A, Kim WG et al (2005) Biological control agent of common scab disease by antagonistic strain *Bacillus* sp. sunhua. J Appl Microbiol 99:213–221
31. Cetina A, Matos A, Garma G, Barba H, Vázquez R, Zepeda-Rodríguez A, Jay D, Monteón V, López AR (2010) Marine bacteria isolated from Gulf of Mexico antimicrobial activity of marine bacteria isolated from Gulf of Mexico Actividad antimicrobiana de bacterias marinas aisladas del Golfo de México. Rev Peru Biol 17:231–236
32. Lu X, Liu X, Long C et al (2011) A preliminary study of the microbial resources and their biological activities of the East china sea. Evid Based Complement Alternat Med 2011:806485
33. Brinkhoff T, Bach G, Heidorn T, Liang L, Schlingloff A, Simon M (2004) Antibiotic production by a Roseobacter clade-affiliated species from the German Wadden Sea and its antagonistic effects on indigenous isolates. Appl Environ Microbiol 70:2560–2565
34. Bruhn JB, Gram L, Belas R (2007) Production of antibacterial compounds and biofilm formation by Roseobacter species are influenced by culture conditions. Appl Environ Microbiol 73:442–450
35. D'Alvise PW, Lillebø S, Prol-Garcia MJ, Wergeland HI, Nielsen KF, Bergh Ø, Gram L (2012) *Phaeobacter gallaeciensis* reduces *Vibrio anguillarum* in cultures of microalgae and rotifers, and prevents vibriosis in cod larvae. PLoS One 7(8):e43996
36. Liu RF, Zhang DJ, Li YG et al (2010) A new antifungal cyclic lipopeptide from *Bacillus marinus* B-9987. Helv Chim Acta 93:2419–2425
37. Chen L, Wang N, Wang X, Hu J, Wang S (2010) Characterization of two anti-fungal lipopeptides produced by *Bacillus amyloliquefaciens* SH-B10. Bioresour Technol 101:8822–8827
38. Oku N, Kawabata K, Adachi K et al (2008) Unnamicins a and C, new antibacterial depsipeptides produced by marine bacterium Photobacterium sp. MBIC06485. J Antibiot (Tokyo) 61:11–17
39. Oku N, Adachi K, Matsuda S et al (2008) Ariakemicins a and B, novel polyketide-peptide antibiotics from a marine gliding bacterium of the genus *Rapidithrix*. Org Lett 10:2481–2484
40. Jones EBG, Sakayaroj J, Suetrong S, Somrithipol S, Pang KL (2009) Classification of marine of marine ascomycota, anamorphic taxa and basidiomycota. Fungal Divers 35:1–187
41. Kohlmeyer J, Kohlmeyer E (1979) Marine mycology. The higher fungi. Academic, New York
42. Jones EBG (2011) Fifty years of marine mycology. Fungal Divers 50:73–112
43. Walker AK, Campbell J (2010) Marine fungal diversity: a comparison of natural and created salt marshes of the north-central Gulf of Mexico. Mycologia 102:513–521
44. Zhou S, Wang M, Feng Q, Lin Y, Zhao H (2016) A study on biological activity of marine fungi from different habitats in coastal regions. SpringerPlus 5:1966
45. Prompanya C, Dethoup T, Bessa L, Pinto M, Gales L, Costa P, Silva A, Kijjoa A (2014) New Isocoumarin derivatives and Meroterpenoids from the marine sponge-associated fungus *Aspergillus similanensis* sp. nov. KUFA 0013. Mar Drugs 12:5160–5173
46. Liu F, Xia J, Wang W (2013) Isolation and identification of two terphenyl compounds from *Aspergillus candidus* metabolites. J Xiamen Univ 52:670–674
47. Fredimoses M, Zhou X, Lin X, Tian X, Ai W, Wang J, Liao S, Liu J, Yang B, Yang X (2014) New prenylxanthenes from the deep-sea derived fungus *Emericella* sp. SCSIO 05240. Mar Drugs 12:3190–3202
48. Bai ZQ, Lin X, Wang Y, Wang J, Zhou X, Yang B, Liu J, Yang X, Wang Y, Liu Y (2014) New phenyl derivatives from endophytic fungus *Aspergillus flavipes* AIL8 derived of mangrove plant *Acanthus ilicifolius*. Fitoterapia 95:194–202
