Review

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Review of bioactive secondary metabolites from marine bryozoans in the progress of new drugs discovery

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Marine bryozoans play an important role for the discovery of novel bioactive compounds among marine organisms. In this review, we summarize 164 new secondary metabolites including macrocyclic lactones, sterols, alkaloids, sphingolipids and so forth from 24 marine bryozoans in the last two decades. The structural features, bioactivity, structure–activity relationship, mechanism and strategies to address the resupply of these scarce secondary metabolites are discussed. The structural and bioactive diversity of the secondary metabolites from marine bryozoans indicated the possibility of using these compounds, especially bryostatin 1 (**1**), bryostatin analog (**BA1**), alkaloids (**50, 53, 127–128** and **134–139**), sphingolipids sulfates (**148** and **149**) and sulfur-containing aromatic compound (**160**), as the starting points for new drug discovery.

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Background

Natural products and their molecular frameworks have a long tradition as valuable starting points for new drug discovery [1]. They serve as an inspiration to discovery of new drug candidates and to illustrate the mechanisms for their complex three-dimensional architecture and exquisite biological activity. Keohane *et al.* surprisingly find that succinate dehydrogenase is the biological target for the natural product of promysalin, using affinity-based protein profiling [2]. A natural product-like compound was discovered as a von Hippel-Lindau mediated HIF1α interaction inhibitor with *in vivo* anti-angiogenic activity using structure-based virtual screening [3]. Moreover, the success of the anticancer compound cisplatin and its analogs inspired the discovery of bioactive leads from metal-based compounds. Leung and coworkers identified an enantiomeric iridum(III) metal-based compound showing potent inhibition against the Ras/Raf interaction and repression of renal cancer xenografts *in vivo* [4]. A novel metal-based rhodium(III) complex was identified as a new lysine-specific demethylase 1 targeting agent and epigenetic modulator [5]. Besides, an iridum(III) complex was reported as the first metal based irreversible inhibitor of bromodomain-containing protein 4, and may serve as a useful scaffold for development of more potent epigenetic agents against cancers [6]. Albada and Metzler-Nolte prepared some active synthetic antimicrobial peptides (AMPs) through rational optimizations in several generations of organometallic AMPs, and analyzed the mode of action for the typical ruthenocene derivative Rc-WRWRW-NH_2. The synthetic AMPs combined with colistin and tobramycin can be used for the treatment of *Pseudomonas aeruginosa* infections that are associated with cystic fibrosis [7].

Marine natural products show a higher incidence of significant bioactivity and structural novelty compared with terrestrial secondary metabolites, which have led a wave for new drug discovery [8]. There are 1340 new compounds

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isolated from marine origin in 429 papers for 2015. Among them, nine new compounds described in five reports were from a bryozoan origin [9]. Marine bryozoans are invertebrates known from tropical to polar regions, and from the intertidal to the deep sea. Habitat-forming bryozoans are abundant and diverse in regions such as New Zealand, the Antarctica, the North Pacific around Japan, the northern Mediterranean and Adriatic, along the southern edge of the North Sea, through the English Channel and around the United Kingdom. Marine bryozoans are often colonial, benthic or epibiotic on algae, seagrass and other marine animals. Bryozoan morphology shows great variation including encrusting uni- and multilaminar colonies, branches of radially arranged zooids and erect uni- and bilaminate colonies [10]. There are more than 6000 species of marine bryozoans, which can be divided into three classes; Phylactolaemata, Stenolaemata and Gymnolaemata. Phylactolaemata is generally considered to be the most primitive class. Molecular sequence data indicate Phylactolaemata is the sister group to Gymnolaemata and Stenolaemata, which is in accordance with morphological phylogenies [11]. Gymnolaemata is the most studied class due to its ease of identification, as well as its abundance in bioactive secondary metabolites. Marine bryozoans are well known producers of bioactive compounds and an important source of marine drug leads, which has attracted researchers' interests due to the remarkable antineoplastic activity of bryostatins discovered by Pettit *et al.* The secondary metabolites and their bioactivities from marine bryozoans are reported every year by the journal Natural Product Reports, within the general subject area of Marine Natural Products. Herein, we discuss the bioactivities of 164 compounds, including macrocyclic lactones, sterols, alkaloids, sphingolipids and other types of tetracyclic terpenoid lactones and sulfur-containing aromatic compounds, from 24 marine bryozoans in the orders of Cheilostomata and Ctenostomata, with the view of their potential as pharmacological probes and/or leads in drug discovery. The 164 metabolites reported since 1996 are classified as six types consisting of macrocyclic lactones, sterols, alkaloids, sphingolipids, sulfur-containing aromatic compounds and tetracyclic terpenoid lactones. These groups were based on the similarity of structural features and the numbers of metabolites identified from marine bryozoans. The structural characteristics, bioactivities and structure–activity relationship (SAR) of the related secondary metabolites are described. In particular, the synthesis, bioactivity and mechanism of action for the new drug candidate bryostatins are highlighted. In addition, strategies to solve the source and resupply of bioactive bryozoan metabolites are also discussed due to their low yield from natural origin.

