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REVIEW

Marine sponges of the genus *Stelletta* as promising drug sources: chemical and biological aspects[☆]



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Abstract Marine sponges of the genus *Stelletta* are well known as rich sources of diverse and complex biologically relevant natural products, including alkaloids, terpenoids, peptides, lipids, and steroids. Some of these metabolites, with novel structures and promising biological activities, have attracted a lot of attention from chemists seeking to perform their total synthesis in parallel to intensive biological studies towards new drug leads. In this review, we summarized the distribution of the chemically investigated *Stelletta* sponges, the isolation, synthesis and biological activities of their secondary metabolites, covering the literature from 1982 to early 2018.

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[☆]Dedication to Prof. Dr. Yue-Wei Guo on the occasion of his 60th birthday, for his pioneer work on Chinese marine natural product chemistry.

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1. Introduction

The ocean is blessed with a great biodiversity bathed in a complex ecological environment with hypersaline, hyperbaric, hypoxia, cryogenic and oligotrophic conditions. Being adapted to such harsh environment, marine organisms have built up unique morphological, ecological and physiological characteristics, along with a capacity to produce diverse and complex functional metabolites, which aroused our deep interest. These compounds, also referred as marine natural products (MNPs), are often characterized by intriguing structures and promising biological properties, which may have a great potential as new drug leads^{1–7}. Therefore, the search for small molecules as potential drug candidates from marine organisms has been an impetus to natural product chemists and biologists, leading to the discovery of at least 9 FDA-approved drugs and more than 20 drug candidates at different stages of clinical trials^{8–10}.

Over the past decades, many species of marine invertebrates (sponges in particular) have been investigated towards potentially bioactive chemical components^{10–15}. Numerous studies have indicated that marine sponges constitute one of the richest sources of MNPs with potential therapeutic applications^{16–19}. Up to now, 4 out of the 9 FDA-approved marine drugs are indeed derived from marine sponges²⁰.

Marine sponges of the genus *Stelletta* (Stellettidae) are widely distributed in the marine ecological systems. Triterpenoids, alkaloids, and peptides are the most prominent bioactive secondary metabolites isolated from the title genus, especially from species of the West Pacific coasts. Widespread biological properties, such as cytotoxic, antimicrobial, anti-HIV, and protein-tyrosine phosphatase 1B (PTP1B) inhibitory activities, enable *Stelletta*-derived MNPs to have a broad range of potential applications and a bright market background in exploiting novel drug leads. Therefore, this review will detail, for the first time, the recent advances of MNPs from *Stelletta* sponges, and provide an insight into their potential as future medicinal drugs. We will cover topics ranging from the

distribution of the sponge to the isolation, synthesis and biological activities of different types of secondary metabolites, with a literature survey from 1982 to early 2018.

2. Structure and properties of MNPs from the *Stelletta* genus

Stelletta sponges are distributed all over the world seas. Many of them were chemically investigated, especially those from the Sea of Japan, the South China Sea, and off the coast of Australia (the Great Australia Bight, Coral Sea, Torres Strait, and Cape Wilberforce) (Fig. 1). A literature survey revealed that alkaloids, terpenoids, peptides, lipids, and steroids have been isolated from *Stelletta*²¹. Alkaloids were mainly discovered from sponges located in Shikine-jima and Shikoku Islands of the Japanese Sea, Keomun Island of the South Sea of Korea, the Great Australia Bight, and Bahamas; triterpenoids were mainly obtained from the Hainan sponges, South China Sea; peptides were isolated from sponges of Oshimashinsone, Southern Japan and Torres Strait, Northwestern Australia; lipids were mainly derived from sponges off the coast of Hainan Island, China and Ullung Island, Korea; steroids were found from South China Sea, China, and Oro Island, Japan (Fig. 1). To date, approximately 131 interesting secondary metabolites (1–131) have been isolated and characterized from *Stelletta* marine sponges, with various biological activities. In this section, the isolation and chemical structures of these compounds will be discussed by different structural types.

2.1. Alkaloids

The chemical investigation of *Stelletta* sponges led to the discovery of alkaloids with novel features. It is worth noting that Fusetani and co-workers²² have made a big contribution to this domain since 1990. From 1990 to 1999, they performed bioassay-guided chemical investigation of the *n*-BuOH fraction of the methanol extract of *Stelletta* sp. (undetermined species) collected off the sea area around Japan.



Figure 1 Global distribution of the chemically investigated *Stelletta* marine sponge according to the structure types of their chemical constituents (red: alkaloids; blue: triterpenoids; green: peptides; brown: lipids; black: steroids).

Stellettamides, the novel alkaloids with a farnesyl moiety connected to an indolizidine skeleton through an amide bond, displaying significant bioactivities, were regarded as marine toxins (Fig. 2). The isolation and structural identification of stellettamide A (**1**), the first indolizidine metabolite, was reported by Fusetani in 1990²². The *Stelletta* sponge was collected in the Shikine-Jima Island (34°19'25'' N, 139°13'19'' E) from Izu Archipelago, Japan. In antifungal and cytotoxic assays, compound **1** showed significant activities against the fungus *Mortierella remaniana* and K562 epithelium cancer cells (IC₅₀ = 5.1 µg/mL)²². Later in 1997, the effect of stellettamide A (**1**) on the contractile system of smooth muscle was investigated by the same group, showing that it inhibited Ca²⁺/calmodulin-dependent myosin light chain (MLC) phosphorylation, and thus could act as a novel inhibitor of calmodulin²³. This interesting activity aroused the interests of chemists to determine the absolute configuration of this natural product by total synthesis, which will be discussed in the synthetic section^{24,25}.

Seven years after the discovery of **1**, stellettamide B (**2**) was obtained from a *Stelletta* sponge (undetermined species), along with **1** (Fig. 2)²⁶. This dark brown sponge was collected at 20–30 m depth along the offshore of Keomun Island, South Sea, Korea. The isolated compounds were also reported to exhibit moderate antifungal and RNA-cleaving activities²⁶. In this paper, the original structure of **2** was established with an *S* configuration at the C-6' position, while the first total synthesis of (–)-stelllettamide B led to the revision of the natural product configuration to 6'*R*²⁷. Stellettamide C (**3**) was isolated from the same extract as **1** by Fusetani in 1999 (Fig. 2)²⁸. In the bioassay, **3** showed moderate activity against *Escherichia coli* with an inhibitory zone of 10.5 mm at 20 µg/disk²⁸. In addition, the first asymmetric synthesis of **3** was reported by Aoyagi and co-workers²⁵ in 2008.

Stellettadine A (**4**), a sesquiterpene amide containing a linear bisguanidinium unit with larval metamorphosis-inducing activity in ascidians, was first isolated from a *Stelletta* sponge of the Gulf of Sagami, Japan, in 1996 (Fig. 3).²⁹ Later in 1999, two Ca²⁺/calmodulin-dependent phosphodiesterase inhibitors, bistelletladines A and B (**5** and **6**), were isolated from a sponge collected off Shikine-jima Island, 200 km South of Tokyo³⁰. It was proposed

that a biosynthetic [4π + 2π] Diels–Alder cycloaddition of two dehydro-stellettadines derivatives would generate the bistelletladines (**5** and **6**) (Fig. 3)³⁰. In 2001, first total synthesis and absolute configuration study of stellettadine A (**4**) was reported by Mori's group³¹. Three antibacterial homosesquiterpene amides, stelletta-zoles A–C (**7**–**9**), bearing an imidazole moiety and a terminal guanidine chain were isolated from the same extract as bistelletladines^{28,32}. In addition, during their study, the acetamide (**10**) was obtained by the acetylation of stellettazole C (**9**) with acetic anhydride (Ac₂O) (Fig. 3).

As for the biological activities, the tested stellettazole A (**7**) and bistelletladines (**5** and **6**) moderately inhibited Ca²⁺/calmodulin-dependent phosphodiesterase (45% and 40% inhibition at 100 µmol/L, respectively). They were also found to have an antibacterial activity against *E. coli* and an antifungal activity against yeast at low concentrations, ranging from 10 to 50 µg/disk³⁰. Stelletta-zoles B and C (**8** and **9**) were active against the growth of *E. coli* at 20 µg/6 mm disk, respectively^{28,32}. Interestingly, stellettadine A (**4**) was found to induce the metamorphosis of the ascidian *Halocynthia roretzi* larvae with an ED₁₀₀ value of 50 µmol/L²⁹.

In 2008, Capon's group³³ reported the discovery of three new terpenyl-pyrrolizidine conjugated alkaloids, bistellettazines A–C (**11**–**13**), and a new cyclic terpenyl-imidazole conjugate, bistellettazole A (**14**), from Southern Australian marine sponge, *Stelletta* sp. CMB-01936 (Fig. 4), collected in the 90 m depth water of the Great Australian Bight. All the four compounds were isolated as salts. Compounds **11**–**13** comprise two pyrrolizidines and two terpene-conjugated amide groups, while **14** contains an *ansa* *N*-methyl imidazole ring and two terpene-conjugated amide groups. It is obvious that these novel compounds might be generated by a Diels–Alder reaction from the two terpene-conjugated amide groups, followed by the coupling with either two pyrrolizidine groups (for **11**–**13**) or one imidazole moiety (**14**). The terpene-conjugated amides may be farnesoic acid derivatives, especially 2,6,10-trimethylundeca-2,4,6,8,10-pentaenoic acid.³³ In fact, the chemistry and biosynthetic studies of natural occurring pyrrolizidine³⁴ and imidazole³⁵ alkaloids were summarized by Robertson³⁴ and Jin³⁵ in 2016, respectively, providing information for further total synthesis of the novel alkaloids such as **11**–**14**.

Except for the above mentioned alkaloids conjugated to linear terpenes, three new cyclic alkaloids, named nordercitin (**15**), dercitamine (**16**), and dercitamide (**17**) with a pyrido[4,3,2-*mn*]thiazolo[3,2-*β*]acridinium-9-ethyl skeleton were isolated in 1989 from a red sponge *Stelletta* sp. from Bahamas, at a depth of 70 m (Fig. 5)³⁶. All the new compounds inhibited the growth of murine P388 leukemia cells *in vitro* with good to moderate IC₅₀ of 4.79, 26.7, and 12.0 µmol/L, respectively³⁶. In 1994, the chemical investigation of the marine sponge *S. maxima*, collected off the Sata Peninsula, Shikoku, resulted into six novel cyclostellettamines (A–F, **18**–**23**) with macrocyclic bis-pyridine structures linked through two C₁₂–C₁₄ alkyl chains³⁷. In the bioassay, the new compounds inhibited the binding of [³H]-methyl quinuclidinyl benzilate (QNB) which is a selective antagonist to muscarinic receptors³⁷. It is worth mentioning that muscarinic acetylcholine receptors were found to play important roles in various physiological functions including memory and learning³⁸. It was reported that muscarinic receptors are known to be correlated with some disease states, and their agonists or antagonists may be potential drugs³⁹.

To date, a variety of marine alkaloids and new derivatives have been isolated and characterized from marine sponges⁴⁰. Some of these interesting bioactive marine alkaloids, represented by marine

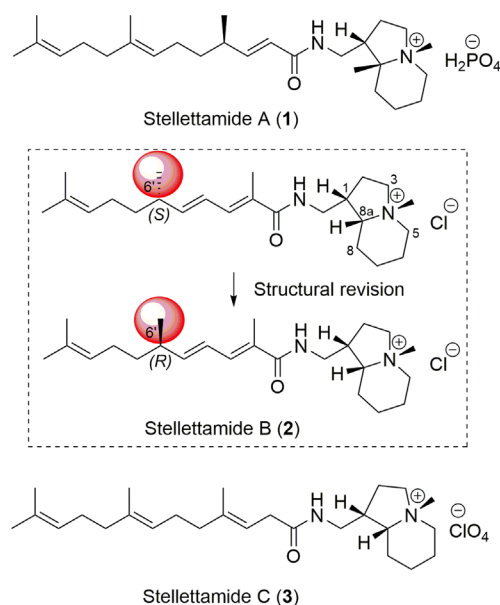


Figure 2 Chemical structures of compounds **1**–**3**.