49. Li C, Blencke HM, Haug T, Jorgensen O, Stensvag K (2014) Expression of antimicrobial peptides in coelomocytes and embryos of the green sea urchin (*Strongylocentrotus droebachiensis*). Dev Comp Immunol 43:106–113
50. Dafemer M, Anke T, Sterner O (2002) Zopfiellamides A and B, antimicrobial pyrrolidinone derivatives from the marine fungus *Zopfiella latipes*. Tetrahedron 58:7781–7784

51. Van Soest RWM, Boury-Esnault N, Hooper JNA, Rützler K, de Voogd NJ, Alvarez de Glasby B, Hajdu E, Pisera AB, Manconi R, Schoenberg C, Klautau M, Picton B, Kelly M, Vacelet J, Dohrmann M, Díaz MC, Cárdenas P, Carballo JL (2017) World Porifera Database. <http://www.marinespecies.org/porifera>. Accessed 1 Feb 2017
52. Bell JJ (2008) The functional roles of marine sponges. *Estuar Coast Shelf Sci* 79:341–353
53. Van Soest RWM, Boury-Esnault N, Hooper JNA, Rützler K, de Voogd NJ, Alvarez de Glasby B, Hajdu E, Pisera AB, Manconi R, Schoenberg C, Klautau M, Picton B, Kelly M, Vacelet J, Dohrmann M, Díaz MC, Cárdenas P, Carballo JL (2011) World Porifera Database. <http://www.marinespecies.org/porifera/porifera.php?p=taxdetails&id=131587>. Accessed 1 Feb 2017
54. Hentschel U, Piel J, Degnan SM, Taylor MW (2012) Genomic insights into the marine sponge microbiome. *Nat Rev Microbiol* (9):641–654
55. Mayer AMS, Rodríguez AD, Taglia-latela-Scafati O, Fusetani N (2013) Marine pharmacology in 2009–2011: marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, and antiviral activities; affecting the immune and nervous systems, and other miscellaneous mechanisms of action. *Mar Drugs* 11 (7):2510–2573
56. Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR (2016) Marine natural products. *Nat Prod Rep* 3:382–431
57. Cheuka P, Mayoka G, Mutai P, Chibale K (2016) The role of natural products in drug discovery and development against neglected tropical diseases. *Molecules* 22:58
58. Urban S, de Almeida LP, Carroll AR, Fechner GA, Smith J, Hooper JNA, QJR (1999) Axinellamines A–D, novel imidazo–azolo–imidazole alkaloids from the Australian marine sponge *Axinella* sp. *OrgChem* 64:731–735
59. Chen C, Ma Z, Wang X, Ma Y (2016) Asymmetric synthesis of axinellamines a and B. *Angew Chem Int Ed* 55:4763–4766
60. Zidar N, Montalvão S, Hodnik Z, Nawrot DA, Žula A, Ilaš J, Kikelj D, Tammela P, Mašič LP (2014) Antimicrobial activity of the marine alkaloids, clathrodin and oroidin, and their synthetic analogues. *Mar Drugs* 12(2):940–963
61. Melander RJ, Liu HB, Stephens MD, Bewley CA, Melander C (2016) Marine sponge alkaloids as a source of anti-bacterial adjuvants. *Bioorg Med Chem Lett* 26(24):5863–5866
62. Zhang Z (2011) Animal biodiversity: an introduction to higher-level classification and taxonomic richness. *Zootaxa* 3148:7–12
63. Crossland CJ, Hatcher BG, Smith SV (1991) Role of coral reefs in global ocean production. *Coral Reefs* 10:55
64. Marques AC, Collins AG (2004) Cladistic analysis of Medusozoa and cnidarian evolution. *Invertebr Biol* 123(1):23–42
65. Mariottini GL (2014) Hemolytic venoms from marine cnidarian jellyfish—an overview. *J Venom Res* 5:22–32
66. Ospina CA, Rodríguez AD, Zhao H, Raptis RG (2007) Bipinnapterolide B, a bioactive oxapolycyclic diterpene from the Colombian gorgonian coral *Pseudopterogorgia bipinnata*. *Tetrahedron Lett* 48:7520–7523
67. Bishara A, Rudi A, Goldberg I, Benayahu Y, Kashman Y (2006) Novaxenicins A–D and xeniolides I–K, seven new diterpenes from the soft coral *Xenia novaebritanniae*. *Tetrahedron* 62:12092–12097