Macrocyclic lactones from marine bryozoans

Discovery of macrocyclic lactones from marine bryozoans

Macrocyclic lactones are a class of common compounds with striking antitumor activity in marine organisms. The size of the lactone ring is relatively large ranging from 10 to 60. Interest of studies on marine bryozoans was initiated from 1968 when Pettit *et al.* found potential cytotoxic constituents from *Bugula neritina* in California. The first macrocyclic lactone, bryostatin 1 (**1**), was finally identified from *B. neritina* by Pettit *et al.* in 1982, due to the limited ability at that time to isolate and elucidate trace amounts of newly active ingredients from nature [12]. After another 14 year endeavor studying macrocylic lactones from marine bryozoans, Pettit and coworkers have identified 18 analogs (bryostatins **1–18**, Figure 1) and evaluated their antitumor activity [13]. During the investigation of antineoplastic constituents from *B. neritina* in the South China Sea, Lin *et al.* isolated a new macrolide bryostatin 19 showing strong cytotoxicity against the U937 cell line with an ED_{50} value of 3.2 nmol/l *in vitro* [14]. Lopanik *et al.* found a novel bryostatin 20 (**20**) that was unpalatable to fish, from the larvae of *B. neritina*. The result represents the first example from marine environment of a microbial symbiont producing an antipredator defense for its host [15]. Recently, four new macrocyclic lactones, bryostatin 21 (**21**) and 9-O-methylbryostatins 4, 16 and 17 were identified from *B. neritina* in the South China Sea. The novelty of bryostatin 21 is the presence of a single methyl group at C-18 compared with the previously isolated bryostatins. However, its cytotoxicity is reduced substantially. Replacement of an α hydroxyl by a methoxyl group at C-9 in bryostatins is also correlated with a loss of inhibitory activity [16]. Thus, the α methyl at C-28 and the α hydroxyl at C-9 play an important role for the potent cytotoxicities for the bryostatins. The predominant structural feature of the 24 isolated bryostatin macrolides is characterized by a 26-membered cyclic skeleton with three imbedded pyran rings, and their main differences were the substituents at C-7 and C-20. The molecular mechanism of the bryostatins antitumor activity has been their ability to selectively bind to the regulatory domain of various individual PKC isozymes within cells. Ruan and Zhu summarized the SAR of bryostatins that a 26-membered macrolactone ring is needed for good PKC binding activity, hydroxyl at C-3 with (*R*)-stereochemistry and a free hydroxyl at C-26 is important for a high enzyme affinity and the structure in the C7-C9 region of the A-ring is critical for the potent inhibition of tumor cells [17].

