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REVIEW

Diversity, abundance and natural products of marine sponge-associated actinomycetes

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Usama Ramadan Abdelmohsen,*[ab] Kristina Bayer[b] and Ute Hentschel[b]

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Actinomycetes are known for their unprecedented ability to produce novel lead compounds of clinical and pharmaceutical importance. This review focuses on the diversity, abundance, and methodological approaches targeting marine sponge-associated actinomycetes. Additionally, novel qPCR data on actinomycete abundances in different sponge species and other environmental sources are presented. The natural products literature is covered, and we are here reporting on their chemical structures, their biological activities, as well as the source organisms from which they were isolated.

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1 Marine sponges

Marine ecosystems consist of taxonomically and biologically diverse macro- and microorganisms which exhibit unique physiological and structural features enabling them to survive under the extremes of pressure, salinity, and temperature. Many marine organisms are further endowed with the ability to produce novel molecules with interesting therapeutic applications not observed in their terrestrial counterparts.[1–3] Sponges (phylum Porifera) are among the oldest multicellular animals with a fossil record dating back to Precambrian times.[4] Sponges populate tropical reefs in great abundance but also the polar latitudes and the deep sea, as well as fresh water lakes and rivers.[5] They are sedentary filter-feeders capable of pumping thousands of liters of water per day.[6] Microorganisms and other food particles are removed from the flowing sea water and are then transported into the mesohyl interior, where they are digested by amoeboid archaeocytes that move freely through the extracellular matrix of the sponge. Sponges feed unselectively on particles up to 50 μm, which is about the maximum size that the pores allow.[7]

The interior of many sponge species is populated by dense and diverse microbial communities, including archaea, bacteria, fungi, and viruses.[8,9] The microbial biomass can occupy up to 35% of the sponge volume.[4] These types of sponges are classified as "high-microbial abundance" (HMA) sponges and harbor microbial concentrations in the range of 10^8–10^{10} cells g^{-1} sponge in their mesohyl matrix, which is two to four orders of magnitude higher than what is typically found in sea water.[10] The other group of sponges whose mesohyl is essentially free of microbial cells were classified as "low-microbial abundance" (LMA) sponges.[10] Here, bacterial numbers are in the range of 10^5–10^6 cells g^{-1} sponge, which is equivalent to the numbers found in natural sea water.[10] The reasons for this sharp dichotomy between HMA and LMA sponges remains unknown. Cultivation-independent techniques including 16S rRNA gene library construction, denaturing gradient gel electrophoresis (DGGE), fluorescence *in situ* hybridization (FISH) and more recently the powerful amplicon tag sequencing provided new insights into the vast microbial diversity of sponges.[5,11] At least 32 bacterial phyla and candidate phyla were described from marine sponges so far; with the most common phyla being Acidobacteria, Actinobacteria, Chloroflexi, Nitrospira, Cyanobacteria, Bacteriodetes, Gemmatimonadetes, Planctomycetes, Spirochaetes and Proteobacteria (Alpha-, Gamma-proteobacteria).[5,12,13]

Many potential benefits were attributed to the microbial symbionts including nutrient acquisition, processing of metabolic waste, and chemical defense, to name a few.[14–16] In return, the symbiotic microbial consortia receive a nutrient-rich habitat with ammonia, carbohydrates and amino acids in abundance. Provided that the symbionts can avoid being digested by the

[a]*Department of Pharmacognosy, Faculty of Pharmacy, Minia University, Minia, Egypt*
[b]*Department of Botany II, Julius-von-Sachs-Institute for Biological Sciences, University of Würzburg, Julius-von-Sachs-Platz 3, 97082 Würzburg, Germany. E-mail: usama.ramadan@uni-wuerzburg.de; Fax: +49-931-3186235; Tel: +49-931-3180297*

host sponge, the mesohyl should be a preferable ecological niche over the nutrient-poor sea water. The high density and diversity of microorganisms in the mesohyl matrix likely promotes various forms of interaction and also communication between the microbial players but also between microorganisms and the animal host. This hypothesis is supported by the identification of quorum sensing signal molecules such as acyl homoserine lactones that were found to play a role in sponge-microbe interactions.[17] Further chemical defense is likely to be of importance for microbial survival within sponges. Indeed, in some cases, the microbial symbionts were shown to be involved in the biosynthesis of defense compounds originally attributed to the host sponge.[18–23] However, the lack of cultivation of any *bona fide* sponge symbiont represents a major bottleneck and we are just beginning to understand the plethora of possible chemical interactions in sponge microcosms. For more information on marine sponges as well as sponges microbiology, review papers by Hentschel *et al.*,[4] Taylor *et al.*,[12] as well as Webster and Taylor,[16] are recommended.

Usama Ramadan Abdelmohsen received his BSc in Pharmaceutical Sciences from Minia University, Egypt, in 2002. He received his PhD with an Egyptian fellowship award from the University of Würzburg, Germany, with his thesis entitled "Antimicrobial activities from plant cell cultures and marine sponge-associated actinomycetes" under the guidance of Professor Ute Hentschel. Since then, he has been working in the Hentschel lab as a postdoctoral researcher. His academic interests are the isolation and structure elucidation of anti-infective secondary metabolites from marine sponge-associated actinomycetes with a particular focus on using spectroscopic, genomic, and metabolomic tools to discover new compounds.

Kristina Bayer received her Diploma and PhD degrees at the University of Würzburg where she studied the physiology and phylogeny of ammonia-oxidizing bacteria and archaea in marine sponges as well as their various functions in the nitrogen cycle. Since 2008 she has been working as a postdoctoral researcher in the lab of Ute Hentschel on the elucidation of community structure and function of microbial symbionts in sponges using a wide range of molecular tools, including single cell genomics, DNA functional gene arrays, real-time PCR, microscopic techniques, and gene cloning.

2 Actinomycete diversity

It has long been known that actinomycetes can be cultured from marine sources,[24] yet it was not clear whether these typically soil-derived bacteria should be considered as terrestrial "contaminants" or as true components of the marine ecosystem.[25] Early evidence for the existence of indigenous marine actinomycete populations came from the description of the first marine species, *Rhodococcus marinonascene* in 1984,[26] the metabolic activity of some *Streptomyces* strains in marine sediments[27] and the isolation of obligate marine strains.[28] A total of 10 400 actinomycete 16S rRNA gene sequences were thus far obtained by isolation from marine sources (Fig. 1). This compares to about 36 000 16S rRNA gene sequences from terrestrial actinomycetes, which, however, have a much longer history of exploration. From the marine environment, actinomycetes were cultivated from sea water[29] and marine

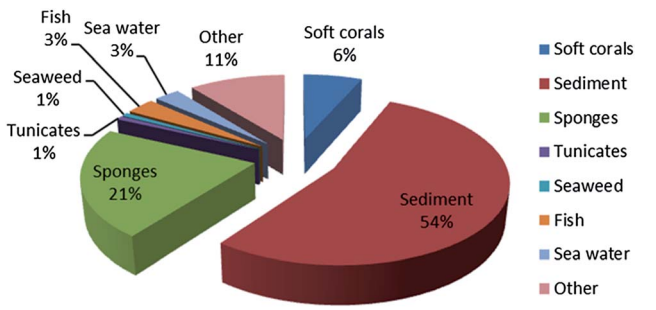


Fig. 1 Distribution of actinomycete-derived 16S rRNA gene sequences (in total 10 400) in the marine environment.

Ute Hentschel obtained her PhD degree in marine biology at Scripps Institution of Oceanography, La Jolla, USA which was followed by postdoctoral research in infection biology at UC Santa Barbara, CA, USA. She became a young investigator group leader in 2004 at the Research Center for Infectious Diseases at the University of Würzburg, Germany. Since 2008, she has been a full professor at the Julius-von-Sachs Institute for Biological Sciences, University of Würzburg, Germany. Her research interests include host-microbe interactions, with a special focus on the diversity, function, and natural products of microorganisms associated with marine sponges.

sediments.[30,31] Actinomycetes were also found in association with different marine invertebrates such as soft corals, tunicates and fish[32–35] but the majority was isolated from sponges.[36–38]

In order to assess the diversity of marine sponge-associated actinomycetes, we constructed a maximum-likelihood phylogenetic tree of all actinomycete 16S rRNA gene sequences with a length of >1300 bp and that were available in the NCBI database in August 2013. One representative sequence of each genus was chosen for the tree construction and altogether 60 different genera were identified as being derived from sponge sources (Fig. 2). The suborder Micrococcineae represents almost half of the genera isolated from marine sponges, among them *Micrococcus*, *Microbacterium*, and *Arthrobacter*, which are readily isolated because of their fast-growing nature. However, their potential for secondary metabolism appears to be limited to few reports.[39,40] On the contrary, the single genus *Streptomyces* is represented by hundreds of sequence entries that were obtained from many different sponge species, and many of which display novel chemistry. Marine sponges are not only a rich source for diverse actinomycetes but also an impressive habitat for new and rare actinomycete genera. Rare genera that have been recovered from sponges include *Actinokineospora*, *Actinomadura*, *Amycolatopsis*, *Knoellia*, *Nonomuraea*, *Pseudonocardia*, *Saccharomonospora*, *Saccharopolyspora*, and *Verrucosispora*, and targeting them could provide novel lead compounds in the future.