68. McCulloch MWB, Haltli B, Marchbank DH, Kerr RG (2012) Evaluation of pseudopteroxazole and pseudopterodin derivatives against *Mycobacterium tuberculosis* and other pathogens. *Mar Drugs* 10:1711–1728
69. Ata A, Win HY, Holt D, Holloway P, Segstro EP, Jayatilake GS (2004) New antibacterial diterpenes from *Pseudopterogorgia elisabethae*. *Helv Chim Acta* 87:1090–1098
70. Rodríguez II, Rodríguez AD (2003) Homopseudopteroxazole, a new antimycobacterial diterpene alkaloid from *Pseudopterogorgia elisabethae*. *J Nat Prod* 66(6):855–857

71. Correa H, Aristizabal F, Duque C, Kerr R (2011) Cytotoxic and antimicrobial activity of pseudopterosins and seco-pseudopterosins isolated from the octocoral *Pseudopterogorgia Elisabethae* of San Andrés and Providencia Islands (Southwest Caribbean Sea). *Mar Drugs* 9(3):334–343
72. Wei MY, Wang CY, Liu QA, Shao CL, She ZG, Lin YC (2010) Five ses-quitertpenoids from a marine-derived fungus *Aspergillus* sp. isolated from a gorgonian *Dichotella gemmacea*. *Mar Drugs* 8(4):941–949
73. El Sayed KA, Bartyzel P, Shen X, Perry TL, Zjawiony JK, Hamann MT (2000) Marine natural products as antituberculosis agents. *Tetrahedron* 56:949–953
74. Liang LF, Lan LF, Taglialatela-Scafati O, Guo YW (2013) Sartrolides AeG and bissartrolide, new cembranolides from the South China Sea soft coral *Sarcophyton trocheliophorum* Marenzeller. *Tetrahedron* 69:7381–7386
75. Shenkarev ZO, Pantelev PV, Balandin SV, Gizatullina AK, Altukhov DA, Finkina EI, Kokryakov VN, Arseniev AS, Ovchinnikova TV (2012) Recombinant expression and solution structure of antimicrobial peptide aurelin from jellyfish *Aurelia aurita*. *Biochem Biophys Res Commun* 429:63–69
76. Jung S, Dingley AJ, Augustin R, Anton-Erxleben F, Stanisak M, Gelhaus C, Gutschmann T, Hammer MU, Podschun R, Bonvin AM, Leippe M, Bosch T, Grötzinger J (2009) Hydramacin-1, structure and antibacterial activity of a protein from the basal metazoan hydra. *J Biol Chem* 284(3):1896–1905
77. Bosch TCG, Augustin R, Anton-Erxleben F, Fraune S, Hemmrich G, Zill H, Rosenstiel P, Jacobs G, Schreiber S, Leippe M et al (2009) Uncovering the evolutionary history of innate immunity: the simple metazoan hydra uses epithelial cells for host defence. *Dev Comp Immunol* 33:559–569
78. Augustin R, Anton-Erxleben F, Jungnickel S et al (2009) Activity of the novel peptide arminin against multiresistant human pathogens shows the considerable potential of phylogenetically ancient organisms as drug sources. *Antimicrob Agents Chemother* 53(12):5245–5250
79. Augustin R, Siebert S, Bosch T (2009) Identification of a kazal-type serine pro-tease inhibitor with potent anti-staphylococcal activity as part of Hydra's in-nate immune system. *Dev Comp Immunol* 33:830–837
80. Bock PE, Gordon DP (2013) Phylum Bryozoa Ehrenberg, 1831. *Zootaxa* 3703(1):67–74
81. Gordon DP (1987) The deep-sea Bryozoa of the New Zealand region. In: Ross JRP (ed) *Bryozoa: present and past*. Washington University, Bellingham, pp 97–104
82. Figuerola B, Sala-Comorera L, Angulo-Preckler C, Vázquez J, Montes MJ, García-Aljaro C, Mercadé E, Blanch AR, Avila C (2014) Antimicrobial activity of Antarctic bryozoans: an ecological perspective with potential for clinical applications. *Mar Environ Res* 101:52–59
83. Prinsep M, Yao B, Nicholson B, Gordon DP (2004) The pterocellins, bioactive alkaloids from the marine bryozoan *Pterocella vesiculosa*. *Phytochem Rev* 3:325–331