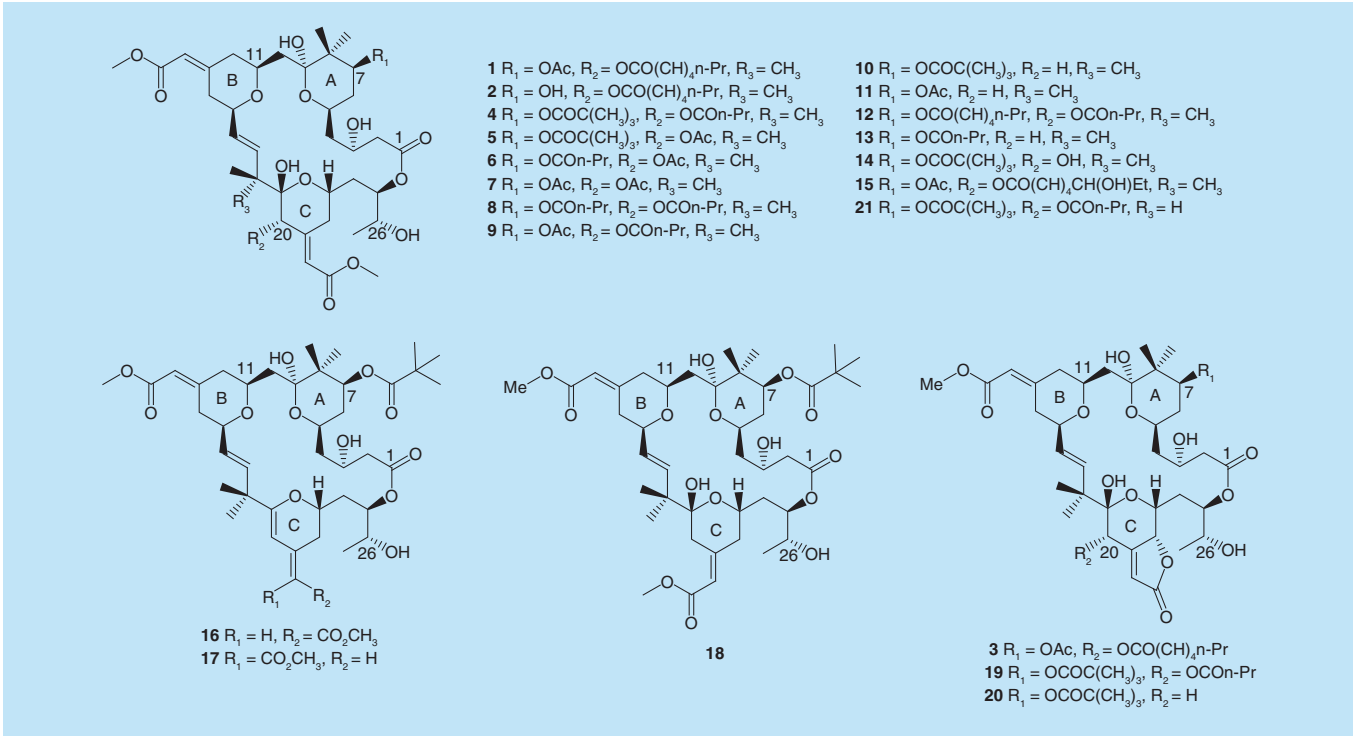

Figure 1. Chemical structures of bryostatins (1–21) from marine bryozoans.

Total synthesis of bryostatins

Total synthesis of bryostatins is an efficient strategy to provide a reliable source of these promising compounds. To date, total synthesis of bryostatins 1, 2, 3, 7, 9 and 16 have been achieved since bryostatin 7 since the first total synthesized reported by Kageyama *et al.* [18]. It's worth noting that Trost *et al.* synthesized bryostatin 16 using a concise atom-economical and chemo-selective approach. The implementation of this strategy allows access to synthesis of various bryostatins and their analogs in the laboratory [19]. However, complicated synthetic routes to the bryostatins make these strategies difficult to adopt for commercial production. For instance, the accomplishment of total synthesis of bryostatin 1 needed 30 steps for the longest linear sequence from commercially available (*R*)-isobutyl lactate [20].