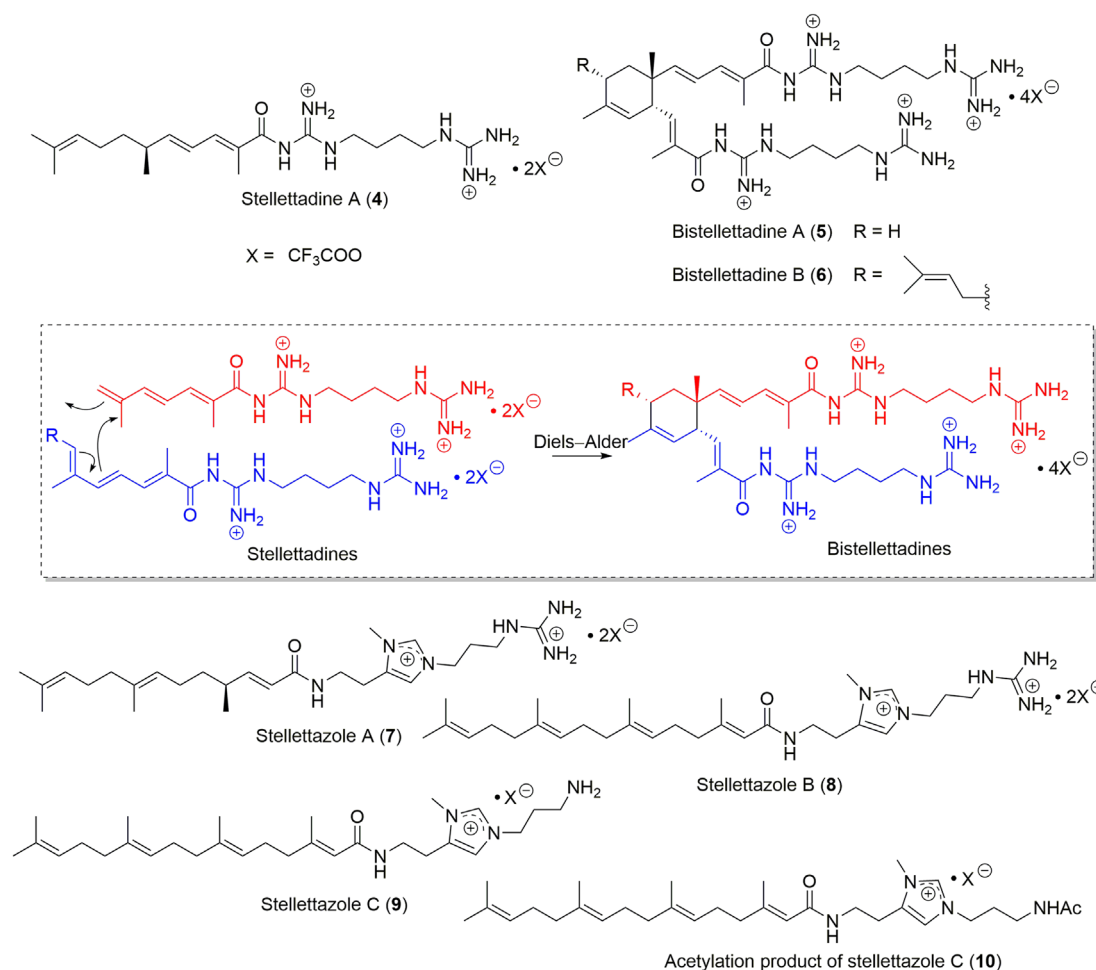


Figure 3 Chemical structures of compounds 4–10, and the proposed biosynthetic pathways of bistellettadines.

guanidines, may be the most promising for future medicinal applications^{41,42}. In the *Stelletta* genus of sponge, 23 novel alkaloids have been discovered and characterized by either extensive spectroscopic analysis or chemical synthesis. The typical *Stelletta*-derived alkaloids were mainly terpene and alkaloid hybrids, featured by a long acyclic terpenyl side chain connected to the alkaloid moieties, such as pyrrolizidine, imidazole, indolizidine, and guanidine groups (Figs. 2–5). The unique structures of such alkaloids might due to the special living ecological system, which also dowered them with significant pharmacological values. It is worth mentioning that the isolation process of alkaloids is always more challenging than the other metabolites due to their high polarity, while most of *Stelletta* alkaloids exist as salt forms. Therefore, *n*-BuOH extracts were mainly investigated in the search for bioactive alkaloids from *Stelletta* sponges and various column chromatography or HPLC conditions were used for their isolation and purification. For instance, the addition of either formic acid or trifluoroacetic acid (TFA) during the reverse phase isolation could optimize the chromatographic peaks to a certain extent³². The novel structures and special isolation methods for these novel alkaloids and their broad biological activities stimulated natural product chemists and biologists for further studies of *Stelletta*-derived natural products, as will be discussed in the following sections.

2.2. Triterpenoids

Isomalabaricane-type triterpenoids are a class of unusual triterpenoids with a 6,6,5-fused tricyclic ring system. The first discovery of such triterpenoid from the sponge *Stelletta* was reported by Clardy and co-workers⁴³ in 1982. The fresh brown sponge was collected off Mogadishu, Somalia. Its chemical investigation resulted into pure yet unstable yellow triterpenoid pigment **24**, of which structure was determined by spectroscopic analysis and X-ray single crystal diffraction (Fig. 6)⁴³. This novel pigment was also obtained 12 years later, in 1994, by Zeng and co-workers⁴⁴ from the Hainan sponge *S. tenuis* Lindgren (Stellettidae). During this work, another new isomalabaricane-type triterpenoid was isolated and designated as stellettin A (**25**), while compound **24** was named stellettin B at the same time (Fig. 6). Both compounds comprise a 6,6,5-fused tricyclic ring system and a terminal 3-methyl-2-pyrone moiety, which are connected *via* a conjugated triene. During biological assays, the authors found that stellettin A (**25**) exhibited significant cytotoxicity against P388 leukemia cells with an ED_{50} value of 0.001 $\mu g/mL$ ⁴⁴.

In 1996, four new cytotoxic isomalabaricane triterpenes, namely stellettins C–F (**26–29**)⁴⁶, along with the three known related compounds, stellettins A (**25**)⁴⁴, B (**24**)⁴³, and G (**30**)⁴⁵ were isolated from the CCl_4 extract of the yellow sponge *Stelletta* sp.

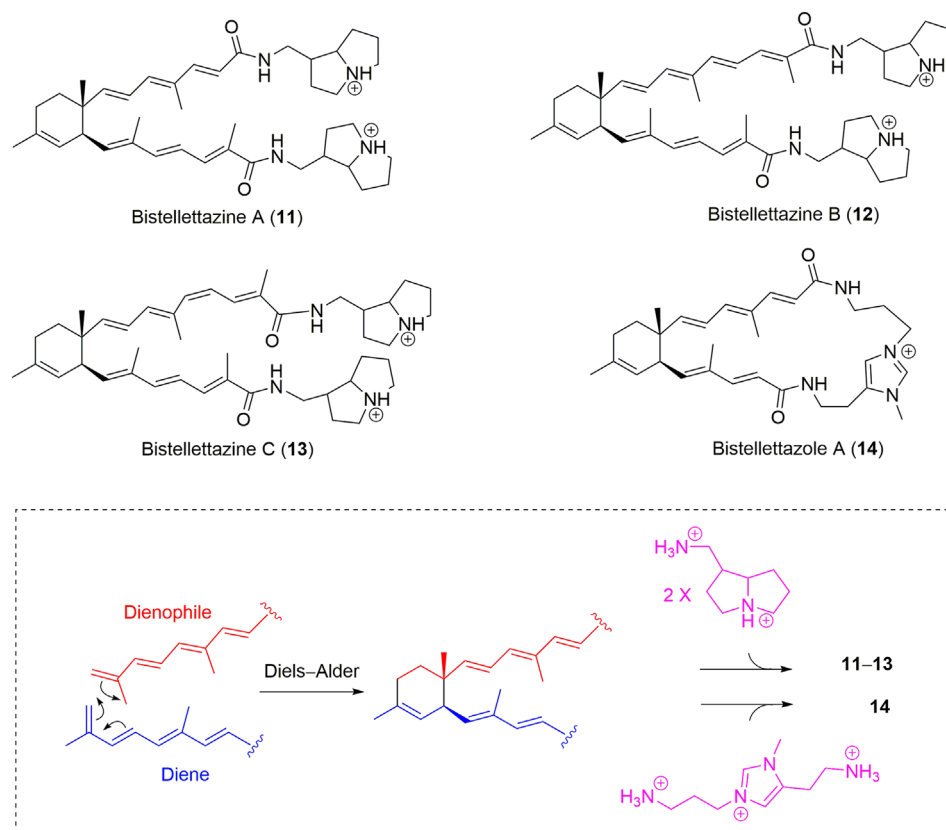


Figure 4 Chemical structures of compounds 11–14, and their possible biosynthetic pathway.

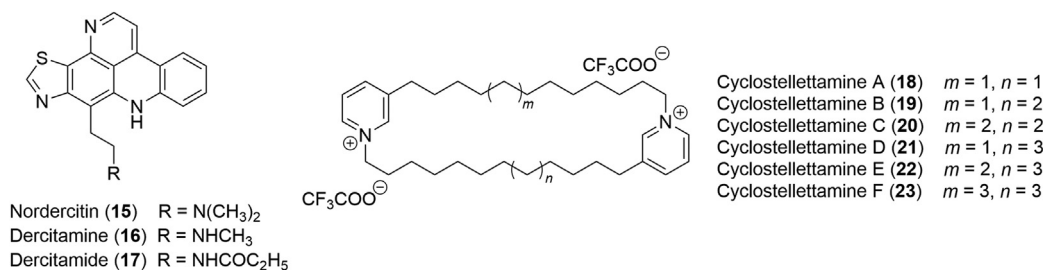


Figure 5 Chemical structures of compounds 15–23.

(Fig. 6), which was guided by the cytotoxicity on human tumor cell lines *in vitro*. The sponge was collected off the shore North of Cape Wilberforce at the depth of 15 m, Australia. In fact, these compounds are all unstable, of which 26 and 30 could be rapidly equilibrate to 27 and 31, respectively, due to the *E/Z* isomerization of the exocyclic olefin⁴⁶. Thus, the cytotoxic potential of the mixtures of each pair of interconverting compounds was evaluated against sensitive cell lines including leukemia, central nervous system (CNS), and renal lines. These mixtures generally showed promising activities with GI_{50} concentrations in the low-to-mid nanomolar range⁴⁶. In fact, during purification, a methylation step of the polar fraction containing stelletins with free carboxylic acid groups made them much easier to be isolated. By using such strategy, compounds 28–30 were initially methylated during the isolation process to afforded compounds 31–33, which could be rapidly isolated by common purification methods⁴⁶.

Eleven years later in 2007, stelletins L and M (34 and 35), two new cytotoxic triterpenes, were isolated by Lin's group⁴⁷ from

S. tenuis collected in Sanya Bay, Hainan Province, China (Fig. 6). In fact, the crude extract of this sponge exhibited obvious activity against the HL-60 human leukemia cell line ($IC_{50} < 0.01 \mu g/mL$). The cytotoxicity-guided fractionation of the CH_2Cl_2 fraction of the EtOH extract led to the isolation and characterization of 34 and 35, which were further tested for their cytotoxicity against human lung (A549), liver (SMC-7721), stomach (AGS), and colon (HT-29) cancer cell lines. Both compounds showed selective cytotoxicity against AGS cancer cells, with IC_{50} values of 3.9 and $2.1 \mu mol/L$, respectively⁴⁷.