84. Till M, Prinsep MR (2009) 5-Bromo-8-methoxy-1-methyl- β -carboline, an alkaloid from the New Zealand marine bryozoan *Pterocella vesiculosa*. *J Nat Prod* 72:796–798
85. Tadesse M, Tabudravu JN, Jaspars M, Strom MB, Hansen E, Andersen JH, Kristiansen PE, Haug T (2011) The antibacterial ent-eusynstyelamide B and eusynstyelamides D, E, and F from the arctic bryozoan *Tegella cf. spitzbergensis*. *J Nat Prod* 74:837–841
86. Brusca RC, Brusca GJ, Martínez FP (2005) *Invertebrados*. McGraw-Hill, Madrid
87. Periyasamy N, Srinivasan M, Balakrishnan S (2012) Antimicrobial activities of the tissue extracts of *Babylonia spirata* Linnaeus, 1758 (Mollusca: Gastropoda) from Thazhanguda, southeast coast of India. *Asian Pac J Trop Biomed* 2:36–40
88. Ruppert EE, Fox RS, Barnes RD (2004) *Invertebrate zoology: a functional evolutionary approach*. *Syst Biol* 53:662–664
89. Dakhil DZ, Tahar AA (2010) Antimicrobial activity of some crude marine Mollusca extracts against some human pathogenic bacteria. *Thi-Qar Med J* 4:142–147

90. Schmutterer H (2005) Mollusca, molluscs. In: Schmutterer H (ed) Neem tree source unique natural. Products for integrated pest management, medicine, industry and other purposes. VCH, Weinheim, pp 151–152
91. Sarumathi G, Arumugam M, Kumaresan S, Balasubramanian T (2012) Studies on bioprospecting potential of a gastropod mollusc *Cantharus tranquebaricus* (Gmelin, 1791). Asian Pac J Trop Biomed 2:759–764
92. He JY, Chi CF, Liu HH (2014) Identification and analysis of an intracellular Cu/Zn superoxide dismutase from *Sepiella maindroni* under stress of *Vibrio harveyi* and Cd²⁺. Dev Comp Immunol 47:1–5
93. He X, Hwang H-M, Aker WG, Wang P, Lin Y, Jiang X, He X (2014) Synergistic combination of marine oligosaccharides and azithromycin against *Pseudomonas aeruginosa*. Microbiol Res 169:759–767
94. Datta D, Nath Talapatra S, Swarnakar S (2015) Bioactive compounds from marine invertebrates for potential medicines—an overview. Int Lett Nat Sci 7:42–61
95. Gustafson K, Andersen RJ (1985) Chemical studies of British Columbia nudibranchs. Tetrahedron 41:1101–1108
96. Kiran N, Siddiqui G, Khan AN, Ibrar K, Tushar P (2014) Extraction and screening of bioactive compounds with antimicrobial properties from selected species of mollusk and crustacean. J Clin Cell Immunol 5:1–5
97. De Petrocellis L, Orlando P, Pierobon P, De Falco M, Ruggiero AM, Stefano GS, Tino A, Grippo P (1999) Kelletinins from the marine mollusc *Buccinum corneum*, promotes differentiation in *Hydra vulgaris*. Res Commun Mol Pathol Pharmacol 103:17–28
98. Orlando P, Carretta F, Grippo P, Cimino G, De Stefano S, Strazzullo G (1991) Kelletinins from the marine mollusc *Buccinum corneum* are inhibitors of eukaryotic DNA polymerase α . Experientia 47:64–66
99. Anbuselvi S, Chellaram C, Jones S, Jayanthi L, Edward JKP (2009) Bioactive potential of coral associated gastropod, *Trochus tentorium* of Gulf of Mannar, southeastern India. J Med Sci 9:240–244
100. Vino AB, Shanmugam V, Shanmugam A (2014) Antimicrobial activity of methanolic extract and fractionated polysaccharide from *Loligo duvauceli* Orbingy 1848 and *Doryteuthis sibogae* Adam 1954 on human pathogenic microorganisms. Afr J Microbiol Res 8:230–236
101. Shanmugam A, Amalraj T, Gnanasekar Devanathan CP, Balasubramanian T (2008) Antimicrobial activity of sulfated mucopolysaccharides [heparin and heparin-like glycosaminoglycans (GAGs)] from Cuttlefish *Euprymna Berryi* Sasaki, 1929. Trends Appl Sci Res 3:97–102