Simplified partial synthesis of bryostatin analogs

Since there are complicated extraction procedures and low yields of bryostatins from their natural source, and there is high cost and low commercial value of total synthesis, the simplified partial synthesis of bryostatin analogs (BA) to replace bryostatins is an attractive way to solve the source problem for bryostatins. The structural difference between bryostatin 1 and BA is the simplified substituent groups at C-7, C-9, C-13, C-20 and C-26 (Supplementary Figure 1). Wender and coworkers synthesized a number of bryostatin analogs for easy synthesis and superior clinical performance since 1980 [21]. The representative analog, termed 'picolog' (BA **1**), exhibited superior growth inhibition of MYC-induced lymphoma *in vitro* compared with bryostatin 1, and it provided the first *in vivo* validation that the bryostatin analog, was a potential therapeutic agent to treat cancer [22]. They also designed a series of synthetically-accessible bryostatin analogs (BA **2–5**), which could serve as superior drug candidates for the eradication of HIV/AIDS [23] and Chikungunya virus [24]. The designed BA retained the active groups and simplified the structure of bryostatins, which made them have more potential to be developed as new drugs.

Bioactivity & mechanism of bryostatins

The bioactivity for bryostatins can be summarized as antitumor, enhancing memory and learning, along with immune modulatory properties. Bryostatins exhibit antitumor activities including inhibition of lymphocytic leukemia P388, histiocytic lymphoma cell U937, prostate cancer cells LNcaP, melanoma B16, reticulum cell sarcoma M5076,

human leukemia HL-60, murine melanoma K1735-M2 – among others. [17,25]. The antitumor mechanisms for bryostatins are linked to the ability to selectively modulate the function of various individual PKC isozymes in cells, while the action of transient duration is the primary mechanism responsible for the unique biology of bryostatin 1. In detail, bryostatin 1 can activate the MEK/ERK path way and some transcription factors including NF-κB, AP1 and EGR1 in prostate cancer cells LNcaP at 60 min, while it fails to induce the late responses including phosphorylation of p65, translocation of cRel and RelB at 6 h and finally induces early termination of the responses in protein and RNA levels [26]. Irie *et al.* design a therapeutic lead (aplog-1) based on bryostatin 1's unique mechanism of activating PKCδ to suppress tumors [27]. Among the 24 macrocyclic lactones, bryostatin 7 shows the most potent binding affinity to PKC in biology, and it was also the first member of the bryostatins to be synthesized [28]. This suggests bryostatin 7 can be an effective surrogate for bryostatin 1 in the development of new drugs. Besides, bryostatins may also be used to treat Alzheimer's disease (AD), depression and traumatic brain injury due to their ability to enhance memory and learning. The mechanisms for bryostatin 1 to enhance memory and learning also involve regulation of PKC. In AD Phase IIa and expanded access trials, the ability of bryostatin 1 to elevate PKCε levels closely tracked cognitive benefits in the first 24 weeks, which suggests the potential to use bryostatin 1 as treatment of AD [29]. Bryostatin 1 is orally active in models of learning and memory. The active effect can be produced in less than 2 weeks while it is not seen with intraperitoneal administration [30]. This suggests the possible application of bryostatin 1 to treat AD in oral administration. Bryostatin 1 can also selectively activate PKCε to treat depressive behavior, immobility and impairment in spatial learning and memory [31] and it affects PKC-α and PKC-ε to protect the brain from severe neurological injury post-middle cerebral artery (post-MCAO) in rats [32]. Last, immune modulatory properties of bryostains make them potentially useful to treat cerebral infarction and HIV brain infection. The combination of exercise and bryostatin 1 administration can induce greater functional recovery than exercise alone in patients with cerebral infarction. This combination of therapy can increase the 5-HT immunoreactivity, affect 5-HT turnover and increase 5-HT concentrations in the perilesional area [33]. Bryostatin can reactivate latent viral infection in normal human astrocytes and human astrocytoma U-87 cells via activation of PKC-α and PKC-δ, and it strongly stimulates long terminal repeat promoter transcription by activating NF-κB [34]. This indicates that bryostatin may be a beneficial adjunct to treat HIV-1 brain infection. A Phase I clinical trial indicated bryostatin 1 is safe to cure HIV infection at a single dose administration, while it does not show any effect on PKC activity or inflammation biomarkers of $sCD14^+$ and IL-6, which may be due to low plasma concentrations [35]. The above analysis for bioactivity and mechanism of bryostatins revealed this class of macrocyclic lactones is the most active secondary metabolites from marine bryozoans, and they have the greatest potential to be developed as new drug candidates.