Despite the rich chemical diversity of these isomalabaricane triterpenes, among various biological activities, only their cytotoxicity had been reported^{44,46,47}. Thus, it was necessary to explore additional biological functions for these metabolites. In 2013, Guo's group⁴⁸ made a breakthrough when searching for MNPs with protein-tyrosine phosphatase 1B (PTP1B) inhibitory activity. Several isomalabaricane triterpenes were isolated from the Hainan sponge *Stelletta* sp. YAL-3, which was collected off Lingshui Bay, Hainan Province, China⁴⁸. After a careful chemical investigation

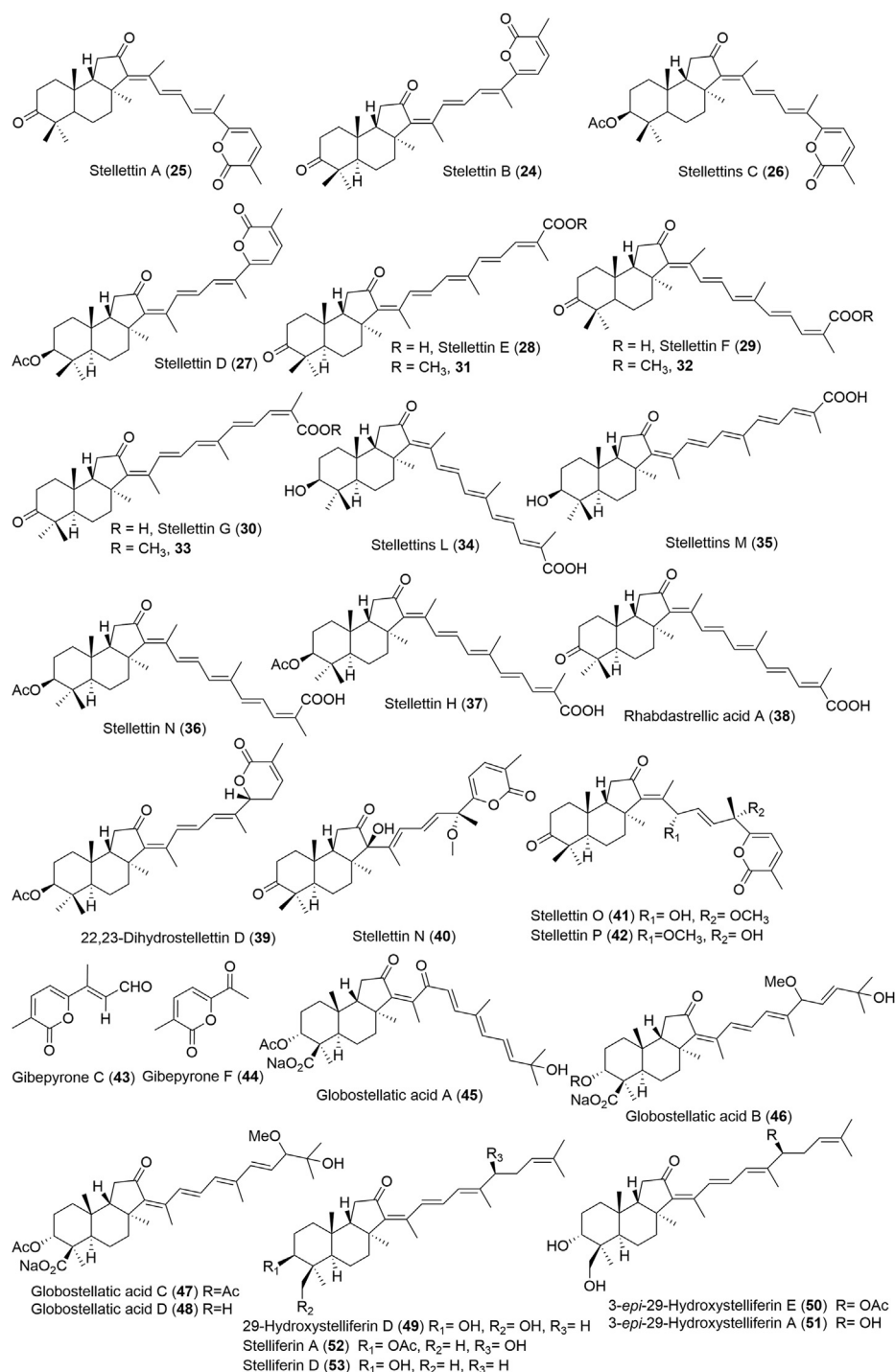


Figure 6 Chemical structures of compounds 24–53.

of the Et₂O fraction of its acetone extract, a new isomalabaricane triterpene, stelletin N (36), was isolated, together with five known analogues, stelletin H (37), rhabdastrellic acid A (38), stelletin G (30), stelletin D (27), and 22,23-dihydrostelletin D (39) (Fig. 6). The biological evaluation revealed that the known compound 27 exhibited significant PTP1B inhibitory activity with an IC₅₀ value of $4.1 \pm 0.9 \mu\text{mol/L}$ ⁴⁸. As discussed above that compounds 26 and 27 could be rapidly equilibrate to each other⁴⁶, the PTP1B inhibitory activity of 27 thus could possibly be the activity of the mixture of these two compounds as well.

The chemical investigation of the marine sponge *S. tenuis* Lindgren, collected in Sanya, Hainan, led to the isolation of three new cytotoxic isomalabaricane triterpenes, named stelletin N (40), stelletin O (41) and stelletin P (42)⁴⁹, and two known related compounds, stelletins C and D (26 and 27)⁴⁶ (Fig. 6). Among all the isolated compounds, only the new ones were tested for cytotoxic activities against three human cancer cell lines (A549, AGS and U-251MG). Biological assays showed that compounds 40–42 were cytotoxic to AGS cells, with IC₅₀ values of 4.52, 9.61 and 7.44 $\mu\text{mol/L}$, respectively⁴⁹. Intriguingly, the authors

identified two naturally occurring α -pyrones, namely gibepyrone C and F (**43** and **44**) (Fig. 6), along with the above triterpenoids⁴⁹. As parts of some isomalabaricane triterpenes, these α -pyrones might be the oxidation products of the co-occurring stelletins. In fact, gibepyrone F had previously been isolated from the fungal plant pathogen *Gibberella fujikuroi*⁵⁰, and occasionally from sponges⁵¹. Therefore, it is questionable if these two compounds are derived from the sponge catabolism or that of associated microorganisms.

Except for alkaloids, Fusetani's group⁵² was also engaged in the discovery of bioactive isomalabaricane triterpenes in 1996. Based on bioassay-guided isolation, four new cytotoxic triterpenes, named globostellatic acids A–D (**45–48**), were isolated from the MeOH extract of the marine sponge *S. globostellata* Carter, 1883 collected at depths of 3–10 m off Mage Island near Kagoshima in southern Japan (Fig. 6). All the four isolated compounds exhibited cytotoxicity against P-388 murine leukemia cells with IC₅₀ values ranging from 0.1 to 0.46 $\mu\text{g/mL}$ ⁵². After their pioneering discovery of bioactive compounds from *Stelletta* sponges, Fusetani and co-workers⁵³ continued their study by investigating metabolites by a different biological assay. The new assay system, using rat 3Y1 fibroblasts, was applied to find compounds involved in the regulation of cytoskeleton formation. It was reported that metabolites from the sponge *S. globostellata* induced unusual morphological changes in the cells. The sponge *S. globostellata* was collected using scuba at a depth of 3 m off Mage-jima Island (30°43' N, 130°52' E). Three new isomalabaricane triterpenes, 29-hydroxystelliferin D (**49**), 3-*epi*-29-hydroxystelliferin E (**50**), and 3-*epi*-29-hydroxystelliferin A (**51**), together with two known ones, stelliferin A (**52**) and stelliferin D (**53**), were isolated from the MeOH fraction of the EtOH extract of *S. globostellata* (Fig. 6)⁵³.

Isomalabaricane-type triterpenoids from *Stelletta* sponges are attractive due to their highly conjugated structures and significant biological activities, which might be influenced by the environment of sponges originated from completely different collections. However, there are still many challenges in the study of such triterpenoids. On the isolation aspect, many highly conjugated isomalabaricane triterpenes were photochemically unstable, and all the extraction and isolation procedures have to be performed under lightproof condition to avoid artifacts. This may cause difficulties in both new compound discovery and biological assays. Therefore, strategies of chemical modification towards more stable compounds could be a future challenge, so as to explore their biological function. In addition, it is interesting to note that sponges from Hainan Island are the main source of new structures of isomalabaricane triterpenoids. The extensive chemical investigation of *Stelletta* collected from this sea area may not only expand the diversity and complexity scope of triterpenes in this genus, but also provide evidence for chemical ecological relationships between *Stelletta* sponges and their marine environment.

2.3. Peptides

2.3.1. Ciliatamides

The chemical diversity and complexity of the genus *Stelletta* was not only exemplified by isomalabaricane triterpenes and alkaloids, but also by novel peptides, such as lipopeptides and depsipeptides. In 2013, two uncommon lipopeptides, ciliatamide D (**54**) containing a *N*-methyl-L-methionine sulfone moiety, and ciliatamide A (**55**), were isolated from the EtOH extract of a marine sponge

Stelletta sp., collected from Oshima-shinsone, southern Japan (Fig. 7)⁵⁴. The absolute configuration of these two peptides were determined by the isolation team by using Marfey's analysis, showing that they contained two L-amino acids residues⁵⁴.

However, after lipopeptides ciliatamides A–C were first reported in 2008 from the sponge *Aaptos ciliata*⁵⁵, Lindsley and co-workers⁵⁶ shortly performed their total synthesis and made a stereochemical revision in the same year. The stereochemistry was thus revised from (12*S*,22*S*) to (12*R*,22*R*). Moreover, Lindsley and co-workers⁵⁶ built up a library of all possible stereoisomers of ciliatamides A–C and the corresponding optical rotations were tested. However, it is surprising that the challenging relative configuration of the naturally occurring ciliatamide A remained incorrect in these two seminal papers. Finally, Matsunaga and co-workers⁵⁷ resolved the problems of the relative and absolute configuration for ciliatamide A, by utilizing NMR and chiral-phase HPLC analyses. Four synthetic isomers were compared with an authentic natural sample of ciliatamide A, allowing its structure to be revised for the second time, with an (12*R*,22*S*) configuration, highlighting the presence of a D-phenylalanine moiety. Furthermore, the absolute configuration of the methionine sulfoxide residue in ciliatamide D (**54**) was also revised to the D form⁵⁴.

Although the HPLC separation of *Stelletta* ciliatamides was carried out by using a bioassay-guided approach, the purified compounds showed no inhibitory activity on cathepsin B nor cytotoxicity against HeLa cells at 50 $\mu\text{g/mL}$ ⁵⁴. Actually, after their first isolation from the sponge *Aaptos ciliata* in 2008⁵⁵, ciliatamides A and C (**55** and **57**) were found to be antileishmanial (50% and 45.5% growth inhibition at 10 $\mu\text{g/mL}$ on *Leishmania major*, respectively). Ciliatamides A–C also inhibited the growth of HeLa cells at IC₅₀ values of 50, 4.5, and 50 $\mu\text{g/mL}$, respectively⁵⁵.

2.3.2. Depsipeptides

Among marine organisms^{58,59}, marine sponges have played an important role in anti-HIV natural products discovery⁶⁰. In particular, depsipeptides (also known as cyclodepsipeptides) may be the most promising molecules⁶¹ as lead compounds in anti-HIV drug research and development^{60–62}. Structurally, these compounds often incorporate unique amino acids in their structures^{63,64}. After literature survey, only seven depsipeptides were found in the genus *Stelletta* by now, thus leaving opportunities to further explore the chemical and biological diversity of *Stelletta*-based depsipeptides.

In 2011, four new HIV-inhibitory depsipeptides, mirabamides E–H (**58–61**), along with the known mirabamide C (**62**) were isolated from the MeOH extract of *S. clavosa*, collected from the interlagoon seabed areas in the Torres Strait (Fig. 8)⁶⁵. This was the first report on depsipeptides from a *Stelletta* sponge. These novel compounds displayed significant inhibitory activities against HIV-1 with potent IC₅₀ values ranging from 42 to 121 nmol/L⁶⁵. In light of these promising activities, the subsequent research reported in 2015 by Gustafson and co-workers⁶⁶ from a *Stelletta* sponge collected from northwestern Australia resulted into two HIV-inhibitory cyclic depsipeptides, named stelletapeptins A and B (**63** and **64**) with potent EC₅₀ values of 23 and 27 nmol/L, respectively (Fig. 8). However, direct cytotoxicity of **63** and **64** against the host cells was observed at low IC₅₀ values of 367 and 373 nmol/L, respectively.