The number of descriptions of new actinomycete species and even genera from sponge sources is continuously rising.[42–45] One example is the obligate marine genus *Salinispora* represented by *S. arenicola*, *S. tropica* and *S. pacifica* which were discovered originally in sediments[46] but were since then also found in sponges such as *Pseudoceratina clavata* from the Great Barrier Reef.[47] Several additional obligate marine new species were isolated, such as *Streptomyces axinellae* sp. nov. from the marine sponge *Axinella polypoides* collected from Banyuls-sur-Mer, France[48] and *Saccharopolyspora cebuensis* sp. nov. isolated from the sponge *Haliclona* sp. collected from Cebu, Philippines.[49] *Micromonospora yangpuensis* sp. nov. (from an unidentified sponge) and *Actinoalloteichus hymeniacidonis* sp. nov. (from *Hymeniacidon perleve*) were both isolated from the Dachan reef, China.[50,45] The novel actinomycete *Tsukamurella*

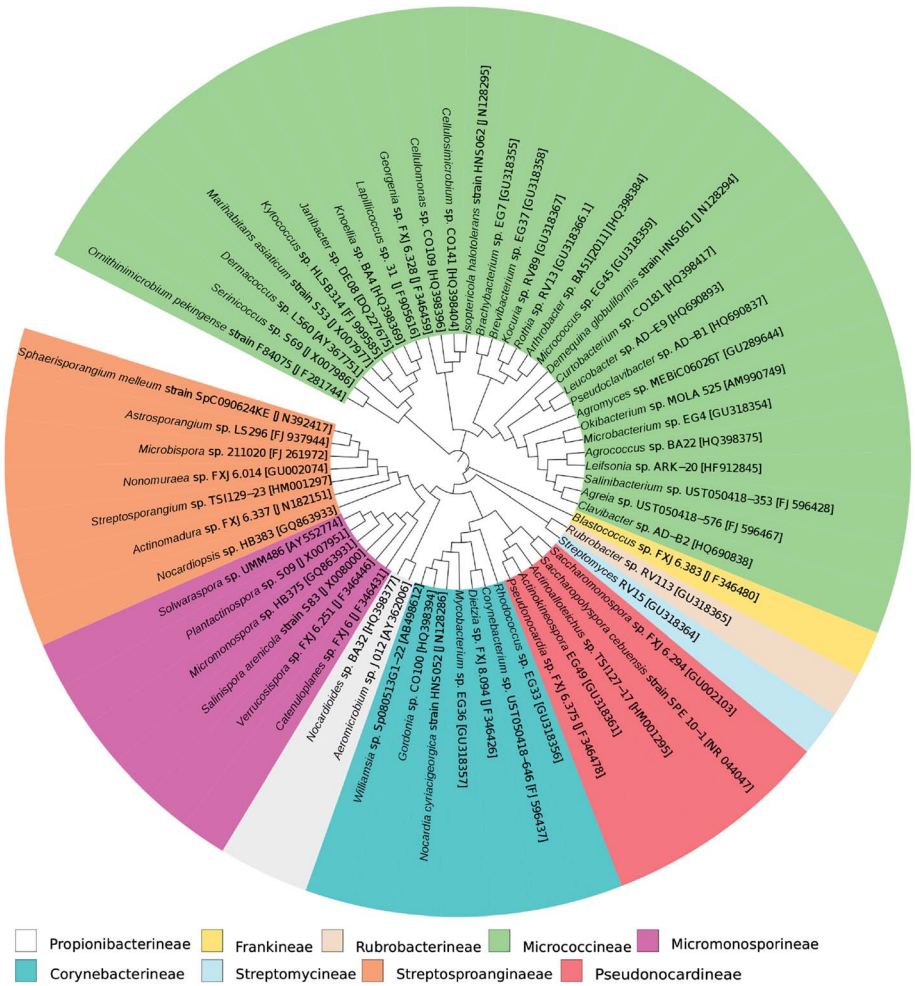


Fig. 2 Maximum likelihood phylogenetic tree based on 16S rRNA gene sequences of sponge-associated actinomycete genera derived from literature and NCBI database. The tree was visualized and labeled using the Interactive Tree of Life V2.[41]

Table 1 Actinomycete genera isolated from different marine sponges

Host sponge	Identified genera	Geographical location	Reference
Agelas sp.	*Micromonospora*	Caribbean, Puerto Rico	64
Agelas sceptrum	*Streptomyces*	Caribbean, Puerto Rico	64
Amphimedon sp.	*Kocuria*, *Microbacterium*, *Micrococcus*	Red Sea, Egypt	33
Aplysina aerophoba	*Gordonia*, *Micromonospora*, *Mycobacterium*, *Rubrobacter*	Mediterranean, Croatia	33
Aplysina fistularis	*Knoellia*, *Streptomyces*	Bahamas	65
Aplysina insularis	*Dietzia*, *Saccharopolyspora*	Colombia	65
Axinyssa sp.	*Catenuloplanes*, *Micromonospora*, *Streptomyces*, *Verrucosispora*	South China Sea, China	66
Axinella sp.	*Micromonospora*, *Nocardiopsis*, *Streptomyces*	South China Sea, China	66
Agelas clathrodes	*Cellulomonas*, *Gordonia*	Bahamas	65
Callyspongia aff. implexa	*Brevibacterium*, *Brachybacterium*, *Corynebacterium*, *Kocuria*, *Mycobacterium*, *Microbacterium*, *Micrococcus*	Red Sea, Egypt	33
Cinachyra sp.	*Actinomadura*, *Microbispora*, *Micromonospora*, *Nocardia*, *Nocardiopsis*, *Nonomuraea*, *Rhodococcus*, *Sphaerosporangium*, *Streptomyces*, *Streptosporangium*	Japan	67
Craniella australiensis	*Streptomyces*, *Pseudonocardia*	Yellow Sea, China	68
Desmapsamma anchorata	*Micromonospora*, *Verrucosispora*	Caribbean, Puerto Rico	64
Dragmacidon reticulata	*Rhodococcus*	Colombia	65
Discodermia dissoluta	*Curtobacterium*, *Mycobacterium*	Colombia	65
Dysidea tupha	*Corynebacterium*, *Kocuria*, *Rothia*, *Streptomyces*,	Mediterranean, Croatia	33
Dysidea avara	*Corynebacterium*, *Micromonospora*, *Nocardiopsis Rhodococcus*	Mediterranean, Croatia	33
Dysidea sp.	*Actinomadura*, *Micromonospora*, *Streptomyces*, *Verrucosispora*	South China Sea, China	66
Erylus formosus	*Arthrobacter*	Bahamas	65
Ectyoplasia ferox	*Micromonospora*, *Salinispora*	Caribbean, Puerto Rico	64
Gelliodes carnosa	*Gordonia*, *Micromonospora*, *Pseudonocardia*, *Rhodococcus*, *Streptomyces*, *Sphaerisporangium*, *Saccharomonospora*, *Verrucosispora*	South China Sea, China	69
Halichondria panicea	*Actinoalloteichus*, *Micrococcus*, *Micromonospora*, *Nocardiopsis*, *Streptomyces*	Baltic Sea, Germany	70
Halichondria rugosa	*Streptomyces*	Yellow Sea, China	68
Hymeniacidon perleve	*Gordonia*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Salinispora*, *Streptomyces*	South China Sea, China	71
Hymeniacidon perleve	*Actinomadura*, *Blastococcus*, *Georgenia*, *Kocuria*, *Micromonospora*, *Pseudonocardia*, *Streptomyces*	Yellow Sea, China	66
Hymeniacidon perleve	*Actinoalloteichus*, *Micromonospora*, *Nocardia*, *Nocardiopsis*, *Pseudonocardia*, *Rhodococcus*, *Streptomyces*	Yellow Sea, China	38
Hyrtios erecta	*Brevibacterium*, *Corynebacterium*, *Microbacterium*, *Micrococcus*, *Rhodococcus*, *Salinispora*	Red Sea, Egypt	33
Hyrtios erectus	*Nocardiopsis sp.*, *Kocuria sp.*, *Curtobacterium sp.*, *Micrococcus sp.*	Red Sea, Egypt	72
Hemimycale columella	*Gordonia*, *Kocuria*, *Mycobacterium*, *Mycobacterium Rhodococcus*	Mediterranean, Croatia	33
Iotrochota birotulata	*Cellulosimicrobium*	Caribbean, Puerto Rico	64
Myrmekioderma styx (new name *Myrmekioderma rea*)	*Micromonospora*	Caribbean, Puerto Rico	64
Mycale laevis	*Micromonospora*	Caribbean, Puerto Rico	64

Table 1 (*Contd.*)