Sterols from marine bryozoans

Sterols are a class of important secondary metabolites in marine organisms. Most sterols of marine origin exhibit biological activities including antitumor, antibacterial and anti-inflammatory and so forth. In addition to a basic cyclopentane parallel hydrogen-phenanthrene nucleus, the structure of ocean-sterols show more novelty in their highly oxidized skeleton and changeable side chains compared with sterols from terrestrial sources. The skeletons of sterols from marine bryozoans are mainly in the cholestane and ergostane class. From the number of hydroxyl-substituents and the position of double bonds, they mainly divided into four types of 3β-hydroxy Δ^5-steroid (A2), 3β-hydroxy Δ^5–7-one-steroid (B), 3β,5α,6β-trihydroxy Δ^7-steroid (C) and 3β,7ζ-dihydroxy Δ^5-steroid (D1-D3), and other types involving 3α-hydroxy Δ^5-steroid (A1) and 3β,6β-dihydroxy Δ^4-steroid (E) (Figure 2). Although the novelty and bioactivity of most bryozoan sterols are inferior compared with macrocyclic lactones, some new active ones have gradually been discovered.

The A2 nucleus is the main type for sterols in marine bryozoans. Kerr *et al.* identified nine sterols with nucleus of A2 from *B. neritina* in California and Florida using GC–MS. The result indicates that cholesterol is the predominant sterol produced by *de novo* biosynthesis, while A2 with 28, 29 and 30 carbons are minor components produced by dietary origin. Besides, the C_{24}-alkylated sterols with A2 nucleus are produced by alkylation of dietary sterols [36]. Five new sterols (**22–26**) were isolated from bryozoan *Cryptosula pallasiana* Moll, found in Huang Island of China [37,38]. Sterol **22**, also identified from *B. neritina* [39], is characterized by a methoxyl group at C-25 and a *trans*-double bond between C-23 and C-24 in its side chain. Compounds **22** and **23** show moderate cytotoxicity against HL-60 cells with IC_{50} of 17.91 and 15.05 μg/ml, respectively. Compounds **24** and **25** were isolated for the first time from a natural source. Compound **26**, which possessed a rare 23*R* methoxyl and a double bond at C-24, showed moderate cytotoxicity against HL-60, Hep-G2 and SGC-7901 cell lines with IC_{50} from 12.34–18.37 μM.