102. Kanagasabapathy S, Samuthirapandian R, Kumaresan M (2011) Preliminary studies for a new antibiotic from the marine mollusk *Melo melo* (Lightfoot, 1786). Asian Pac J Trop Med 4:310–314
103. Dolashka P, Dolashki A, Voelter W, Van Beeumen J, Stevanovic S (2015) Antimicrobial activity of peptides from the hemolymph of *Helix lucorum* snails. Int J Curr Microbiol App Sci 4:1061–1071
104. Dolashka P, Moshanska V, Borisova V, Dolashki A, Stevanovic S, Dimanov T, Voelter W (2011) Antimicrobial proline-rich peptides from the hemolymph of marine snail *Rapana venosa*. Peptides 32:1477–1483
105. Castillo MG, Salazar KA, Joffe NR (2015) The immune response of cephalopods from head to foot. Fish Shellfish Immunol 46:145–160
106. Boyle P, Rodhouse P (2007) Cephalopods: ecology and fisheries. Wiley, Oxford
107. Rajasekharan Nair J, Pillai D, Joseph SM et al (2011) Cephalopod research and bioactive substances. Indian J Mar Sci 40:13–27
108. Ramasamy P, Subhapradha N, Srinivasan A et al (2011) In vitro evaluation of antimicrobial activity of methanolic extract from selected species of cephalopods on clinical isolates. Afr J Microbiol Res 5:3884–3889
109. Shanmugam A, Mahalakshmi TS, Barwin Vino A (2008) Antimicrobial activity of polysaccharide isolated from the cuttlebone of *Sepia aculeata* (Orbingy, 1848) and *Sepia brevimana* (Steenstrup, 1875): an approach to selected antimicrobial activity for human pathogenic microorganisms. Fish Aquat Sci 3:268–274

110. Lavelle P (1996) Diversity of soil fauna and ecosystem function. *Biol Int* 33:3–16
111. Nosrati H, Nosrati M, Karimi R (2013) The phylum annelida: a short introduction. *Agric Sci Dev* 2:28–30
112. Cuvillier-Hot V, Boidin-Wichlacz C, Tasiemski A (2014) Polychaetes as annelid models to study ecoimmunology of marine organisms. *J Mar Sci Technol* 22:9–14
113. Otero-González AJ, Magalhães BS, Garcia-Villarino M, López-Abarrategui C, Sousa DA, Dias SC, Franco OL (2010) Antimicrobial peptides from marine invertebrates as a new frontier for microbial infection control. *FASEB J* 24:1320–1334
114. Tasiemski A (2008) Antimicrobial peptides in annelids. *Invertebr Surviv J* 5:75–82
115. Maltseva AL, Kotenko ON, Kokryakov VN, Starunov VV, Krasnodembskaya AD (2014) Expression pattern of arenicins—the antimicrobial peptides of polychaete *Arenicola marina*. *Front Physiol* 5:1–11
116. Anderson RS, Chain BM (1982) Antibacterial activity in the coelomic fluid of a marine annelid, *Glycera dibranchiata*. *J Invertebr Pathol* 40:320–326
117. Chain BM, Anderson RS (1983) Antibacterial of the coelomic fluid from the polychaeta, *Glycera dibranchiata*. II. Partial purification and biochemical characterization of the active factor. *Biol Bull* 164:41–49
118. Pan W, Liu X, Ge F, Han J, Zheng T (2004) Perinerin, a novel antimicrobial peptide purified from the clamworm *Perinereis aibuhitensis* Grube and its partial characterization. *J Biochem* 135:297–304
119. Ovchinnikova TV, Aleshina GM, Balandin SV, Krasnodembskaya AD, Markelov ML, Frolova EI, Leonova YF, Tagaev AA, Krasnodembsky EG, Kokryakov VN (2004) Purification and primary structure of two isoforms of arenicin, a novel antimicrobial peptide from marine polychaeta *Arenicola marina*. *FEBS Lett* 577:209–214
120. Tasiemski A, Schikorski D, Le Marrec-Croq F, Pontoire-Van Camp C, Boidin-Wichlacz C, Sautière PE (2007) Hedistin: a novel antimicrobial peptide containing bromotryptophan constitutively expressed in the NK cells-like of the marine annelid, *Nereis diversicolor*. *Dev Comp Immunol* 31:749–762