Host sponge	Identified genera	Geographical location	Reference
Negombata magnifica	*Microbacterium*, *Rhodococcus*	Red Sea, Egypt	33
Petrosia weinbergi	*Micromonospora*	Caribbean, Puerto Rico	64
Plakortis sp.	*Kocuria*	Bahamas	65
Plakinastrella onkodes	*Cellulosimicrobium*	Colombia	65
Prosuberites laughlini	*Microbacteriaceae*, *Micromonospora*, *Salinispora*, *Solwaraspora*	Caribbean, Puerto Rico	64
Pseudocorticium sp.	*Micromonospora*, *Salinospora*	Caribbean, Puerto Rico	64
Reniochalina sp.	*Streptomyces*	Yellow Sea, China	68
Reniochalina sp.	*Gordonia*, *Micromonospora*, *Nonomuraea*, *Streptomyces*	Yellow Sea, China	66
Rhopaloeides odorabile	*Gordonia*, *Pseudonocardia*	Great Barrier Reef, Australia	56
Scopalina ruetzleri	*Agrococcus*, *Lapillicoccus*, *Microbacterium*, *Micromonospora*, *Salinispora*	Bahamas	65
Scopalina ruetzleri	*Micromonospora*, *Streptomyces*	Caribbean, Puerto Rico	64
Spheciospongia vagabunda	*Actinokineospora*, *Arthrobacter*, *Brevibacterium*, *Micrococcus*, *Microbacterium*	Red Sea, Egypt	33
Spongia sp.	*Micromonospora*, *Streptomyces*	South China Sea, China	66
Unidentified sponge sp.	*Nocardia*, *Pseudonocardia*, *Streptomyces*	Yellow Sea, China	68
Stelletta tenuis	*Pseudonocardia*, *Streptomyces*	Yellow Sea, China	68
Xestospongia sp.	*Micromonospora*, *Streptomyces*, *Saccharomonospora*, *Verrucosispora*	South China Sea, China	66
Xestospongia muta	*Verrucosispora*	Caribbean, Puerto Rico	64

spongiae sp. nov. was isolated from a deep-water marine sponge collected off the coast of Curaçao, Netherlands Antilles.[51] Three novel actinomycetes, *Streptomyces tateyamensis* sp. nov., *S. marinus* sp. nov., and *S. haliclonae* sp. nov., were isolated from *Haliclona* sp. offshore of Tateyama City, Japan.[52] Another recent example is *Verrucosispora andamanensis* sp. nov., isolated from *Xestospongia* sp. collected from the Andaman Sea, Thailand.[42]

The success in the discovery of rare and novel actinomycete species from marine sponges relies, besides the exclusiveness of sponges as a niche, on the use of appropriate isolation protocols.[43,53] For example, heat shock is frequently used to reduce the numbers of Gram negative bacteria from sea water to prevent overgrowth.[54] Pretreatment with UV radiation and high frequency waves was shown to effectively stimulate spore germination.[55] Cultivation media are frequently supplemented with antibiotics (cycloheximide, nystatin) to inhibit fungal growth and to inhibit Gram negative bacteria.[56] Media with a low-nutrient composition enhance the growth of oligotrophic bacteria that are in abundance in the marine environment.[57] The addition of aqueous sponge extract to M1 medium resulted in the isolation of a new species, *Rubrobacter aplysiniae*, which showed only moderate sequence similarity to other members of the genus *Rubrobacter*.[58] This serves as one example for the effectiveness of new methods to recover actinomycete diversity from sponges and other sources.[33,59] New approaches such as encapsulation of cells in gel microdroplets[60] or the employment of diffusion chambers,[61] microbial traps,[62] and isolation chips[63] were recently deployed in terrestrial environments and resulted in the isolation of rare and unusual actinomycete species.[43,53]

The distribution of actinomycetes in host sponges does not reveal any patterns that would point to a specific host-symbiont relationship. Rather, the actinomycetes appear to be distributed randomly in the host sponges investigated (Table 1). The sponge species *Hymeniacidon perleve* was repeatedly examined from the two locations offshore of China using different media formulations.[38,66,71] With the exception of *Streptomyces*, most other actinomycete genera were variably present in the three sponges investigated. Similarly, there is no consistent pattern among the three closely related *Aplysina* sponges and neither among the three *Dysidea* sponge species. However, since a systematic study with replicate sampling over space and time aiming to resolve patterns of host specificity is still lacking, it is too early to draw any conclusions. A comprehensive study by Vicente and coworkers also revealed no evidence for a specific relationship between actinomycetes and the host sponges from which they were isolated. Rather sedimentation rate was identified as a determining factor in that sedimentation rich habitats provided more actinomycete diversity and higher numbers of isolation than pristine waters.[64] In contrast to the sponge-specific microbial consortia consisting of other phyla that are vertically transmitted through the reproductive stages and permanently associated with their host sponge,[4] the actinomycetes are very likely taken up from the environment by filtration and appear to persist in the mesohyl matrix.

3 Actinomycete abundance

Although several studies were carried out on actinomycete diversity from sponges, quantitative data on their abundances in the sponge ecosystem are rare. We therefore tested the copy numbers of 16S rRNA genes of Actinobacteria in different sponge species and compared them to other environmental

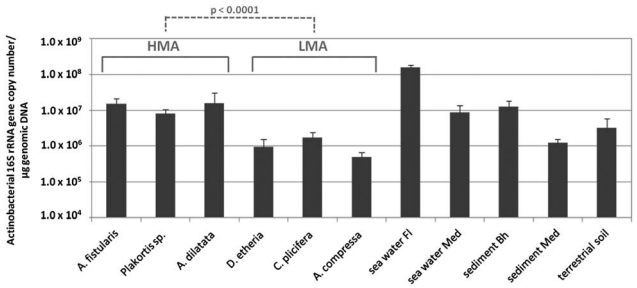


Fig. 3 Copy number of actinobacterial 16S rRNA genes per µg genomic DNA in sponges, sea water, marine sediments and terrestrial soil. Fl = Florida, Med = Mediterranean, Bh = Bahamas. Each bar represents three sponge individuals per species and each assay was performed in triplicates with amplification efficiencies between 95.0–97.3%. Based on accurate concentration measurements and product length, a calculation of copy number was performed as described previously.[73] Statistics were performed by Mann–Whitney-*U* test using GraphPad Prism version 6.01 for windows.

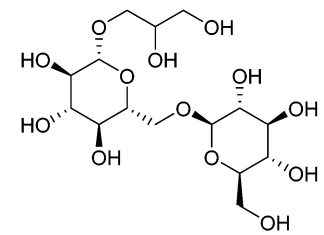
samples (sea water, marine sediment, terrestrial soil) using quantitative real time PCR (qPCR) (see supplemental material for methodological details). Previously described primers which showed a high coverage of 91.0% *in silico* for the order Actinomycetales using the ARB-Silva database (http://www.arb-silva.de/search/testprime/) were adapted for this purpose. Copy numbers ranging from 8.25×10^6–1.60×10^7 per µg genomic DNA were found in the HMA sponges *Aplysina fistularis*, *Plakortis* sp. and *Agelas dilatata*. The numbers of actinobacterial 16S rRNA genes were significantly lower (5.00×10^5–1.72×10^6) in the LMA sponges *Dysidea etheria*, *Callyspongia plicifera*, and *Amphimedon compressa* (Fig. 3). The calculated copy numbers are 2–3 orders of magnitude higher than described previously by Noyer *et al.*[73] for the two Mediterranean species, *A. aerophoba* and *Spongia lamella*. This is not surprising since Noyer *et al.* used a primer pair which showed coverage of only 2% for the phylum Actinobacteria using *in silico* PCR. In sea water, marine sediments and terrestrial soil, we detected 1.25×10^6–1.56×10^8 actinobacterial 16S rRNA gene copy numbers per µg genomic DNA. This implies that marine sponges are better sources for Actinobacteria since larger volumes of sea water (approx. 3 L) and marine sediments (approx. 250 mg) are needed to extract the same amount of genomic DNA when compared to sponges (7.5–20 mg).