2.4. Lipids

Modified lipids are among the most abundant secondary metabolites found in sponges⁶⁷. During the last 35 years, over 30 lipids

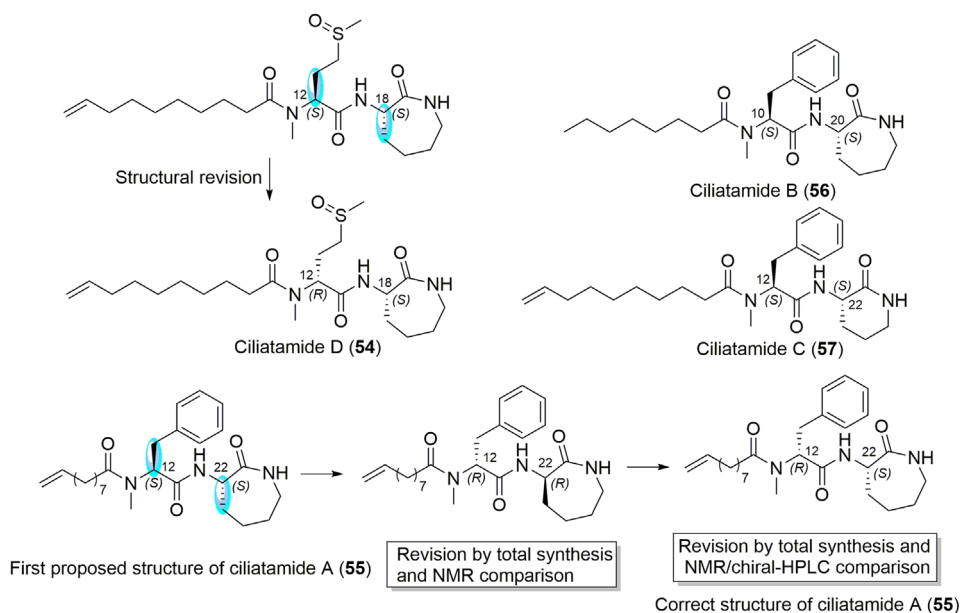


Figure 7 Chemical structures of compounds **54–57**.

were discovered from marine *Stelletta* sponges. These lipids showed both novel structural features and significant biological activities.

Chemical study of the EtOAc fraction of EtOH extract from the sponge *S. tenuis* (Lindgren) collected off Hainan Island led to the isolation of a new *N*-nervonoyl-D-sphingosine derivative (**65**, the first example of lipids found in the title sponge) and a saturated amide (**66**), respectively (Fig. 9)⁶⁸. Actually, compound **65** possesses the two hydroxyl groups of sphingosine at C-1 and C-3, respectively, and an additional uncommon one at C-4. As reported, from a biogenetical point of view, the hydroxyl group at C-4 would possibly be introduced by hydration of sphingosine⁶⁸.

Acetylenic acids are a series of metabolites featuring an acetylenic or an enyne moiety, and usually, a terminal double bond. New acetylenic acids including stelletic acid A (**67**), its methyl ester (**68**) (*Z*-stelletic acid B (**69**), its sodium salt (**70**), (*E*)-stelletic acid B (**71**), (*Z*)-stelletic acid C (**72**) and its sodium salt (**73**), were obtained from the MeOH extract of a *Stelletta* sponge by Jung in 2003 (Fig. 9)⁶⁹. The sponges were collected off the coast of Ullung Island, Korea. Since the MeOH extract displayed toxicity to brine shrimp larvae ($LD_{50} = 296 \mu\text{g/mL}$), bioactivity guided isolation was used during the whole separation procedure. *In vitro* cytotoxic activities against five human tumor cell lines, including human solid tumor cells, human lung cancer cell A549, human ovarian cancer cell SK-OV-3, human skin cancer cell SK-MEL-2, human CNS cancer cell XF498, and human colon cancer cell HCT15, indicated the significant cytotoxicity of the aforementioned purified compounds with ED_{50} values ranging from 3.4 to $30 \mu\text{g/mL}$. In fact, compound **72** was found to be the most cytotoxic metabolite against above described cancer cell lines, with ED_{50} values ranging from 3.4 to $4.8 \mu\text{g/mL}$ ⁶⁹. Interestingly, three years later, the potential of compound **72** to induce apoptosis in human leukemic U937 cells and its inhibition on telomerase activity were further investigated due to its significant cytotoxicity⁷⁰.

In 2003, Shin and co-workers⁷¹ reported the discovery of three new acetylenic metabolites, 12-methyloctadeca-17-ene-5-ynoic acid (**74**), 2-methoxy-12-methyloctadeca-17-ene-5-ynoic acid

(**75**), and 2-methoxy-12-methyloctadeca-17-ene-5-ynoic anhydride (**76**), from the marine sponge *Stelletta* sp., which was collected off the shore of GaguDo Island, Korea (Fig. 9). Although the crude extract of these sponges only showed weak cytotoxicity against the human leukemia cell-line K562 with LC_{50} value of $490 \mu\text{g/mL}$, the great potential of sponges from Korean water to produce novel metabolites encouraged the authors to further chemically investigate these animals. Thus, the authors introduced the ^1H NMR spectra guided isolation for new lipid-type compound discovery and finally obtained acetylenic acids **74–76** (Fig. 9). However, although their chemical structures were close to acetylenic acids reported by Jung's group⁶⁹ (**67–73**), unlike compounds **67–73** showing potent cytotoxic properties, compounds **74–76** displayed no significant cytotoxic effects. They only exhibited weak cytotoxic activity against the human leukemia cell-line K562 with LC_{50} values of 43.5, 51.3, and $62.5 \mu\text{g/mL}$, respectively⁷¹.

From the same extract, Jung and co-workers⁷² characterized two new lysophosphatidylcholines (**77** and **78**) and four new monoglycerides (**79–82**), along with two known monoglycerides (**83** and **84**) in 2003 (Fig. 10). These metabolites were also evaluated for their cytotoxicity against the same five human tumor cell lines as mentioned above. Unfortunately, only **77** and **78** exhibited moderate cytotoxicity (ED_{50} values ranging from 4.8 to $30 \mu\text{g/mL}$), while the new monoglycerides were all inactive (ED_{50} value over $30 \mu\text{g/mL}$).⁷² The same year, the bioactivity-guided isolation of the same marine sponge extract further gave a new glycerol ether (**85**) with significant cytotoxicity (Fig. 10)⁷³. Compound **85** was established as the first reported glycerol ether from a natural source. The cytotoxicity evaluation of this compound against the same five tumor cell lines, showed that **85** exhibited a potent cytotoxic effect with ED_{50} values ranging from 4.4 to $8.7 \mu\text{g/mL}$ ⁷³.

In 2003, the Jung's group⁷⁴ reported the isolation of norsarco-tride A (**86**), another new cyclitol derivative, along with three known related compounds, sarcotrides A–C (**87–89**) (Fig. 10). Since the previously reported compounds showed promising cytotoxicity against several human cell lines, the new compound

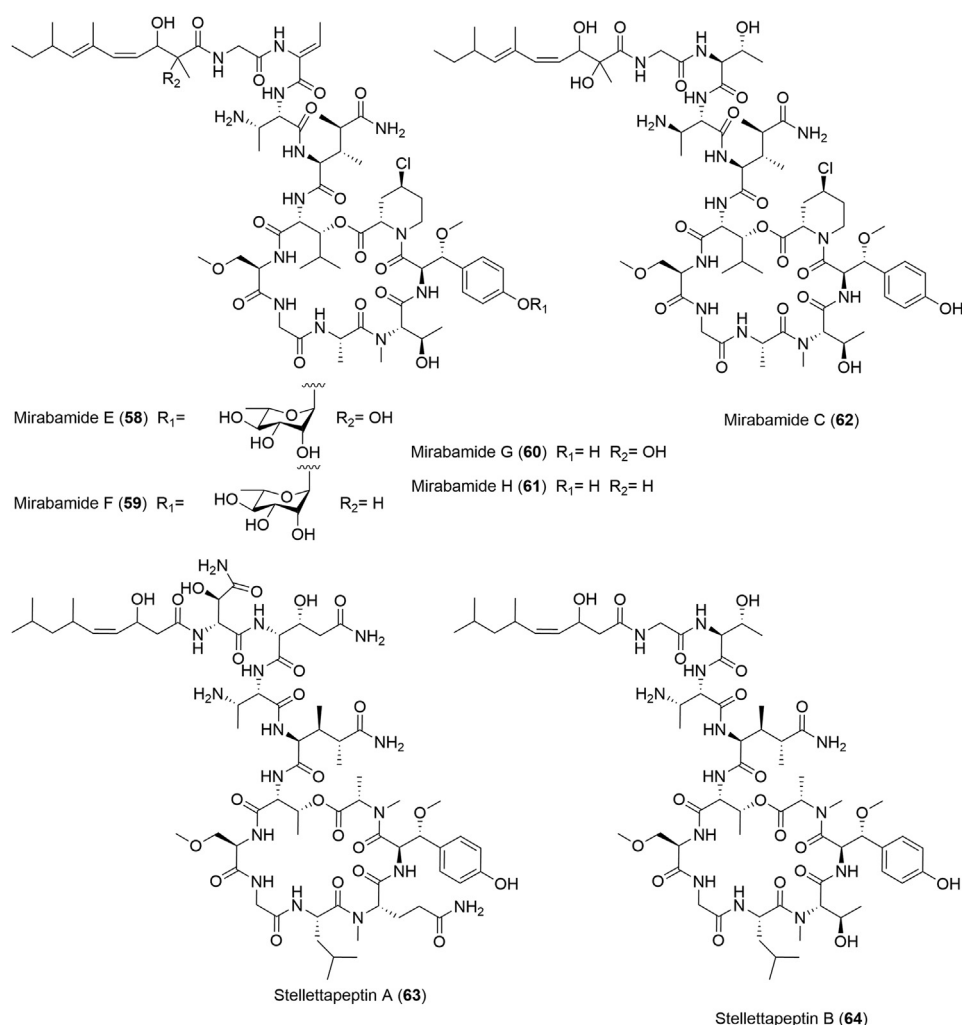


Figure 8 Chemical structures of compounds 58–64.

was also tested for its cytotoxicity and showed significant effects against previously described five human solid tumor cells, with ED_{50} values of 4.3, 5.1, 5.3, 4.4, and 3.9 $\mu\text{g/mL}$, respectively⁷⁴.

The isolation from the MeOH extract of a marine sponge *Stelletta* sp. by Hong and co-workers⁷⁵ gave five new monoacylglycerols (MAGs, **90–94**) (Fig. 11). As for the isolation procedure, the animals were extracted by MeOH and the resulting extract was partitioned between H_2O and CH_2Cl_2 , and the organic solvent layer was further partitioned between MeOH and *n*-hexane to yield two portions. The MeOH portion was subjected to HPLC for isolation, with the four purified metabolites being identified as MAGs **90–94** by positive ion fast atom bombardment mass spectrometry (FAB-MS)⁷⁵.

Glycolipids from natural origins, such as rhamnolipids are regarded as promising bioactive molecules due to their novel structures and diverse biological functions.⁷⁶ In 2015, Matsunaga and co-workers⁷⁷ reported the chemical investigation of a *Stelletta* sponge, which was collected at a depth of 170 m at the seamount “Oshima-shinsonne” in 2008. In this research, LC–MS analysis combined with cytotoxicity assays of the extract resulted in the isolation of several glycosylated fatty acid derived amides, including two isomers, stellettosides A1 (**95**) and A2 (**96**), as well as stellettosides B1–B4 (**97–100**) (Fig. 11). The stellettosides contain an *N,N*-dimethylputrescine moiety as the amine fragment

and an erythro diol on the fatty acid moiety, which is acetylated and glycosylated. Besides, stellettosides comprise *L*-arabinose units as the sugar components. The biological assays indicated that the mixture of stellettosides B1–B4 exhibited moderate cytotoxic activity against HeLa cells with an IC_{50} value of 9 $\mu\text{mol/L}$, whereas the mixture of stellettosides A1 and A2 was not active at a concentration of 10 $\mu\text{mol/L}$ ⁷⁷.