121. Bulet P, Stöcklin R, Menin L (2004) Anti-microbial peptides: from invertebrates to vertebrates. *Immunol Rev* 198:169–184
122. Zhou Q, Li M, Xi T (2009) Cloning and expression of a clamworm antimicrobial peptide perinerin in *Pichia pastoris*. *Curr Microbiol* 58:384–388
123. Elayaraja S, Murugesan P, Vijayalakshmi S, Balasubramanian T (2010) Antibacterial and antifungal activities of polychaete *Perinereis cultrifera*. *Indian. J Mar Sci* 39:257–261
124. El-Gamal MI, Abdel-Maksoud MS, CH O (2013) Recent advances in the research and development of marine antimicrobial peptides. *Curr Top Med Chem* 13:2026–2033
125. Kondo M, Akasaka K (2012) Current status of echinoderm genome analysis—what do we know? *Curr Genomics* 13:134–143
126. Li C, Blencke HM, Haug T, Stensvåg K (2015) Antimicrobial peptides in echinoderm host defense. *Dev Comp Immunol* 49:190–197
127. Motuhi S-E, Mehiri M, Payri C et al (2016) Marine natural products from new Caledonia—a review. *Mar Drugs* 14:58
128. Uthicke S, Schaffelke B, Byrne M (2009) A boom–bust phylum? Ecological and evolutionary consequences of density variations in echinoderms. *Ecol Monogr* 79:3–24
129. Li C, Haug T, Stensvåg K (2010) Antimicrobial peptides in Echinoderms. *Invertebr Surviv J* 7:132–140
130. Solstad RG, Li C, Isaksson J, Johansen J, Svenson J, Stensvåg K, Haug T (2016) Novel antimicrobial peptides EeCentrocins 1, 2 and EeStrongylocin 2 from the Edible sea urchin *Echinus esculentus* have 6-br-tp post-translational modifications. *PLoS One* 11:1–25
131. Loker ES, Adema CM, Zhang SM, Kepler TB (2004) Invertebrate immune systems—not homogeneous, not simple, not well understood. *Immunol Rev* 198:10–24

132. Adibpour N, Nasr F, Nematpour F, Shakouri A, Ameri A (2014) Antibacterial and antifungal activity of *Holothuria leucospilota* isolated from Persian gulf and Oman Sea. *Jundishapur J Microbiol* 7:1–4
133. García-arrarás JE, Ramirez-gomez FJ (2010) Echinoderm immunity. *Invertebr Surviv J* 7:211–220
134. Abubakar L, Mwangi C, Uku J, Ndirangu S (2012) Antimicrobial activity of various extracts of the sea urchin *Tripneustes gratilla* (Echinoidea). *Afr J Pharmacol Ther* 1:19–23
135. Canicatti C, Roch P (1989) Studies on *Holothuria polk* (Echinodermata) antibacterial proteins. I. Evidence for and activity of a coelomocyte lysozyme. *Experientia* 45:756–759
136. Stabili L, Licciano M, Pagliara P (1994) Evidence of antibacterial and lysozyme-like activity in different planktonic larval stages of *Paracentrotus lividus*. *Mar Biol* 119:501–505
137. Leonard LA, Strandberg JD, Winkelstein JA (1990) Complement-like activity in the sea star, *Asterias forbesi*. *Dev Comp Immunol* 14:19–30
138. Beauregard KA, Truong NT, Zhang H, Lin W, Beck G (2001) The detection and isolation of a novel antimicrobial peptide from the *Echinoderm Cucumaria* Frondosa. In: Beck G, Sugumaran M, Cooper EL (eds) *Phylogenetic perspectives on the vertebrate immune system*. Springer US, Boston, pp 55–62
139. Service M, Wardlaw AC (1984) Echinochrome-a as a bactericidal substance in the coelomic fluid of *Echinus esculentus* (L.). *Comp Biochem Physiol B Biochem* 79:161–165
140. Ageenko NV, Kiselev KV, Dmitrenok PS, Odintsova NA (2014) Pigment cell differentiation in sea urchin blastula-derived primary cell cultures. *Mar Drugs* 12:3874–3891
141. Li C, Haug T, Styrvold OB, Jørgensen TØ, Stensvåg K (2008) Strongylocins, novel antimicrobial peptides from the green sea urchin, *li*. *Dev Comp Immunol* 32:1430–1440