4 Actinomycete natural products

Among the different microbial phyla in marine ecosystems, actinomycetes produced the major fraction of natural products,[74–79] with bioactivities including antibacterial, antifungal, antiparasitic, antimalarial, immunomodulatory, anti-inflammatory, antioxidant, and anticancer activities.[80–84] These diverse bioactivities are mediated by several classes of compounds including polyketides, alkaloids, fatty acids, peptides and terpenes.[82,83,85–88]

Four unusual glycoglycerolipids and one diphosphatidylglycerol were isolated from *Microbacterium* sp. strain HP2, cultivated from the sponge *Halichondria panacea* collected from the Adriatic coast, Rovinj, Croatia. These compounds were identified as GGL.1 1,2-*O*-diacyl-3-[β-glucopyranosyl-(1–6)-β-glucopyranosyl)]glycerol (**1**), GGL.2 1-*O*-acyl-3-[α-glucopyranosyl- (1–3)-(6-*O*-acyl-α-mannopyranosyl)]glycerol (**2**), GGL.3 1-*O*-acyl-3-[6-*O*-acetyl- α-glucopyranosyl-(1–3)-(6-*O*-acyl-α-mannopyranosyl)] glycerol (**3**) and GGL.4 1,2-*O*-diacyl-3-[β-galactofuranosyl)]glycerol (**4**).[89] The major compound GGL.2 showed antitumor activity by inhibiting growth of the tumor cell lines HM02 and Hep G2 with GI_{50} values of 0.38 and 2.7 µg ml^{-1}, respectively.[40]

Lutoside (**5**), an acyl-1-(acyl-6′-mannobiosyl)-3-glycerol was isolated from *Micrococcus luteus*, cultivated from the sponge *Xestospongia* sp. which was collected by scuba diving off Noumea, New Caledonia. The known synthetic 2,4,48-trichloro-28-hydroxydiphenylether was isolated from the same strain and it was active against *Staphylococcus aureus*, *Vibrio anguillarum* and *Candida albicans*.[39]



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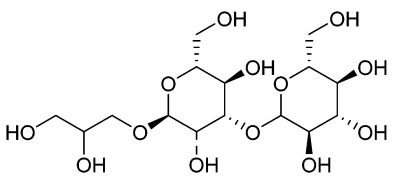

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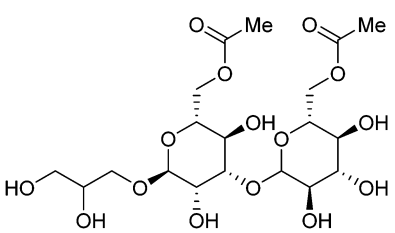

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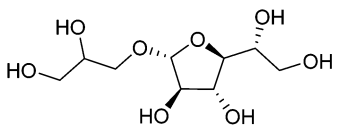

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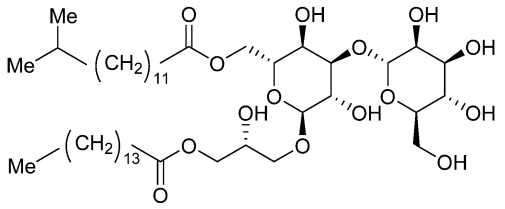

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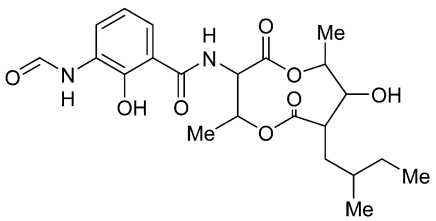
Two new antimycins urauchimycin A and B (**6**,**7**) were isolated from the ethyl acetate extract of *Streptomyces* sp. strain NI80 obtained from an unidentified sponge sp. collected at Urauchicove, Iriomote, Japan. These compounds consist of a 9-membered dilactone moiety which is characteristic for antimycin antibiotics. Both compounds showed antifungal activity against *Candida albicans* at a concentration of 10 μg ml^{-1}.[90] In 2006, two new urauchimycin derivatives urauchimycin C and D (**8**,**9**) were isolated from *Streptomyces* sp. isolate B1751.[91] They were tested against *Escherichia coli* and *Staphylococcus aureus* as well as fungi including *Candida albicans* and *Mucor miehei*, but in contrast to urauchimycins A and B, urauchimycins C and D were inactive.

The new nucleoside derivative 3-acetyl-5-methyl-2′-deoxyuridine (**10**) and two known compounds 3,5-dimethyl-2′-deoxyuridine (**11**) and 3-methyl-2′-deoxyuridine (**12**), were purified from the ethyl acetate extract of the broth culture of *Streptomyces microflavus* associated with the sponge *Hymeniacidon perlevis* (Dalian, Yellow Sea, China).[92] Interestingly, 3-methyl-2′-deoxyuridine had previously been obtained from the sponge *Geodia baretti*,[93] collected in Swedish waters, suggesting that the sponge might not be the actual producer.

Rifamycins are a group of antibiotics that belong to the ansamycin family with pronounced activities against Gram positive bacteria. They were previously isolated from terrestrial actinomycetes such as *Amycolatopsis mediterranei*. Rifamycins B and SV (**13**,**14**) were found to also be produced by the *Salinispora* sp. strain M403, cultivated from the marine sponge *Pseudoceratina clavata*.[94] It was first predicted by phylogenetic analysis of the ketosynthase (KS) gene sequences of *Salinispora* M403, which indicated that the polyketide synthase (PKS) gene sequence is most closely related to that of the rifamycin B synthase of *Amycolatopsis mediterranei*. Liquid chromatography-tandem mass spectrometry analysis demonstrated that the *Salinispora* sp. strain M403 produces rifamycins B and SV.

Four new cyclic lipopeptides, cyclo-(AFA-Ser-Gln-Asn-Tyr-Asn-Ser-Thr), cyclodysidins A–D (**15–18**) were isolated from the fermentation culture of the *Streptomyces* strain RV15, associated with the marine sponge *Dysidea tupha*. They have the same amino acid composition but differ in the fatty acid side chain part.[95] The compounds were inactive when tested against bacteria, fungi, and parasites. The absolute stereostructures were determined by Marfey's analysis followed by HPLC, showing that the α-amino acid building blocks were L in all



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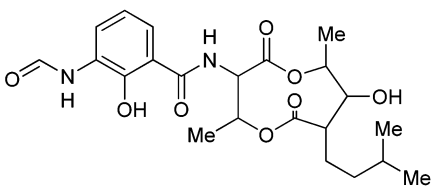

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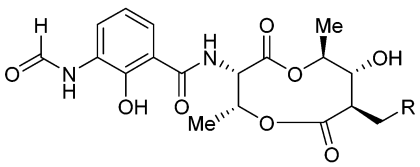
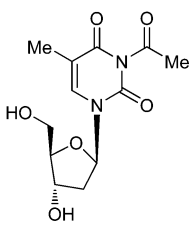


8: R= Me; **9**: R= H



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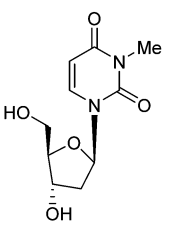

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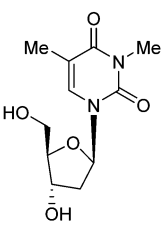

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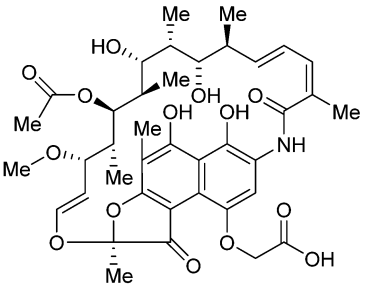

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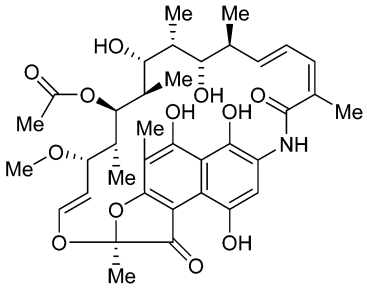

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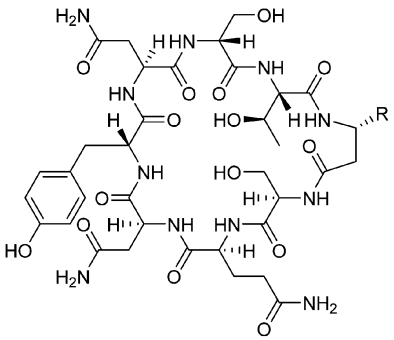


15: R= $-(CH_2)_9-CH_3$

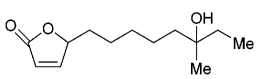
16: R= $-(CH_2)_{10}-CH_3$

17: R= $-(CH_2)_{11}-CH_3$

18: R= $-(CH_2)_{13}-CH_3$

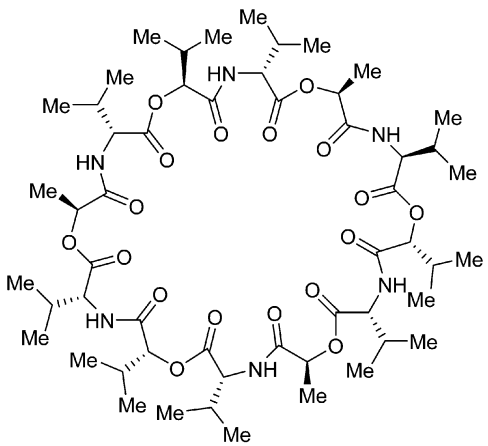
cases except for tyrosine and serine, which were found to possess the D-configuration. The β-amino acid blocks were assigned to a D-configuration.