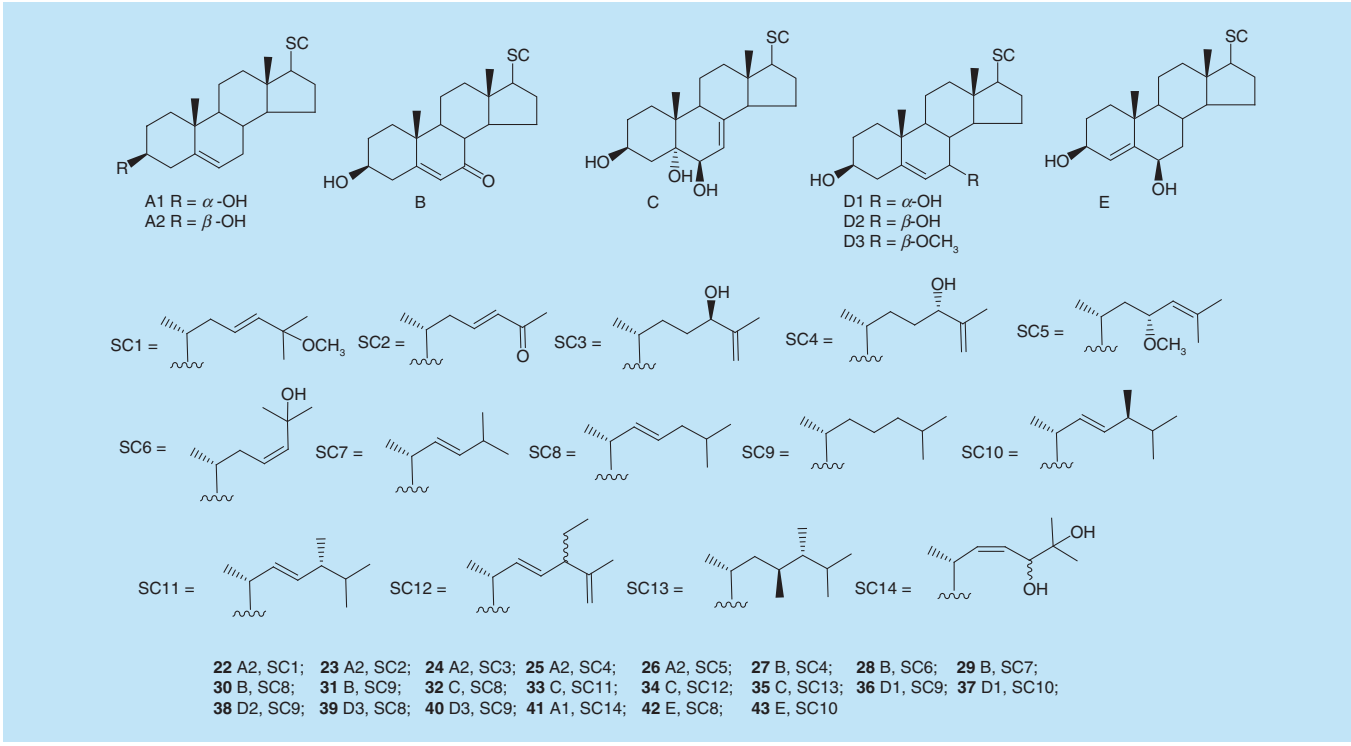

Figure 2. Sterols (22–43) from marine bryozoans (SC means side chain of sterols).

The novelty of this type of sterol is based on the branched chain at C-17, and most of them show moderate cytotoxic activity. The nucleus of B is another typical of sterols discovered from marine bryozoans. Sterols **27** and **28**, isolated from *B. neritina*, have shown cytotoxicity to HepG2, HT-29 and NCI-H460 with IC_{50} values in the range of 22.58–53.42 μg/ml [40]. Sterols (**29–31**) with B nucleus were also discovered from *C. pallasiana*. Compounds **29** and **30** show cytotoxicity against HL-60 cells with IC_{50} values of 15.12 and 14.73 μg/ml, respectively. However, sterol **31** does not show any apparent cytotoxicity, which may be connected with the loss of a *trans* double bond at C-22 in the side chain [37]. Besides, sterols **29–31** were also previously isolated from marine sponges *Cliona copiosa* and *Stelodoryx chlorophylla* [41,42]. Sterols with C nucleus are also featured in marine bryozoans. For example, three new sterols (**32–34**) with C nucleus were isolated for the first time from the marine bryozoan *Myriapora truncate* collected along the Mediterranean Coasts [43]. Several known sterols and a new one (**35**) with the same skeleton were found in the search for bioactive secondary metabolites from *B. neritina* in the South China Sea. Cytotoxicity evaluations of those sterols demonstrated that they are inactive constituents against tumor cell lines HepG2, NCI-H460 and SGC7901 [44]. SAR analysis suggested that the double bond at C-7 in the nucleus plays an important role in the lower cytotoxicity for this type of sterol [45]. The nucleus of 3β,7ζ-dihydroxy Δ^5-steroid is a precursor of B. Three sterols with D1 nucleus (**36–37**) and a D2 nucleus (**38**) were first isolated from the marine bryozoan *Biflustra grandicella* collected in Huang Island of China [46]. It was shown that 3β,7ζ-dihydroxy Δ^5-steroids were relatively common secondary metabolites from marine bryozoans since sterols **36** and **38** were subsequently obtained from *C. pallasiana*. Two new sterols (**39–40**) with D2 nucleus were also isolated from *C. pallasiana*, which displayed moderate cytotoxicity with IC_{50} values of 21.30 and 22.11 μg/ml to HL-60 cells [37]. Moreover, compound **40**, obtained by chemical synthesis, could inhibit cholesterol acyltransferase effectively [47].