2.5. Steroids

It has been widely reported that steroids are among the most frequently discovered metabolites from marine organisms^{78,79}. In sponges of the genus *Stelletta*, 30 new sterols were isolated and characterized. The first sterols and sterones were discovered from a sponge *Stelletta* sp., collected at depth of 700 m in the deep Coral Sea, southeast of Noumea in 1988⁸⁰. In fact, these compounds are the first examples of stigmastane steroids with a $\Delta^{24,25}$ from marine origin. The structures were elucidated as stigmasta-4,24(25)-dien-3-one (**101**), stigmasta-4,6,24(25)-trien-3-one (**102**), stigmasta-4,24(25)-diene-3,6-dione (**103**), 6 β -hydroxystigmasta-4,24(25)-dien-3-one (**104**), acetate of compound **101** (**105**), stigmasta-5,24(25)-dien-3 β -ol (**106**), and compound **107**. In addition, two ketone analogues were also obtained probably by chemical oxidation

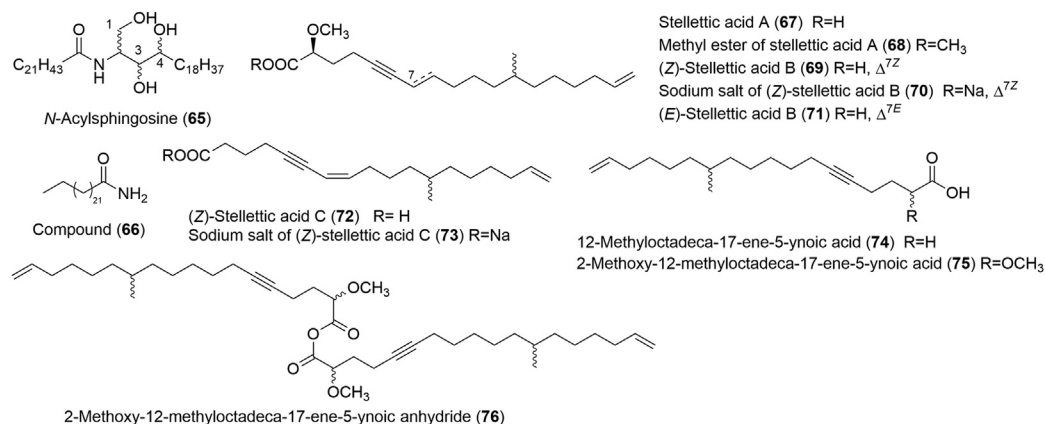


Figure 9 Chemical structures of compounds 65–76.

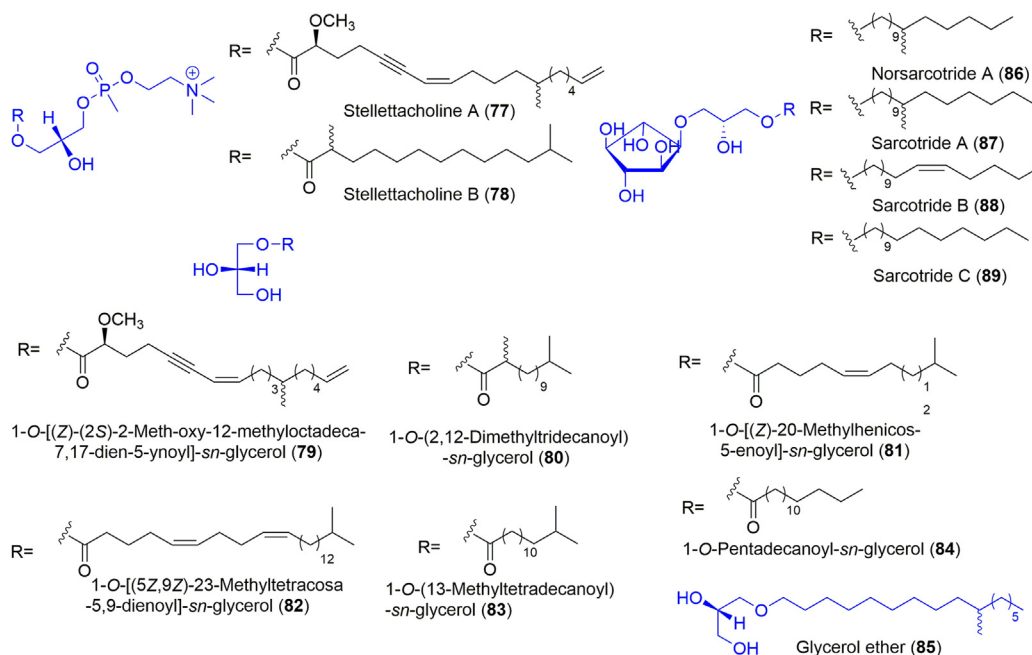


Figure 10 Chemical structures of compounds 77–89.

(**108** and **109**) (Fig. 12). As demonstrated by the authors, the stigmastanes are the only steroids of this sponge, which were more likely to function as stabilizers of sponge cell walls⁸⁰.

In 1992, 24-methylene-27-methylcholesterol (**110**) was identified from a marine *Stelletta* collected in the South China Sea by Deng and co-workers⁸¹. Although this compound was a known one, its structural assignment was accomplished for the first time. Stellettasterol (**111**), a new 9,11-secosterol, was isolated from a Japanese marine sponge, *Stelletta* sp.⁸², collected off Shikine-jima Island of the Izu Archipelago, Japan. In fact, this new compound was the C-3 isomer of known ichthyotoxic *secosterol* **112** isolated from the marine sponge *Dysidea herbacea*⁸³. It was found to have an antifungal activity against *M. remanians* with an MIC value of 12.5 µg/mL, while not cytotoxic against P388 leukemia cells at 2 µg/mL⁸². In 2001, Su and co-workers⁸⁴ carried out the chemical investigation of the South China Sea derived sponge *S. tenuis* Lindgren, which was collected off Xisha Island. The sponge gave two known steroids, 3β-hydroxy-stigmasta-5-en-7-one (**113**) and 3β-hydroxy-cholesta-5-en-7-one (**114**). Besides, eleven steroids

(**115**–**125**) were also detected by GC–MS from the same extract (Fig. 12). During the search for natural anti-cancer drugs from marine invertebrates, Higuchi and co-workers⁸⁵ reported a novel sterol named orostanol (**126**) and its synthetic analog, KPN-2001 (**127**), which induced apoptosis in HL-60 cells at 10 µg/mL. Moreover, the cytotoxic activity indicated that compounds **126** and **127** were highly toxic with IC₅₀ values of 1.7 and 2.2 µmol/L, respectively. Bioactive compound **126** was isolated from a Japanese marine sponge, *S. hiwasaensis*, which was collected off Oro Island, Fukuoka Prefecture, Japan. Structurally, the absolute configuration of **126** was determined by comparison of its CD spectra with that of a synthetic analogs, KPN-2001 (**127**)⁸⁵. Such uncommon sterol with 6/5/6/5 fused rings had only been previously found in taiwaniasterols, which were isolated from the leaves of *Taiwania cryptomerioides*⁸⁶.

In 2007, Lin and co-workers⁴⁶ isolated six steroid derivatives from the marine sponge *S. tenuis*, including three new ones, 24-methylene-27-methylcholesta-3β,5α,6β-triol (**128**), 24-methylene-27-methylcholesta-5-ene-3α,7R-diol (**129**), and

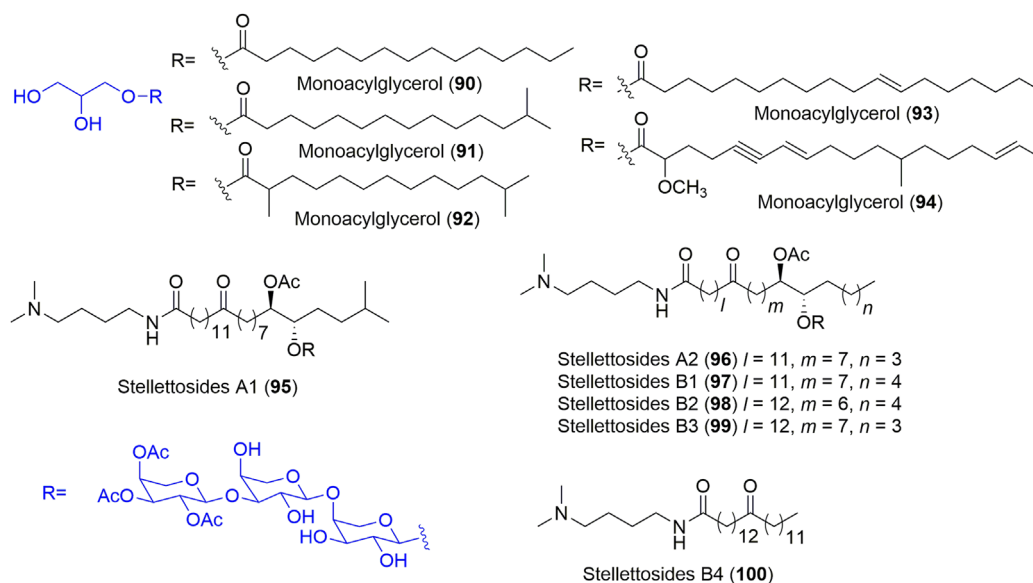


Figure 11 Chemical structures of compounds 90–100.

24-methylene-27-methylcholest-5-ene-3 α ,7 α -diol (**130**) (Fig. 12). The structure determination of the new sterols and the structural revision of a known steroid (**131**) were reported in this paper, but no biological assay⁴⁶.

2.6. MNPs from *Stelletta*-derived microorganisms

As we know that chemistry drives many biological interactions between the microorganisms and their host animals, yet it is often challenging to identify the chemicals involved. Many of the sponge-microbial associates are symbionts involved in nutrient cycling and may also play a role in the sponge's chemical defense mechanisms^{87,88}. Probably by this reason, several *Stelletta*-derived microorganisms have been isolated and chemically studied, leading to the discovery of a total of 28 secondary metabolites (**132**–**159**) from four marine-derived strains (two bacteria and two fungi), which were isolated from the inner tissues of the sponge *Stelletta* sp. (Figs. 13 and 14)^{89–92}. Among them, the most intriguing compounds were the anti-inflammatory sesquiterpenoids (**135**–**148**) from *Stelletta*-derived fungus *Acremonium* sp.⁹¹ Acremofuranones A (**136**) and B (**137**) possess unprecedented cyclic skeleton and were evaluated by measuring the inhibitory effects on the production of pro-inflammatory mediators (NO, IL-6, and TNF- α) in RAW 264.7 murine macrophage cells. Among the tested compounds, **141** and **143** inhibited the production of NO and TNF- α at the concentration of 100 μ mol/L, while compounds **145** and **146** showed selective inhibition of NO production at the same concentration⁹¹.

Over comparison of the secondary metabolites from *Stelletta* sponge and their associate microorganisms, the most possible chemicals involved between them might be depsipeptides, since they shared the similar structure skeletons. While, the typical and intriguing alkaloids or triterpenoids from *Stelletta* sponge have not yet been found any possible relation to their associate microorganisms.

3. Synthetic achievements on *Stelletta* MNPs

The complex structures and extensive biological activities of MNPs from *Stelletta* sponges have attracted many synthetic

chemists toward their total syntheses, which either allowed the determination or revision of their absolute configuration or provided sufficient amounts of compounds for further biological evaluation. In this section, all synthetic intermediates will be numbered as **S n** ($n = 1, 2, 3...$).

3.1. Stellettadine A

In light of the interesting biological activity of stellettadine A (**4**), the total synthesis of **4** and its enantiomer (*ent*-**4**) was achieved by Mori and co-workers³¹ in 2000, ending with structure revision of **4**. As shown in Scheme 1, for the synthesis of (*S*)-stellettadine A and (*S*)-citronellal (**S1**) was used as the starting material, which underwent five steps, including a Wittig reaction, a palladium-catalyzed oxidation, a second Wittig reaction, an ester hydrolysis, and a chlorination to afford conjugated acyl chloride **S2**, with 36% overall yield. Meanwhile, after some synthetic attempt, the authors selected the commercially available agmatine sulfate (**S3**) as the guanidine starting material to be reacted with **S2**, after *t*-butoxycarbonyl (Boc) protection of the non-guanidine primary amino group towards **S4**. The key acylation of **S4** in the presence of two equivalents of **S2** gave rise to diacylated product **S5** in 42% yield over two steps. As for the acylation conditions, the authors did many attempts, and finally used Ottenheim's method⁹³ by treating **S4** with trimethylsilyl chloride in the presence of diisopropylethylamine (DIPEA) to yield *N*-silylated intermediate, which then reacted with **S2** in the presence of DIPEA to afford **S5**. The Boc group was removed by *p*-TsOH in 2-methyl-2-butene and MeOH towards **S6** in 84% yield, and the second guanidine group at the *N*-terminal position was installed by treating **S6** with aminoimino-methanesulfonic acid in MeOH to give **S7**. The extra acyl group was removed by methanolysis in the presence of KOH to afford *ent*-**4** as its dihydrochloride after HCl treatment, in 67% yield over two steps, with $[\alpha]_D^{25} +39.4$ (*c* 1.01, MeOH). Therefore, the total synthesis of (*S*)-stellettadine A was achieved in 10 steps from *S*-citronellal, with an overall yield of 8.5%. By using the same sequence, and starting from *R*-citronellal (**S8**), (*R*)-stellettadine A (**4**) was obtained with $[\alpha]_D^{25} -45.6$ (*c* 1.37, MeOH) (Scheme 1).