The lactone-derived compound, butenolide (**19**) was produced by a *Streptomyces* sp. obtained from the sponge *Tethya* sp., as well as from the marine sediment-derived *Streptomyces* sp. strain M027750.[96] Butenolide exhibited antitrypanosomal activity against *Trypanosoma brucei brucei* with an IC_{50} value of 0.022 μM.[80] Butenolide and several analogues were reported from various marine sources including sponges,[97] soft corals,[98] bacteria,[99] fungi[100] as well as terrestrial sources including plants[101] and endophytic fungi,[102] A range of biological activities was detected for butenolides including antimicrobial, anti-fouling, estrogenic, serotonergic, anticancer and anti-HIV activities.[98,103,104]



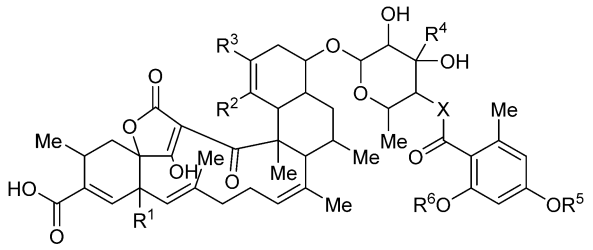
19

The cyclic depsipeptide, valinomycin (**20**) was purified from a *Streptomyces* sp. recovered from the sponge *Aplysina aerophoba*, as well as from previous terrestrial actinomycetes.[105] Valinomycin exhibited significant inhibitory activities against the parasites *Leishmania major* (IC_{50} < 0.11 μM) and *Trypanosoma brucei brucei* (IC_{50} 0.0032 μM).[80]



20

The new tetronic acid derivatives, tetromycins 1–4 (**21–24**), were isolated from *Streptomyces axinellae* Pol001T, which had been cultivated from the Mediterranean sponge *Axinella polypoides*. Tetromycins 3–4 showed protease inhibition activities against several cysteine proteases.[106] The compounds inhibited cathepsin-L like enzymes in time-dependent manner and this inhibition was observed neither with cathepsin B nor with the coronaviral protease PLpro. They exhibited pronounced activity against Gram positive bacteria including methicillin-resistant *Staphylococcus aureus*.[107]



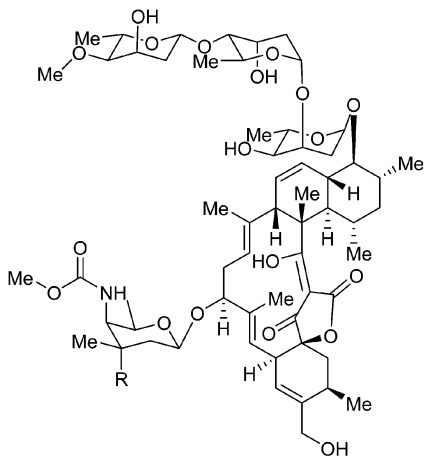
21: R^1=R^3=R^4=R^5= Me, R^2=R^6= H, X= NH

22: R^1=R^3=R^5=R^6= Me, R^2=R^4= H, X= O

23: R^2=R^6= Me, R^1=R^3=R^4=R^5= H, X= O

24: R^1=R^3=R^6= Me, R^2=R^4=R^5= H, X= O

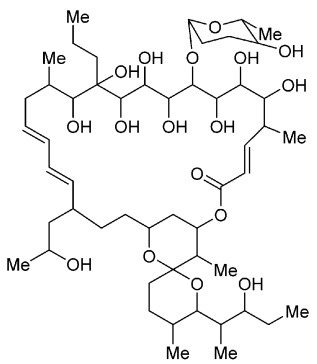
Bioassay-guided isolation and purification from the isolate *Streptomyces carnosus* strain AZS17 cultivated from the sponge *Hymeniacidon* sp. yielded two new kijanimicin derivatives, lobophorin C (**25**) and D (**26**). Kijanimicin has a unique tetronic acid structure and was first isolated from the terrestrial strain *Actinomadura kvaniata* nov. sp. SCCl256. Lobophorin C displayed potent cytotoxic activity against the human liver cancer cell line 7402 with IC_{50} values of 0.6 μg mL^{-1}, while lobophorin D showed a good inhibitory effect on the growth of human breast cancer cells MDA-MB 435 with an IC_{50} value of 7.5 μM, which might suggest a selective cytotoxicity against these cancer cell lines.[108] Lobophorins C and D have similarity in their structures to the other kijanimicin derivatives which did not exhibit antibiotic activities, but potent anti-inflammatory activities which was likely attributed to the differences in the stereochemistry of the two groups of compounds.



25: R= NO_2, **26**: R= NH_2

The indolocarbazole alkaloid, staurosporine (**27**) was isolated from *Streptomyces* sp. strain 11 cultivated from the sponge *Tedania* sp. It showed significant anti-parasitic activity against *Leishmania major* with IC_{50} 5.30 μM and *Trypanosoma brucei brucei* with IC_{50} 0.022 μM.[80] The parent molecule staurosporine was first isolated from the fermentation broth of the soil actinomycete *S. staurosporeus*.[109] Staurosporine showed inhibition of protein kinases through the prevention of ATP binding to the kinase with dissociation constant value, K_d < 3 μM.[110] Later, two staurosporine derivatives, 4′-*N*-methyl-5′-hydroxystaurosporine (**28**) and 5′-hydroxystaurosporine (**29**) and the known staurosporine, were isolated from *Micromonospora* sp. L-31-CLCO-002, cultivated from the sponge *Clathrina coriacea* which was collected offshore of Fuerteventura (Canary Islands).[111] Staurosporine, 4′-*N*-methyl-5′-hydroxystaurosporine and 5′-hydroxystaurosporine showed cytotoxic activities against murine macrophague P388D1 (IC_{50} of 0.01, 0.02 and 0.04 μg mL^{-1}), human lung adenocarcinoma A549 (IC_{50} of 0.0005, 0.002 and 0.004 μg mL^{-1}), colon adenocarcinoma HT-29 (IC_{50} of 0.02, 0.004 and 0.004 μg mL^{-1}) and melanoma SK-MEL-28 cell lines (IC_{50} of 0.001, 0.002 and 0.004 μg mL^{-1}).

IB-96212 (**30**), is a 26-membered spiroketal macrolide which was purified from the mycelial extracts of *Micromonospora* sp. L-25-ES25-008, isolated from an unidentified sponge (Indian Ocean, Mozambique).[112] IB-96212 showed cytotoxic activity against mouse leukemia P-388, human lung non-small carcinoma A-549, colon adenocarcinoma HT-29 and melanoma MEL-28 cell lines.[113]



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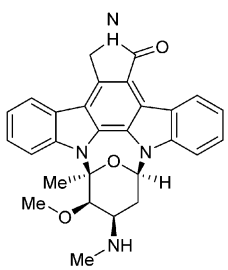
Bioassay-guided fractionation of chloroform extracts from the fermentation broth of *Saccharopolyspora* sp. nov., isolated



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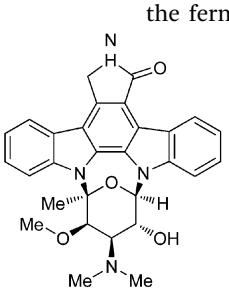

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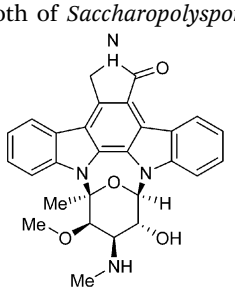


29

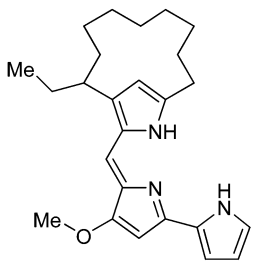
from the sponge *Mycale plumose* (offshore of Qingdao, China), yielded metacycloprodigiosin (**31**) and undecylprodigiosin (**32**). They exhibited significant cytotoxic activities *in vitro* against five cancer cell lines; mouse lymphoma P388, human peripheral blood promyeloblast HL60, lung carcinoma A- 549 and SPCA4 and hepatic carcinoma BEL-7402 with IC_{50} values between 0.007 and 7.52 μM for metacycloprodigiosin and 0.013 to 0.11 μM for undecylprodigiosin.[114] Prodigiosin (**33**) is a polypyrrole red pigment and was first identified from *Serratia marcescens* and exhibited anticancer and immunosuppressive activities.[115,116] Metacycloprodigiosin was also isolated from fermentation broth of *Streptomyces spectabilis* BCC 4785 using bioassay-guided fractionation. Metacycloprodigiosin showed significant antiplasmodial activity against human malaria parasite *Plasmodium falciparum* K1, with an IC_{50} of 0.005 μg ml^{-1}.[117]

The benzo[α]naphthacene quinine SF2446 A2 (**34**) was isolated from the broth culture of *Streptomyces* sp. strain RV15, which was cultivated from the sponge *Dysidea tupha*. It was previously reported that SF2446 A2 exhibited strong activity against Gram positive bacteria and mycoplasmas including macrolide-resistant strains such as *Mycoplasma gallisepticum* with little activity against Gram negative bacteria and fungi.[118] In our study, it showed new activity against *Trypanosoma brucei* with an IC_{50} value of 3.05 μM which was not previously described (unpublished data).

The two new macrolactams cebulactams A1 and A2 (**35**,**36**) were isolated from the ethyl acetate extract of the obligate marine *Saccharopolyspora cebuensis* type strain SPE 10-1, derived from the sponge *Haliclona* sp. that was collected from Cebu, Philippines.[119] The compounds were tested against bacteria, fungi and parasites but no activity was detected.