In addition to the above-mentioned four main types of sterols from marine bryozoans, there are other rare types of sterols including those with nuclei of A1 and E. One new sterol (**41**), possessed A1 nucleus with a unique 22*Z*-24ζ,25-dihydroxy side chain at C-17, was identified from *B. grandicella*. The sterol with the similar side chain has only been prepared by chemical synthesis [46]. Two new sterols (**42–43**) with nucleus of E are also discovered from *B. neritina* [44]. Compounds **42** and **43** showed selective growth inhibition against the HepG2 cancer cell line with IC_{50} values of 36.5 and 52.1 μM, respectively, while they were inactive against NCI-H460 and SGC7801 cell lines. Interestingly, sterols with E nucleus from marine bryozoans are also frequently discovered from marine

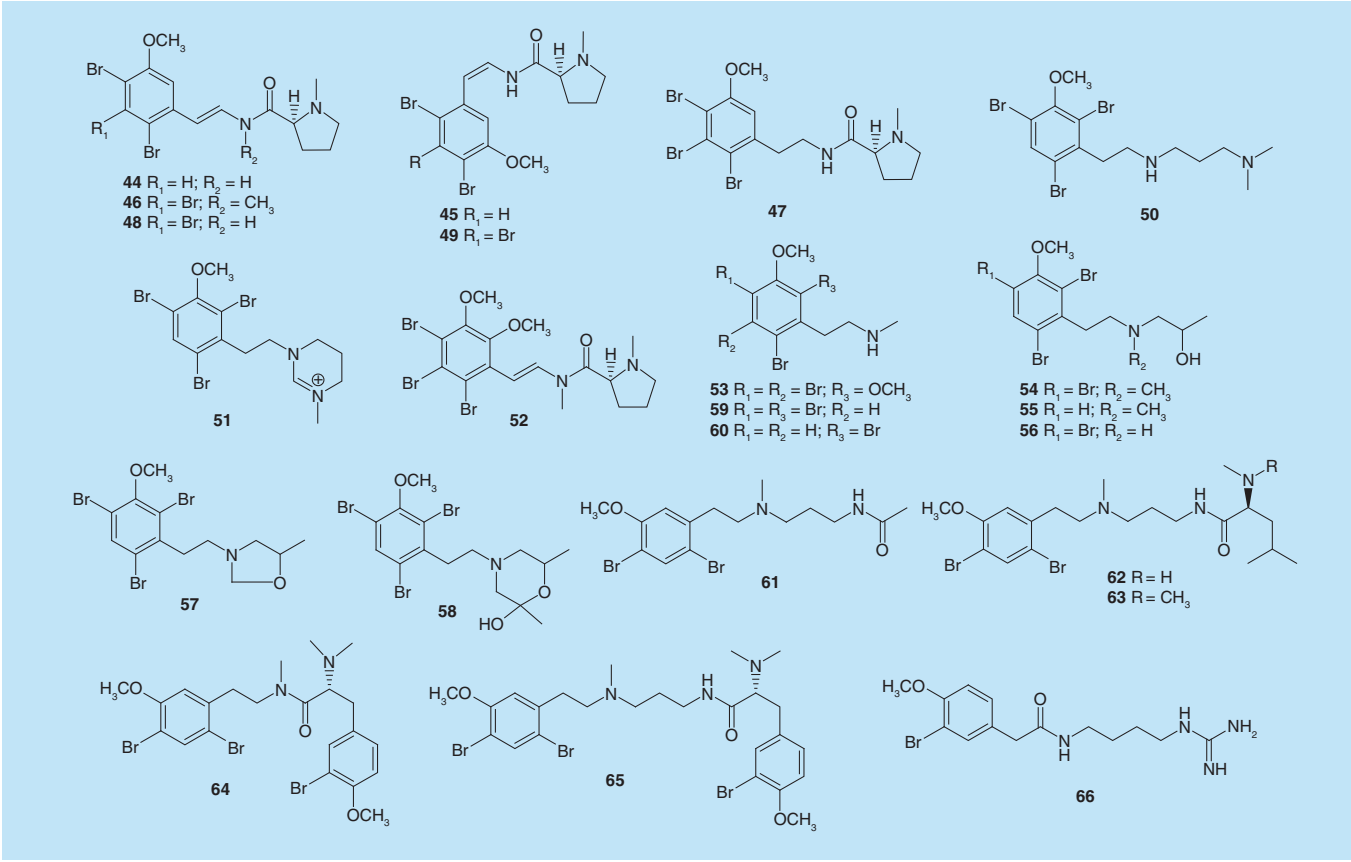

Figure 3. β-Phenylethylamine alkaloids (44–66) from marine bryozoans.

microalgae, which provided possible chemical evidence that secondary metabolites found in marine bryozoans may be produced by an epizoic source, which has important ecological implications [48]. In summary, while the structural diversity of the steroidal nucleus of compounds from marine bryozoans is less extensive compared with sterols from other marine organisms such as sponges, starfishes and coral and so on, the position and stereochemistry of hydroxyl or methoxyl groups between C-23 and C-25 (sterols **26** and **41**) in the side chain are novel and characteristic for marine bryozoans. As for bioactivities, studies just focused on the simple evaluation of cytotoxicity for the isolated sterols, a systematic evaluation of their biological properties should be undertaken to facilitate potential new drug discovery.

Alkaloids from marine bryozoans