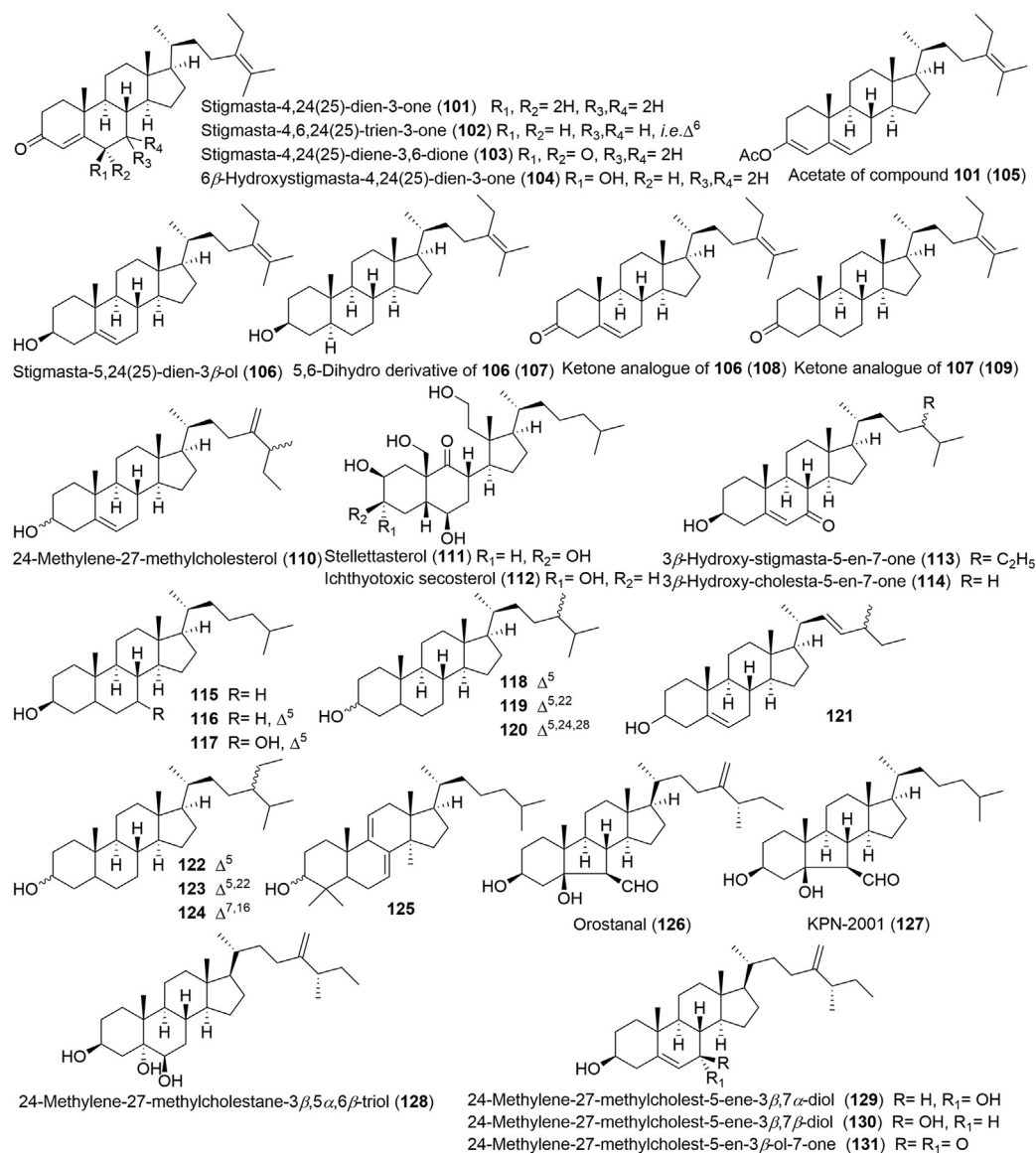


Figure 12 Chemical structures of compounds **101**–**131**.

By comparing the specific rotations, the natural product $\{[\alpha]_D^{25} - 42.7$ (c 0.092, MeOH) $\}$ was revised to be (*R*)-stellettadine A.

3.2. Stellettamides A–C

As discussed in the isolation section, stellettamides A–C (**1**–**3**) exhibited strong cytotoxicity and antifungal activities, which attracted the attention of synthetic chemists. In 1997, Carreira and co-workers²⁴ reported an enantioselective synthesis of *ent*-stellettamide A (*ent*-**1**), involving an asymmetric diazoalkane dipolar cycloaddition, to establish its absolute stereochemistry (Scheme 2). They first used dipolarophile **S9** to react with a solution of 2.2 equiv. of Me_3SiCHN_2 , affording the pyrazoline cycloadducts as a 93:7 mixture of C2/C3 diastereomers. The unpurified mixture was further treated with EtO_2CCl and $AgOTf$ towards **S10** and **S11**, with 71% and 6% yield, respectively. **S10** was then reduced to the corresponding primary alcohol with $LiAlH_4$ in 91%, followed by Swern oxidation with 93% yield to

produce aldehyde **S12**. It was further treated by the acetylide derived from $TBSOCH_2C \equiv CH$ to afford a secondary propargyl alcohol. Without purification, this alcohol underwent mesylation to obtain **S13** with 68% yield over two steps. **S13** was converted to *cis*-alkene **S14** by the hydrogenolysis of the propargyl mesylate followed by partial hydrogenation of the alkyne, with 72% yield over two steps. **S14** was treated by aqueous $Ba(OH)_2$ to hydrolyze its ethyl carbamate and the silyl ether, to give a pyrazoline which was selectively protected towards the *N*-Boc-carbamate, followed by mesylation of the remaining alcohol to afford **S15** with 80% overall yield. Dissolution of **S15** in 10% concentrated H_2SO_4 /dioxane resulted in hydrolysis of the carbamate, and the reaction mixture was then added to a 2 mol/L NaOH solution to give bicyclic intermediate **S16** (83%, two steps). This compound was then treated with Raney Ni/ H_2 to reduce C=C and C=N unsaturations and cleave the N–N bond to afford a diamine, which was further selectively protected as the corresponding *N*-trifluoroacetamide (**S17**) with 85% yield over two steps. Deprotection of the benzyl ether followed by treatment of the resulting primary

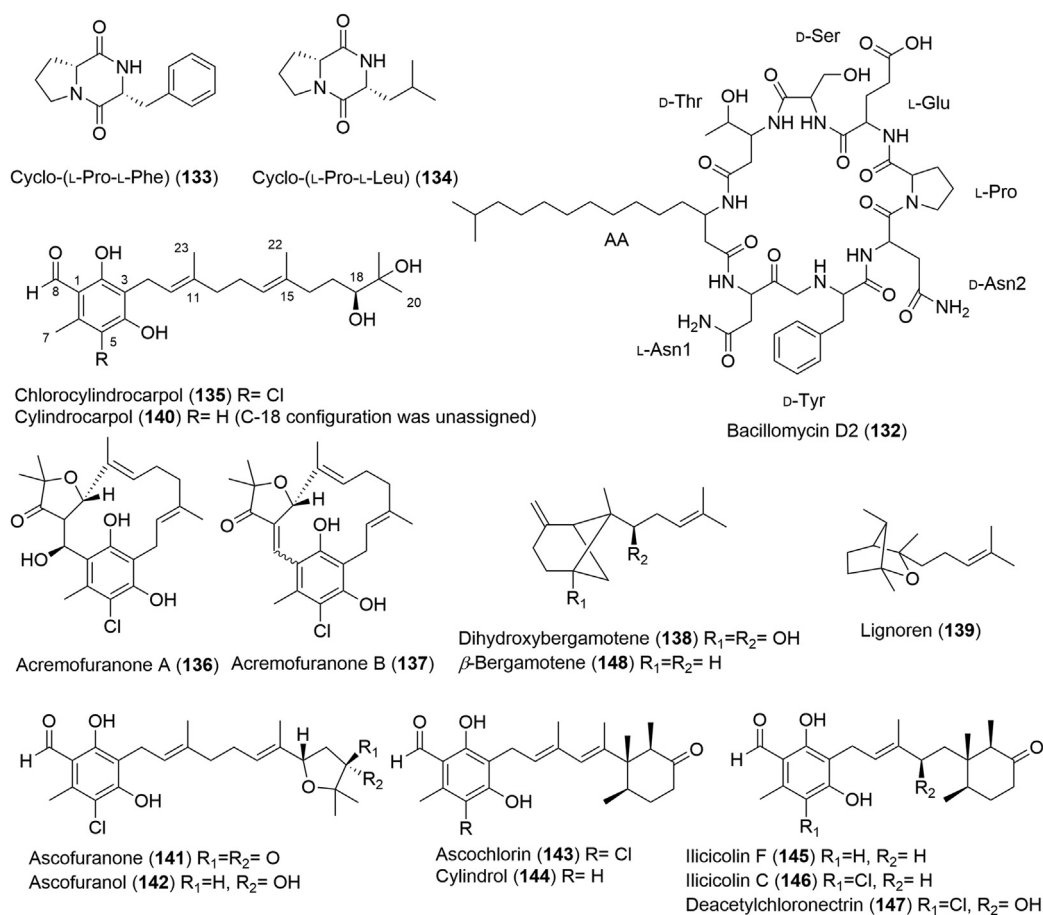


Figure 13 Chemical structures of compounds 132–148.