Three diketopiperazines (**37**–**38**) were reported from *Micrococcus* sp., cultivated from the sponge *Tedania ignis*. These diketopiperazines were previously isolated from the sponge itself and this was the first evidence that a bacterium associated with a sponge might produce secondary compounds ascribed to the host sponge.[120]

Fractionation of *Actinomadura* sp. SBMs009 cultivated from the sponge *Suberites japonicus* lead to the discovery of 3-keto sterols named bendigoles D–F (**40**–**42**).[121] The purification was based on their NF-κB inhibition and glucocorticoid receptor–protein binding properties targeting the anti-inflammatory activity. Bendigole F showed the highest activity against translocation of GFP-labeled NF-κB into the nucleus of hamster ovary CHO cells *in vivo* with an IC_{50} of 71 μM. The three sterols displayed activity against the glucocorticoid receptor translocation and bendigole D was the most potent. The MTT assays showed that bendigoles D and E are clearly nontoxic to the L929 murine aneuploid fibrosarcoma, while bendigole D displayed mild cytotoxicity against the L929 cell line with an IC_{50} of 30 μM.



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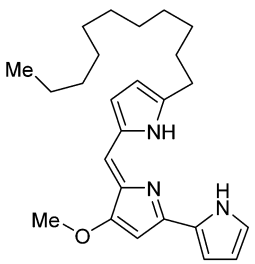

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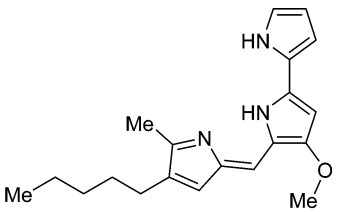

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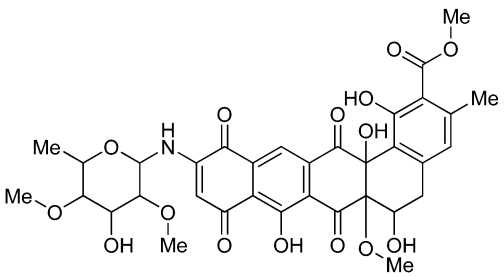

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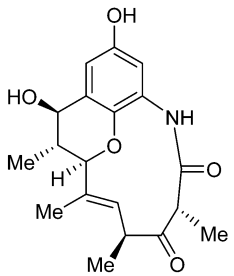

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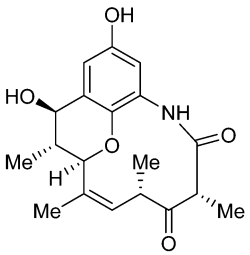

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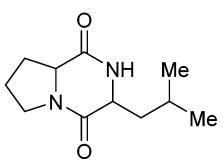

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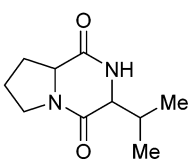

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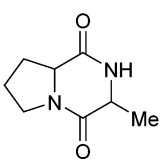

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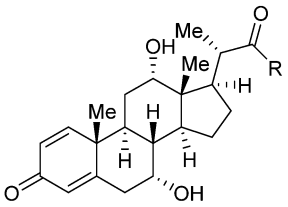
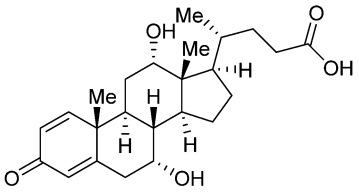


40: R= OH, **41**: R= Me



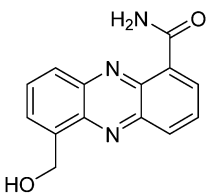
42

Two new antibacterial phenazines, 6-hydroxymethyl-1-phenazine-carboxamide (**43**) and 1,6-phenazinedimethanol (**44**) were isolated from the culture broth of *Brevibacterium* sp. KMD 003, isolated from *Callyspongia* sp. (Kyeongpo, Gangwondo, Korea). The two compounds showed antibacterial activities against *Enterococcus hirae* and *Micrococcus luteus* with an IC_{50} value of 5 μM.[122]

The farnesylated dibenzodiazepinone alkaloid diazepinomicin (**45**) was isolated from the ethyl acetate extract of the *Micromonospora* sp. RV115, cultivated from the Mediterranean sponge *Aplysina aerophoba*. Diazepinomicin showed a broad-spectrum antitumor activity against cancer cells *in vitro* and in tumor xenografts *in vivo*, including leukemia and solid tumors such as melanoma and glioma, and it is currently in phase II clinical trials for Thallion pharmaceuticals.[123] We reported new antioxidant activity for diazepinomicin using both chemical and cellular approaches against human kidney (HK-2) and human promyelocytic (HL-60) cell lines. Moreover, diazepinomicin inhibited the proteases rhodesain and cathepsin L at an IC_{50} of 70–90 μM. It also showed antiparasitic activity against trypomastigote forms of *Trypanosoma brucei* with an IC_{50} of 13.5 μM.[83]

Manzamines are a class of alkaloids with a β-carboline moiety and an unusual polycyclic system in their structure. They are promising lead compounds and have demonstrated activities against malaria, tuberculosis and HIV.[124] They were isolated from several marine sponges.[125] The *Micromonospora* sp. strain M42, cultivated from the Indonesian sponge *Acanthostrongylophora* sp., was found to produce manzamine A (**46**) and 8-hydroxy manzamine (**47**).[18]

A new indole alkaloid, streptomycindole (**48**), in addition to a known related synthetic compound, *N*-phenylacetyl-L-tryptophan (**49**) were isolated from *Streptomyces* sp. DA22, cultivated from the sponge *Craniella australiensis* (South China Sea).[126]



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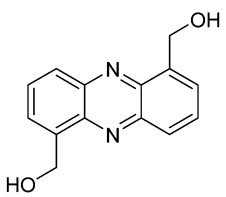

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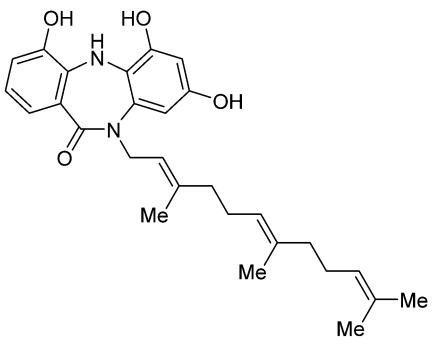

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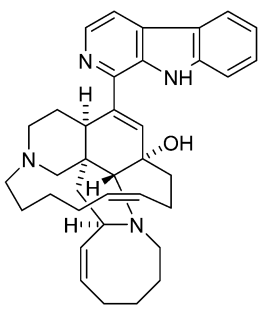

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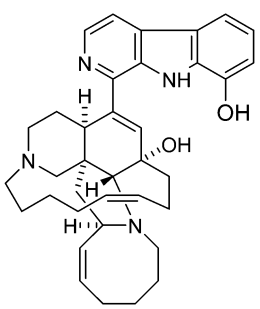

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The compounds were tested for cytotoxicity against tumor cell lines HL-60 leukemic, HCT-116 colon carcinoma, HO-8910 ovarian epithelial carcinoma, and HepG2 human hepatocarcinoma, but no activity was reported.

Four new γ-pyrones, nocapyrones A–D (**50–53**) and a synthetic known diketopiperazine, 2*E*/5*Z*-2-[(4-methoxyphenyl) methylene]-5-(2-methylpropylidene)-3,6-piperazinedione) (**54**), were isolated from the culture broth of the *Nocardiopsis* strain HB383, which was cultivated from the marine sponge *Halichondria panicea* that was collected from the Baltic Sea (Germany). *In vitro* cytotoxicity testing showed no activity against the mouse fibroblast cell line NIH-3T3 as well as the human hepatocellular carcinoma cell line HepG2 and the human colon adenocarcinom cell line HT-29.[127]

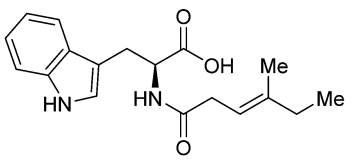
The potent cytotoxic thiodepsipeptide thiocoraline (**55**) was first isolated in 1997 from the mycelia of *Micromonospora marina*[128] and five new analogs of thiocoraline (**56–60**) were identified from the *Verrucosispora* sp. strain WMMA107, cultivated from the sponge *Chondrilla caribensis* f. *caribensis* (Florida Keys, USA). 22′-Deoxythiocoraline, thiochondrilline C (**56**), and 12′-sulfoxythiocoraline (**57**) exhibited significant cytotoxicity against the A549 human cancer cell line with EC_{50} values of 0.13, 2.86, and 1.26 μM, respectively.[129]

The known cyclic peptide, nocardamine (**61**) and two new dehydroxy and desmethylenyl derivatives (**62,63**) were isolated from the culture broth of *Streptomyces* sp. strain M1087, isolated from an unidentified marine sponge found offshore of Jaeju Island, Korea.[130] The new compounds exhibited weak inhibition activity against the recombinant enzyme sortase B with EC_{50} values of 88.3 and 126.4 μg mL^{-1} for the new derivatives, respectively, while nocardamine was inactive.