142. Li C, Haug T, Moe MK, Styrvold OB, Stensvåg K (2010) Centrocin: isolation and characterization of novel dimeric antimicrobial peptides from the green sea urchin, *Strongylocentrotus droebachiensis*. *Dev Comp Immunol* 34:959–968
143. Björn C, Håkansson J, Myhrman E, Sjöstrand V, Haug T, Lindgren K, Blencke HM, Stensvåg K, Mahlapuu M (2012) Anti-infectious and anti-inflammatory effects of peptide fragments sequentially derived from the antimicrobial peptide centrocin 1 isolated from the green sea urchin, *Strongylocentrotus droebachiensis*. *AMB Exp* 2:67
144. Schillaci D, Arizza V, Parrinello N, Di Stefano V, Fanara S, Muccilli V, Cunsolo V, Haagensen JJA, Molin S (2010) Antimicrobial and antistaphylococcal biofilm activity from the sea urchin *Paracentrotus lividus*. *J Appl Microbiol* 108:17–24
145. Stabili L, Pagliara L, Roch P (1996) Antibacterial activity in the coelomocytes of the sea urchin *Paracentrotus lividus*. *Comp Biochem Physiol B* 113:639–644
146. Zou Z, Yi Y, Wu H et al (2005) Intercedensides D-I, cytotoxic triterpene glycosides from the sea cucumber *Mensamaria intercedens* lampert. *J Nat Prod* 68:540–546
147. Haug T, Kjuul AK, Styrvold OB, Sandsdalen E, Olsen ØM, Stensvåg K (2002) Antibacterial activity in *Strongylocentrotus droebachiensis* (Echinoidea), *Cucumaria frondosa* (Holothuroidea), and *Asterias rubens* (Asteroidea). *J Invertebr Pathol* 81:94–102
148. Wang H, Liu Y, Li M, Huang H, Xu HM, Hong RJ, Shen H (2010) Multifunctional TiO₂ nanowires-modified nanoparticles bilayer film for 3D dye-sensitized solar cells. *Optoelectron Adv Mater Rapid Commun* 4:1166–1169
149. Pereira DM, Valento P, Andrade PB (2014) Marine natural pigments: chemistry, distribution and analysis. *Dyes Pigments* 111:124–134
150. Babenkova IV, Teselkin IO, Makashova NV, Guseva MR (1999) Antioxidative activity of histochrome and some other drugs used in ophthalmology. *Vestn Oftalmol* 115:22–24
151. Heilmann C, Hussain M, Peters G, Götz F (1997) Evidence for autolysin-mediated primary attachment of *Staphylococcus epidermidis* to a polystyrene surface. *Mol Microbiol* 24:1013–1024
152. Schillaci D, Cusimano MG, Russo D, Arizza V (2014) Antimicrobial peptides from echinoderms as antibiofilm agents: a natural strategy to combat bacterial infections. *Ital J Zool* 81:312–321

153. Chen JY, Huang DY, Peng QQ, Chi HM, Wang XQ, Feng M (2003) The first tunicate from the early Cambrian of South China. *Proc Natl Acad Sci USA* 100:8314–8318
154. Stolfi A, Brown F (2015) Evolutionary developmental biology of invertebrates 6: Deuterostomia. Springer, Vienna, pp 135–204
155. Sings H, Rinehart K (1996) Compounds produced from potential tunicate-blue-green algal symbiosis: a review. *J Ind Microbiol* 17:385–396
156. Yankova L (2014) Chemical profiling and biological activity of two tunicate-associated marine bacteria. Honors Scholar Theses, p 336
157. Aassila H, Bourguet-Kondracki ML, Rifai S, Fassouane A, Guyot M (2003) Identification of Harman as the antibiotic compound produced by a tunicate-associated bacterium. *Mar Biotechnol* 5:163–166
158. Sikorska J, Parker-Nance S, Davies-Coleman MT, Vining OB, Sikora AE, McPhail KL (2012) Antimicrobial rubrolides from a south African species of *Synicum tunicate*. *J Nat Prod* 75:1824–1827
159. Karak M, Acosta JAM, Barbosa LCA, Boukouvalas J (2016) Late-stage bromination enables the synthesis of rubrolides B, I, K, and O. *Eur J Org Chem* 22:1099–0690