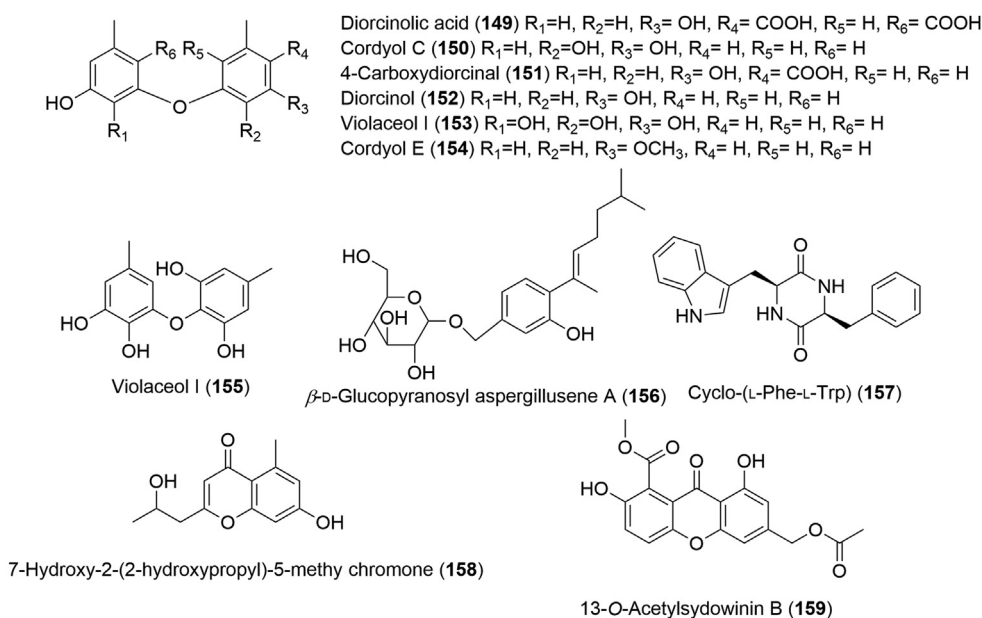
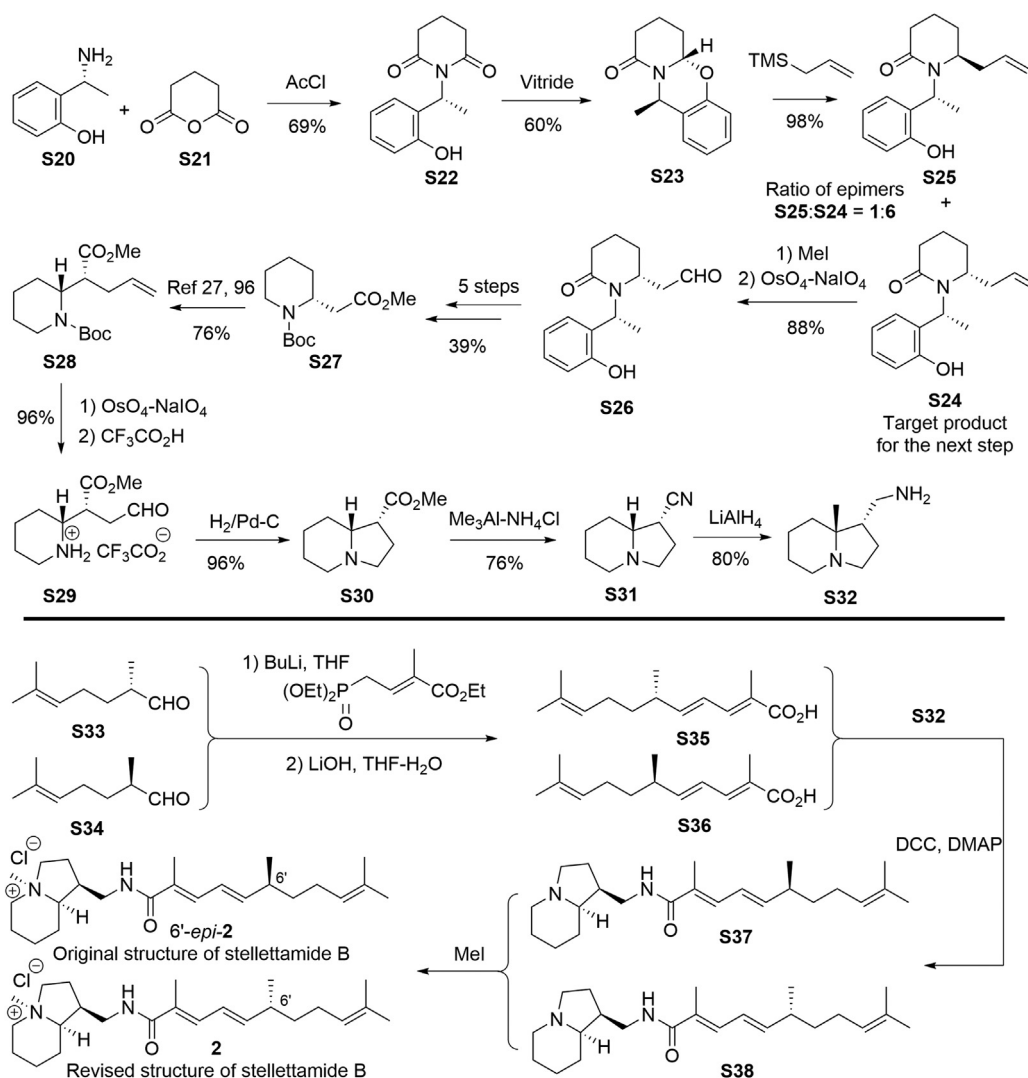


Figure 14 Chemical structures of compounds 149–159.

alcohol with CBr₄ and Ph₃P led to ring closure⁹⁴, of which the alkaline hydrolysis of the trifluoroacetamide gave the indolizidine core of stelletamide **S18**. Since the isolation report did not

describe the relative configuration of the side chain²², both (*R*)- and (*S*)-trienic acids **S19** and *ent*-**S19** were synthesized⁹⁵. Each of these two carboxylic acids was coupled with **S18** in the presence



Scheme 3 Total synthesis of (-)-stellettamide B.

synthesis of the indolizidine ring started by the treatment of enantiomeric 2-(1-aminoethyl)phenol (**S20**) with glutaric anhydride (**S21**) towards glutarimide **S22**, which was then reduced to tricyclic N,O-acetal **S23** as a single diastereomer by Vitride® followed by HCl treatment. **S23** was further submitted to allylation with allyltrimethylsilane (3 equiv.) and TiCl_4 (3 equiv.) at 50 °C to afford the allylated products **S24** and **S25** in a ratio of 6:1 favoring the desired (6*R*)-allyl-2-piperidinone **S24**. This intermediate underwent methylation of the phenolic hydroxyl group, followed by Lemieux-Johnson oxidative cleavage of the olefin (OsO_4 , NaIO_4) to give aldehyde **S26** (Scheme 3). It was further functionalized by lactam reduction, chiral auxiliary cleavage, N-Boc protection, PDC oxidation of the aldehyde, and esterification to give 2-[(2*R*)-piperidinyl] acetate **S27**. Then, the diastereoselective allylation of ester **S27** was performed by using allyl bromide after deprotonation by LiHMDS, to obtain the desired product **S28** (84% de, 76% yield)^{27,96}. Another oxidative cleavage of **S28**, followed by N-Boc deprotection led to compound **S29** as a TFA salt, which underwent an intramolecular reductive amination in the presence of $\text{Pd-C}/\text{H}_2$ in MeOH to promote ring closure towards indolizidine **S30** as a single isomer. It was then

converted into nitrile **S31** by treatment with trimethylaluminum- NH_4Cl , which was further transformed into aminomethylindolizidine **S32** by LiAlH_4 reduction in diisopropyl ether. After completion of the cyclic part, the linear moiety **S35** was achieved by the Horner-Emmons olefination of linear aldehyde **S33** with the appropriate 4-phosphono-2-methylcrotonate, followed by hydrolysis of the ester. The two fragments **S32** and **S35** were coupled by using DCC and DMAP, furnishing the desired amide **S37** in 83% yield, which further underwent quaternization of the indolizidine part with iodomethane. Final exposure to AgCl resulted into methylammonium chloride 6'-*epi*-**2** in 84% yield. However, the NMR data of 6'-*epi*-**2** did not completely match those of the natural product stellettamide B, and more strikingly, the values of their specific rotation were of the opposite sign. Therefore, 6'-*epi*-**2** should be an isomer of natural stellettamide B. Finally, the authors performed the same synthetic sequence from **S34** to achieve the total synthesis of (-)-stellettamide B (**2**). It was confirmed to be identical to the natural product by NMR and displayed similar specific rotation. In conclusion, Kibayashi and co-workers²⁵ reported another synthetic strategy for the construction of the indolizidine ring and further

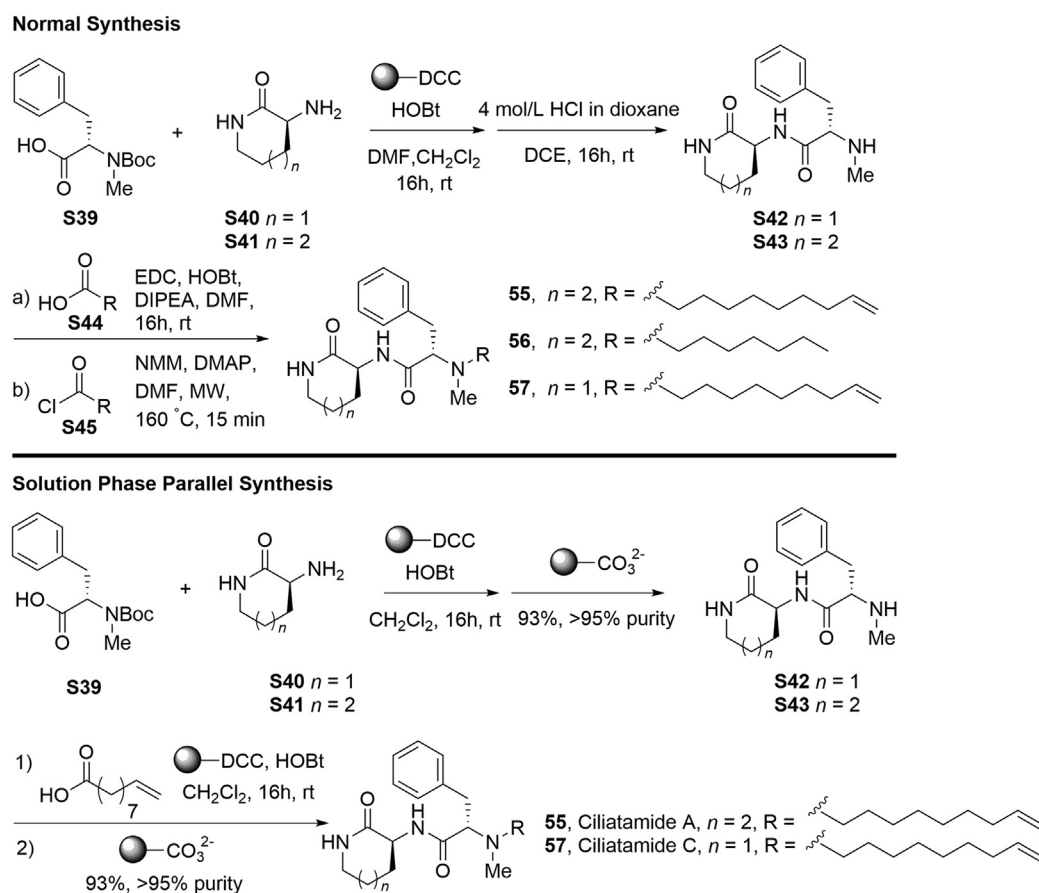
achieved the total synthesis of natural (–)-stellettamide B and its structure revision. In 2008, by using a similar synthetic route, Yamazaki and co-workers²⁵ achieved the asymmetric total synthesis of (+)-stellettamide A and (–)-stellettamide C.

3.3. Ciliatamides A–C

Ciliatamides A and B (**55** and **56**) exhibited significant antileishmanial activity, which led them to become valuable targets for total synthesis and further biological evaluation towards new and structurally less complex antileishmanial drugs. With this aim, in 2008, Lindsley and co-workers⁵⁵ reported the total synthesis of ciliatamides A–C (**55–57**) and their structure revision (Scheme 4). They started from *N*-Boc-*N*-Me-L-Phe **S39**, by an amide coupling with either (*S*)-3-aminoazepan-2-one **S41** (for **55** and **56**) or (*S*)-3-aminopiperid-2-one **S40** (for **57**) in the presence of PS-DCC and HOBT, followed by Boc-deprotection with HCl in dioxane, leading to cyclic precursors **S43** and **S42**, respectively. **S43** underwent a second amide coupling either with fatty acid **S44** in the presence of EDC, HOBT and DIPEA in DMF, or with acyl chloride **S45** in the presence of NMM, DMAP, in DMF under microwave at 160 °C to afford **56**, in 56% and 58% yield, respectively. With the same procedure as for **55**, ciliatamide C (**57**) was achieved by coupling **S42** and **S44**. Due to the physicochemical properties of **55–57** and the poor chromatographic performance of such lipopeptides, the overall yields were lower than expected. Therefore, the authors developed a solution phase parallel synthesis approach to synthesize ciliatamides A (**55**) and C

(**57**), involving only polymer supported reagents, scavengers and ion-exchange chromatography to avoid normal and reverse-phase chromatography.⁹⁷ This strategy improved the synthesis, giving **55** and **57** in overall yields of more than 75% for the three steps with over 95% purity (Scheme 4). The NMR data of synthetic ciliatamides A–C overlaid with those of the natural products, while their optical rotations were of opposite sign. On the basis of this fact, the authors synthesized all stereoisomers (*S,S*), (*S,R*), (*R,S*), and (*R,R*) of ciliatamides A–C, employing the solid phase route depicted in Scheme 4. Finally, **55–57** were determined to be (*R,R*)-ciliatamide A, (*R,R*)-ciliatamide B, and (*R,R*)-ciliatamide C, respectively, by comparing their NMR data and optical rotation with those reported for the natural products. It is worth mentioning that the authors further synthesized more than 50 unnatural ciliatamide analogs in order to evaluate their antileishmanial activity and the structure-activity relationships, while the biological results were not reported in that paper.