PM070747 (**64**) is a new angucyclinone, produced by *Saccharopolyspora taberi* strain PEM-06-F23-019B, cultivated from a marine sponge collected near the coast of Tanzania.[131] The antitumor activity was tested against human breast adenocarcinoma MDA-MB-231, human colorectal adenocarcinoma HT-29 and human lung carcinoma A-549 cells showing growth inhibition with GI_{50} values of 0.71, 1.42 and 3.28 μM, respectively.

Streptomyces sp. strain Sp080513GE-26, cultivated from the marine sponge *Haliclona* sp. (Tateyama, Japan), produced two new anthracyclines, tetracenoquinocin (**65**) and 5-iminoaranciamycin (**66**), in addition to the known compounds aranciamycin and the antibiotic SM 173B.[132] Aranciamycin showed cytotoxicity against human cervical carcinoma HeLa cells and human acute myelogenous leukemia LH-60 cells with IC_{50} values of 2.7 and 4.1 μM, respectively, while tetracenoquinocin and 5-iminoaranciamycin were inactive.

Mayamycin (**67**) is a new benz[α]anthracene derivative that was isolated from *Streptomyces* sp. strain HB202, cultivated from the marine sponge *Halichondria panicea*. Mayamycin showed potent *in vitro* cytotoxicity against eight human cancer cell lines with IC_{50} values of 0.13 to 0.33 μM. Interestingly, mayamycin revealed strong inhibitory activity against several clinically relevant bacteria, including *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) with IC_{50} values of 0.31 to 31.2 μM.[133]



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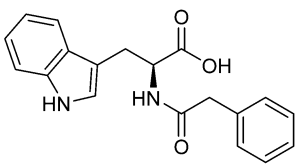

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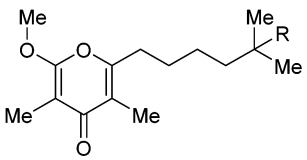
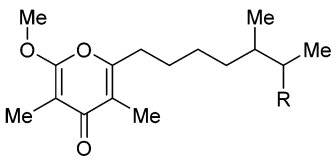


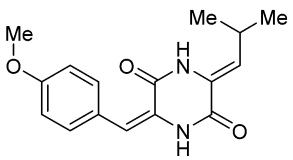
50: R= -OH

51: R= -H



52: R= -OH

53: R= =O



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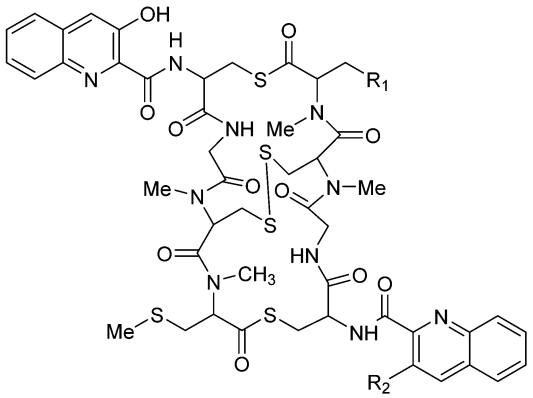
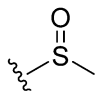
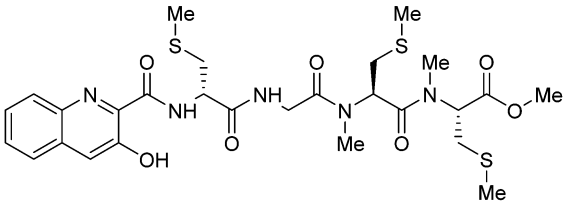


55 (R_1= SMe, R_2= OH); **56** (R_1= SMe, R_2= H); **57** (R_1=  , R_2= OH)



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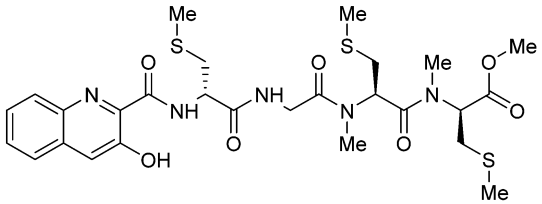

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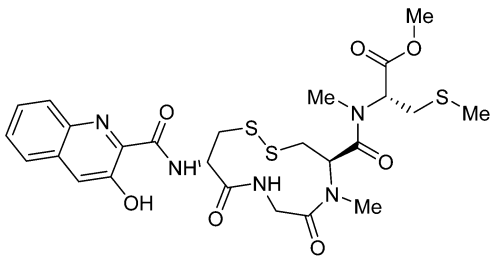

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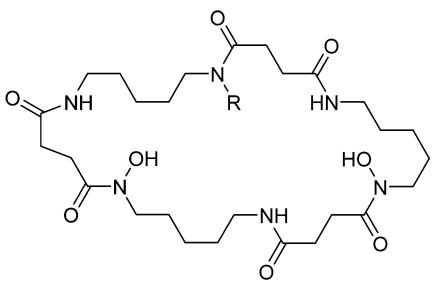
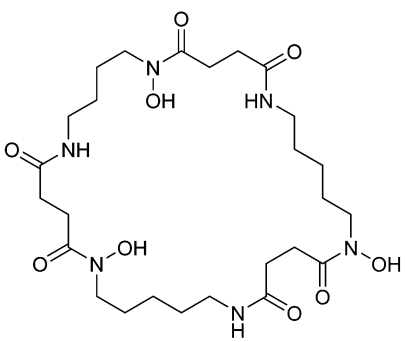
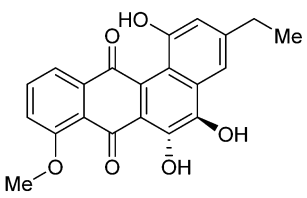


61: R= OH; **62**: R= H



63



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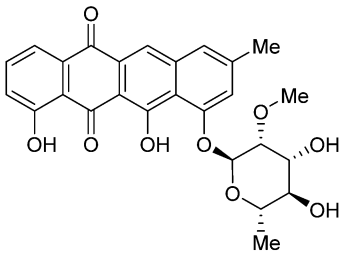

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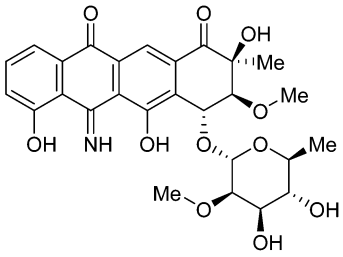

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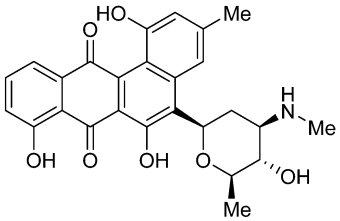


67

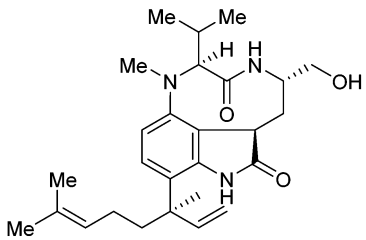
The new teleocidin analog JBIR-31 (**68**) was isolated from the obligate marine *Streptomyces* sp. NBRC 105896, cultivated from the marine sponge *Haliclona* sp. that was collected offshore of Tateyama City (Chiba Prefecture, Japan).[134] The compound showed weak cytotoxic activity against human cervical carcinoma HeLa cells with IC_{50} value of 49 μM.

Two new tetrapeptides with a modified indole moiety, JBIR-34 and JBIR-35, were isolated from the butanol extract of *Streptomyces* sp. strain Sp080513GE-23, cultivated from *Haliclona* sp. (Tateyama, Japan).[135] JBIR-34 (**69**) and JBIR-35 (**70**) exhibited weak DPPH radical scavenging activity with IC_{50} values of 1.0 and 2.5 mM, respectively.

Three novel isoprenoids JBIR 46–48 (**71–73**) were isolated from *Streptomyces* sp. strain SpC080624SC-11, cultivated from the marine sponge *Cinachyra* sp. which was collected from the sea shore at Nagura Bay, Ishigaki, Japan.[136] Interestingly, the compounds were identified after PCR screening for 3-hydroxy-3-methyl glutaryl coenzyme A reductase, which is a key enzyme in the mevalonate pathway for the biosynthesis of isoprenoid compounds.

Two new peptides, JBIR-56 (**74**) and JBIR-57 (**75**), were described from the broth culture of the new isolate *Streptomyces* sp. strain SpD081030SC-03, cultivated from an unidentified sponge collected from Ishigaki, Okinawa, Japan.[137]

JBIR-58 (**76**), a new salicylamide derivative, was isolated from *Streptomyces* sp. strain SpD081030ME-02, cultivated from an unidentified sponge collected offshore of Ishigaki Island, Japan.[138] JBIR-58 showed cytotoxic activity against human cervical carcinoma HeLa cells with an IC_{50} value of 28 μM.