160. Bontemps N, Bry D, López-Legentil S, Simon-Levert A, Long C, Banaigs B (2010) Structures and antimicrobial activities of pyridoacridine alkaloids isolated from different chromotypes of the ascidian *Cystodytes dellechiaiei*. *J Nat Prod* 73:1044–1048
161. Tadesse M, Strøm MB, Svenson J, Jaspars M, Milne BF, Tørfoss V, Andersen JH, Hansen E, Stensvåg K, Haug T (2010) Synoxazolidinones A and B: novel bioactive alkaloids from the ascidian *Synicum pulmonaria*. *Org Lett* 12:4752–4755
162. Taylor SW, Craig AG, Fischer WH, Park M, Lehrer RI (2000) Styelin D, an extensively modified antimicrobial peptide from ascidian hemocytes. *J Biol Chem* 275:38417–38426
163. Woong SJ, Kyu NK, Young SL, Myung HN, In HL (2002) Halocidin: a new antimicrobial peptide from hemocytes of the solitary tunicate, *Halocynthia aurantium*. *FEBS Lett* 521:81–86
164. Wyche TP, Hou Y, Vazquez-Rivera E, Braun D, Bugni TS (2012) Peptidolipins B-F, antibacterial lipopeptides from an ascidian-derived *Nocardia* sp. *J Nat Prod* 75:735–740
165. Weber T, Laiple KJ, Pross EK, Textor A, Grond S, Welzel K, Pelzer S, Vente A, Wohlleben W (2008) Molecular analysis of the kirromycin biosynthetic gene cluster revealed β -alanine as precursor of the pyridone moiety. *Chem Biol* 15:175–188
166. Shannon E, Abu-Ghannam N (2016) Antibacterial derivatives of marine algae: an overview of pharmacological mechanisms and applications. *Mar Drugs* 14(81):1–23
167. Blunt JW, Munro MHG, Copp BR, Keyzers RA, Prinsep MR (2015) Marine natural products. *Nat Prod Rep* 32:116–211
168. Cardozo KHM, Guaratini T, Barros MP, Falcão VR, Tonon AP, Lopes NP, Campos S, Torres MA, Souza AO, Colepicolo P, Pinto E (2007) Metabolites from algae with economical impact. *Comp Biochem Physiol C Toxicol Pharmacol* 146(1–2):60–78
169. Holanda ML, Melo VM, Silva LM, Amorim RC, Pereira MG, Benevides NM (2005) Differential activity of a lectin from *Solieria filiformis* against human pathogenic bacteria. *Braz J Med Biol Res* 38:1769–1773
170. Lee SH, Kim SK (2015) Biological phlorotannins of *Eisenia bicyclis*. In: Kim SK, Chojnacka K (eds) Marine algae extracts: processes, products, and applications. Wiley, Oxford, pp 453–464
171. Besednova NN, Zaporozhets TS, Somova LM, Kuznetsova TA (2015) Review: prospects for the use of extracts and polysaccharides from marine algae to prevent and treat the diseases caused by *Helicobacter pylori*. *Helicobacter* 20:89–97
172. Kadam SU, O'Donnell CP, Rai DK, Hossain MB, Burgess CM, Walsh D, Tiwari BK (2015) Laminarin from Irish brown seaweeds *Ascophyllum nodosum* and *Laminaria Hyperborea*: ultrasound assisted extraction, characterization and bioactivity. *Mar Drugs* 13:4270–4280
173. Deyab MA, Abou-Dobara MI (2013) Antibacterial activity of some marine algal extracts against most nosocomial bacterial infections. *Egypt J Exp Biol* 9:281–286

174. Rajauria G, Abu-Ghannam N (2013) Isolation and partial characterization of bioactive fucoxanthin from *Himanthalia elongata* Brown seaweed: a TLC-based approach. *Int J Anal Chem* 2013:802573
175. Nguyen LT, Haney EF, Vogel HJ (2011) The expanding scope of antimicrobial peptide structures and their modes of action. *Trends Biotechnol* 29:464–472
176. Pierre G, Sopena V, Juin C, Mastouri A, Graber M, Maugard T (2011) Antibacterial activity of a sulfated galactan extracted from the marine alga *Chaetomorpha aerea* against *Staphylococcus aureus*. *Biotechnol Bioprocess Eng* 16:937–945

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