In fact, the total synthesis of ciliatamide A (**55**) and its three isomers were also accomplished by Takada and co-workers⁵⁶ with a similar synthetic strategy as that of Lindsley. However, through the comparison of NMR data, optical rotation, and more convincingly chiral HPLC of the four synthetic compounds with those of the natural product, the authors also revised the structure of natural ciliatamide A **55** to be of 12*R*,22*S* configuration. In addition, by using Marfey's method, the absolute configuration of the methionine sulfoxide residue in ciliatamide D (**54**) was also revised to be D-form. This research reminds us that, in certain cases, the direct comparison of synthetic compounds with the natural product for their absolute configuration determination is crucial.



Scheme 4 Total synthesis of ciliatamides A–C.

In summary, synthetic efforts were mainly focused on *Stelletta* alkaloids or peptides and, in most cases, led to structure revision of the natural products. Some synthetic methodologies have also been developed during these syntheses, such as Carreira's asymmetric [3+2] cycloaddition for the rapid access to optically active pyrazolines. However, the authors do not seem to have taken advantage of the large quantity of natural products obtained from total synthesis to gain more deepened biological results. Future work should thus be conducted toward the intensive biological evaluation of these natural products and their analogs derived by rapid synthesis.

4. Bioactivities of stellettins A and B

Among all the metabolites identified in marine *Stelletta* sponge, the triterpenoids stellettins A and B (**25** and **24**) have been intensively studied for their anticancer activities by several research groups, which is worth to be detailed discussed herein so as to understand how the bioactive *Stelletta*-derived compounds being well studied towards potential drug leads.

Although the selective cytotoxicity of both stellettins A and B were reported from 1997 to 2002, the underlying mechanism of stellettin A (**25**) was only reported in 2006 by Liu's group⁹⁸. It was suggested that **25** was able to induce oxidative stress and apoptosis in HL-60 human leukemia and LNCaP prostate cancer cell lines. More specifically, they found that **25** induced more potent oxidative stress in HL-60 cells than in LNCaP cells, resulting into cell death through a FasL-caspase-3-apoptotic pathway (Fig. 15). In 2009, Folmer et al.⁹⁹ investigated the cytotoxic effects of several natural products, including stellettins A and B, and their inhibition of TNF- α -induced NF- κ B activation. After structure-activity relationship (SAR) analysis, they suggested that for both compounds **24** and **25**, the α,β -unsaturated lactone rings might be responsible for their interference with IKK β and with the binding of NF- κ B to DNA⁹⁹. In 2012, Liu's group¹⁰⁰ reported that stellettin A (**25**) could act as an endoplasmic reticulum stressor to inhibit the growth of B16 melanoma cells by induction of abnormal protein glycosylation and autophagy. They also

demonstrated that **25** was able to induce the upregulation of the unfolded protein chaperone, glucose-regulated protein 78, in a dose-dependent manner (Fig. 15).

Undoubtedly, due to the highly similar structures of stellettins A and B, stellettin B (**24**) was also found to be active on several cell lines. Liu and co-workers¹⁰¹ investigated the apoptotic activity of stellettins A and B. The study indicated that a ketone group at the C-3 position of isomalabaricane triterpenes **24** and **25** was necessary for free radical production and dissipation of mitochondrial membrane potential during the induction of leukemia HL60 cell death. Recently, a more systematical *in vitro* cytotoxicity study of stellettin B on human glioblastoma cancer SF295 cells was reported (Fig. 15)¹⁰². The authors investigated the cytotoxic activities on 39 human cancer cell lines, showing significant cytotoxic effects against the human glioblastoma cell line SF295, with a GI₅₀ value of 0.01 μ mol/L. Moreover, compared to the cytotoxic activity against several normal human cell lines including HMEC, RPTEC, NHBE and PrEC with GI₅₀ values of 10 μ mol/L, compound **24** possessed relatively selective cytotoxicity against human cancer cells. Further flow cytometric analysis showed that stellettin B induced apoptosis of SF295 cells in a concentration-dependent manner. The treatment of SF295 cells with stellettin B also increased ROS production, caspase 3/7 activity, and cleavage of PARP, all of which were known to be involved in apoptosis. To better understand the molecular mechanism for cell proliferation inhibition and apoptosis induction by stellettin B, its effect on the phosphorylation of several signal proteins of PI3K/Akt and RAS/MAPK pathways was examined. The targeted compound inhibited the phosphorylation of Akt, without any activity on p-ERK and p-p38, indicating that inhibition of PI3K/Akt pathway might be involved in the antiproliferative and apoptosis-inducing effect (Fig. 15). However, homogenous time-resolved fluorescence (HTRF) assays indicated that stellettin B did not inhibit PI3K, suggesting that the direct target might be a signal protein upstream of Akt pathway other than PI3K.

Another recent biological study of stellettin B isolated from the marine sponge *Jaspis stellifera* demonstrated that stellettin B induced G1 arrest, apoptosis and autophagy at low concentrations

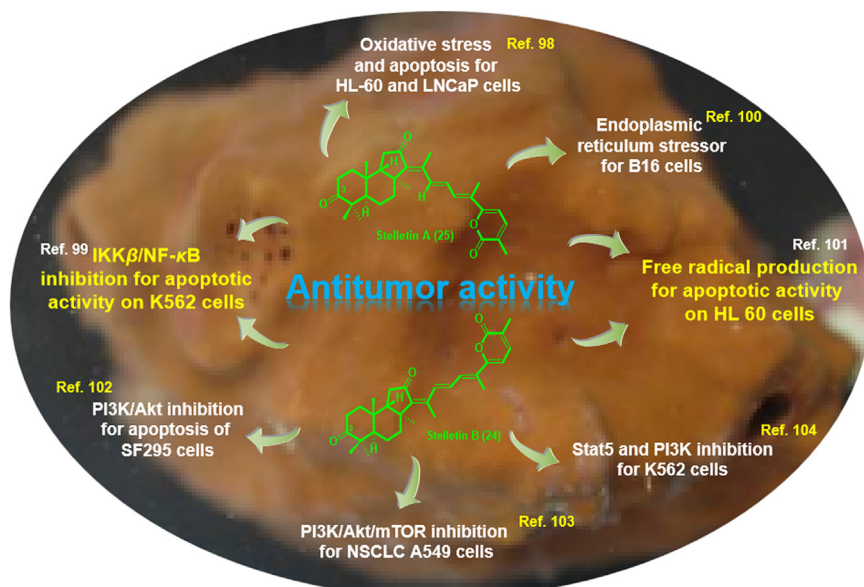


Figure 15 Summary of cytotoxicity mechanism studies of stellettins A and B (**25** and **24**).

in human non-small cell lung cancer (NSCLC) A549 cells¹⁰³. The findings indicated that the cytotoxicity of stellettin B on NSCLC was triggered by targeting PI3K/Akt/mTOR pathway (Fig. 15). G1 arrest by stellettin B might be attributed to the reduction of cyclin D1 and enhancement of p27 expression. Several assays including monodansylcadaverine (MDC) staining, transmission electron microscopy (TEM), tandem mRFP GFP-LC3 fluorescence microscopy, and western blot detection of the autophagy markers of LC3B, p62 and Atg5 were used to demonstrate autophagy inducing ability of stellettin B in A549 cells. The apoptosis induction of stellettin B in A549 cells might be related to the cleavage of PARP and increase of ROS generation. Meanwhile, stellettin B inhibited the expression of PI3K-p110, and the phosphorylation of PDK1, Akt, mTOR, p70S6K, as well as GSK-3 β , suggesting a correlation with PI3K/Akt/mTOR pathway inhibition in the above cytotoxicity. Thus, the cytotoxic effects of stellettin B on human NSCLC A549 cells, including G1 cell cycle arrest, apoptosis and autophagy induction was well investigated during the research.

The most recent biological research on stellettin B was reported by Kong and co-workers¹⁰⁴ in 2017. They investigated *in vitro* anti-leukemia activity of stellettin B on human CML K562 and KU812 cells. **24** inhibited K562 and KU812 cell proliferation with IC₅₀ as 0.035 and 0.95 μ mol/L respectively. After treatment by stellettin B, apoptosis was induced in K562 cells and it was described that stellettin B-induced apoptosis was possibly related in mitochondrial pathway to increased BAD and BAX, decreased BCL-2 and caspase-9 activation. In addition, the authors demonstrated that the inhibition of Stat5 and PI3K might be involved in the apoptosis-inducing effect based on a set of experimental results, including the inhibition of Stat5 phosphorylation, inhibition of the expression of 4 PI3K catalytic isoforms, and inhibition of the phosphorylation of downstream effectors including PDK1 and Akt (Fig. 15). Furthermore, the synergistic effect of stellettin B and Imatinib occurred with ratio as IC₅₀ (stelllettin B): 5 $\times$ IC₅₀ (Imatinib), which made stellettin B a promising candidate against chronic myeloid leukemia therapy alone or together with Imatinib. As for the SAR analysis of these two stellettins, although **24** and **25** shared similar structural features and bioactivities, their mechanism action are different. It seems that the configuration at $\Delta^{13,14}$ significantly altered the binding sites of the compounds.

In summary, the mechanisms of anticancer activity of **24** and **25** were studied by several groups. They provided clues for the understanding of the signal pathway or target protein of both compounds towards cancer cells. Further *in vivo* study should be conducted to evaluate the druggability of these two potential cytotoxicity natural products.

5. Conclusion and perspectives

Great efforts have been made for the chemical investigation of marine *Stelletta* sponges all over the world sea from 1982 to early 2018. During the 35 years, more than 130 diverse secondary metabolites, including alkaloids, terpenoids, peptides, lipids and steroids, were discovered and characterized with different ratio of structure types varied by years (Fig. 16), and broad biological activities, such as antimicrobial, RNA cleavage activating, cytotoxic and anti-HIV effects. It is worth noting that, as have been demonstrated in Fig. 1, the skeletons of *Stelletta* metabolites also differed according to the collection locations. For instance, steroids

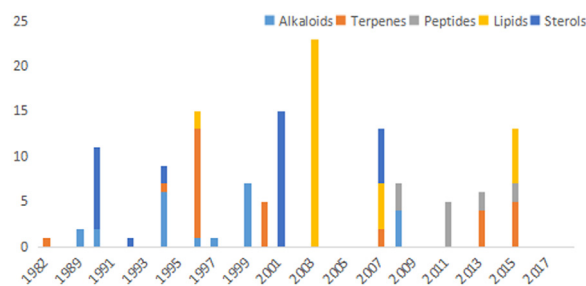


Figure 16 *Stelletta*-derived secondary metabolites from 1982 to 2017.

and triterpenoids were mainly discovered in South China Sea, whereas alkaloids and lipids were mostly isolated from Japanese Sea. Such observation indicated that marine *Stelletta* sponges were capable of producing different kind of secondary metabolites to adapt to the various living environments. On this chemo-ecological point of view, future work would be interesting to focus on the discovery of targeted bioactive molecules, such as highly cytotoxic triterpenoids, from certain sea areas. Another outlook worth to mention is the possible biogenetic relationship between some metabolites in this genus. It could inspire biomimetic syntheses and compound interconversion, to scale up the available amounts for further pharmacological and toxicological evaluation. Furthermore, we also hold the belief that symbiotic microbes in *Stelletta* sponges have taken part in the generation of some metabolites, which could be another direction for the future study.

As we all know that the research of natural products is a labor and resource intensive work, novel and more powerful chromatographic methods should be developed in accelerating the discovery process toward new drug leads from Nature, especially from marine environments which have yet seldom been developed. For example, the LC-MS-based metabolomics approach is a promising way to recognize already known metabolites from marine sponges¹⁰⁵. It may further become crucial in *Stelletta*-derived drug candidate discovery. Since the high chemical diversity in *Stelletta*-derived MNPs was established, tandem mass spectrometry could be applied to build molecular networks for new compound searching. Data analysis of MSⁿ fragmentation can be searched and assigned based on the open access databases, such as Global Natural Products Social Molecular Networking (GNPS)¹⁰⁶. All these efforts would speed up the discovery of functional metabolites, not only from *Stelletta* sponges, but also from all the precious Ocean resources.

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