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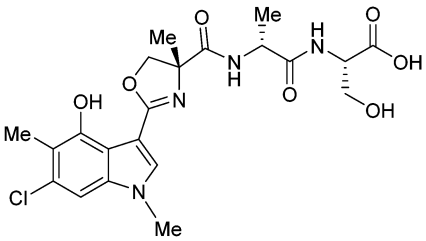

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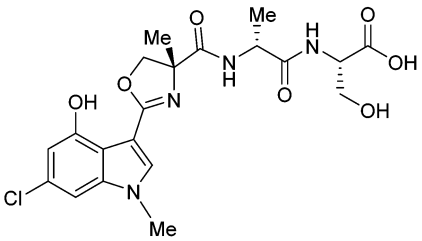

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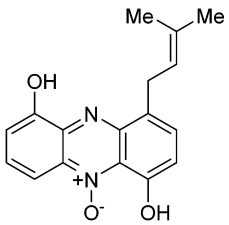

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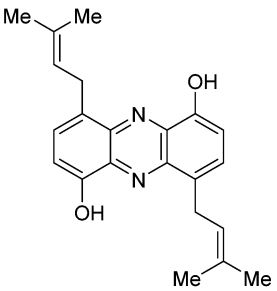

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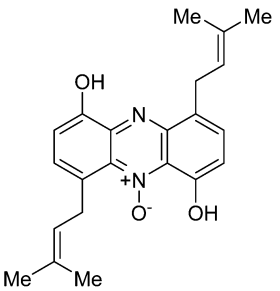


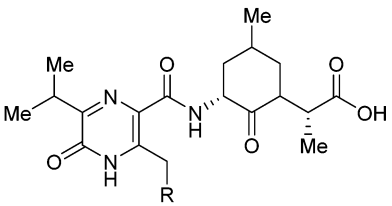
73

The new diterpene JBIR-65 (**77**) was isolated from the acetone extract of *Actinomadura* sp. SpB081030SC-15, cultivated from an unidentified marine sponge collected offshore of Ishigaki Island, Japan.[139] JBIR-65 showed weak protection of neuronal hybridoma N18-RE-105 cells from L-glutamate toxicity with an EC_{50} value of 31 μM.

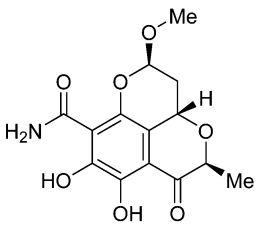
Three new trichostatin analogues, JBIR-109 (**78**), JBIR-110 (**79**) and JBIR-111 (**80**), in addition to trichostatin A and trichostatic acid were reported from *Streptomyces* sp. strain RM72, cultivated from an unidentified marine sponge collected near Takara Island, Japan. The compounds were tested for their histone deacetylase inhibitory activities but no significant activity was detected.[140]

Four diketopiperazines (**81–84**) were isolated from *Streptomyces* sp. DA18, cultivated from the marine sponge *Craniella australiensis* which was collected from the South China Sea.[141]

The actinomycete strain *Streptomyces* sp. strain KM86-913 with topoisomerase I inhibition activity was isolated from an unidentified marine sponge collected from Keomun Island (Korea). Bioassay-guided isolation resulted in identification of seven iso- and anteiso-fatty acids.[142] These fatty acids were 14-methylpentadecenoic acid (iso-16 : 1), hexadecenoic acid (16 : 1), 12-methyltetradecanoic acid (anteiso-15 : 0), cyclopropane fatty acid, 14-methylpentadecanoic acid (iso-16 : 0), hexadecanoic acid (16 : 0) and 14-methylhexadecanoic acid (anteiso-17 : 0). Cyclopropane and 14-methylhexadecanoic fatty acids were found to be responsible for the antitumor activity. Last but not least, sponge-associated actinomycetes are also sources of clinically important enzymes such as the antifungal ChiC type chitinase produced by *Streptomyces* sp. DA11 isolated from the South China sponge *Craniella australiensis*.[143]



74: R= Me; **75**: R= H



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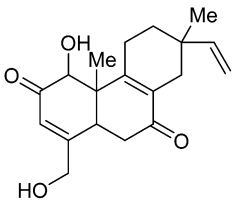

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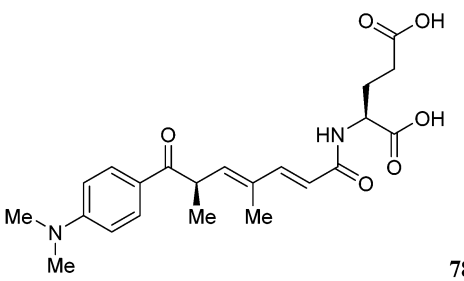

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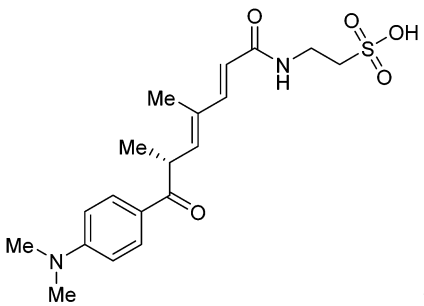

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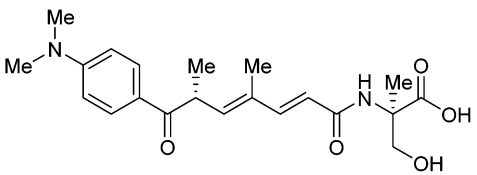

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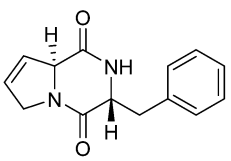

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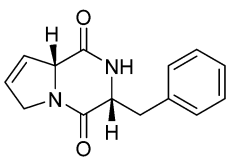
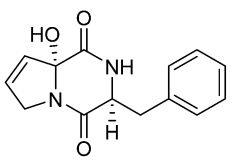


82



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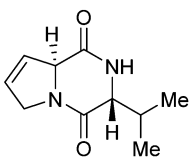

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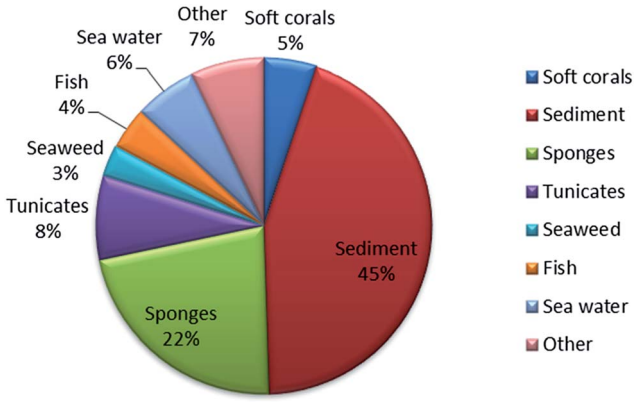

Fig. 4 Percentage distribution of natural products from marine actinomycetes (data collected from MarinLit 2013 and literature).

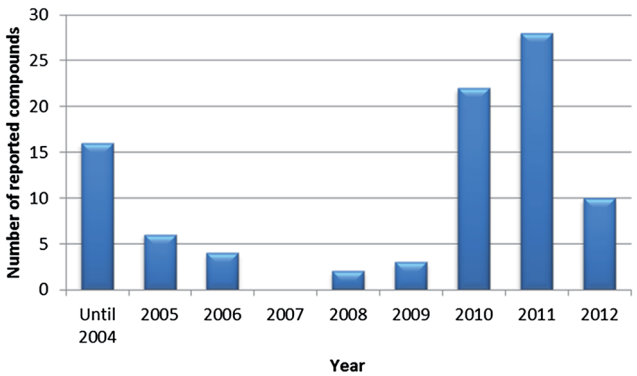

Fig. 5 Natural products from marine actinomycetes according to the year of publication (data collected from MarinLit 2013 and literature).

5 Conclusions

A total of 411 natural products from marine actinomycetes were reported in the MarinLit database in 2013 (J. Blunt, MarinLit, University of Canterbury, New Zealand) of which 22% were obtained from marine sponge-associated actinomycetes (Fig. 4). The number of natural products discovered from marine sponge-associated actinomycetes is reported in Fig. 5. In comparison to actinomycetes from other environmental sources, the sponge-associated actinomycetes were remarkably rich, with higher chances of finding new chemotypes and less problems with rediscovery. Among the several actinomycete genera, *Streptomyces*, *Rhodococcus*, *Salinispora*, and *Micromonospora* were the most prolific producers of secondary metabolites which displayed broad chemical diversity and diverse pharmaceutically and medically relevant bioactivities. The recovery of rare genera along with the frequent description of new actinomycete species and even genera illustrates that there is room for discovery. With regard to diversity, abundance and the natural product repertoire of marine sponge-associated actinomycetes, we envision that only the tip of the iceberg has been reached. Future interdisciplinary efforts combining the fields of microbiology, genomics, metabolomics, natural products chemistry, and pharmacology are needed to fully explore this still largely untapped natural resource.

6 Acknowledgements

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