Alkaloids are the major components of marine bryozoans. Most alkaloids in marine bryozoans are rich in halogen substitution, which is a characteristic functional group for marine natural products. Alkaloids in marine bryozoans also show unique structural diversity and bioactive diversity, which increases the possibility to develop them as pharmacological leads in the new drug discovery process. Herein, we give a systematic summary of structural diversity and bioactive diversity for alkaloids from bryozoans in recent years.

β-Phenylethylamine alkaloids

In general, marine bryozoans of the genus *Amathia* from different geographic areas contain a large number of bromine-containing alkaloids with β-phenylethylamine skeleton (Figure 3). The possible biosynthetic pathway of this type of alkaloids is derived from a processor of 2-(2,4-dibromo-5-methoxyphenyl) ethanamine, with related amino acids followed by introduction of a double bond, or methyl, methoxyl or bromine substituents [49]. Five brominated β-phenylethylamine and proline related alkaloids, amathamides A–F (**44–49**), are discovered from *A. wilsoni* in the Australian island of Tasmania [50,51]. The structures of these alkaloids differ from each other by the degree of bromination and methylation, as well as the presence or stereochemistry of a double bond. Two other

bryozoans, *A. tortusa* and *A. convoluta* in Tasmania were also abundant in this type of alkaloids. Convolutamines I (**50**) and J (**51**), two new β-phenylethylamine alkaloids, were identified in the discovery of new antitrypanosomal leads from *A. tortusa* in Tasmania. Compounds **50** and **51** were shown to be active toward the parasite *Trypanosoma brucei* with IC_{50} values of 1.1 and 13.7 μM, respectively. They also exhibit cytotoxicity against HEK293 with IC_{50} of 22.0 and 41.0 μM [52]. Interestingly, β-phenylethylamine alkaloids from the bryozoan *A. convoluta* show special structural and bioactive diversity in different geographic areas. Amathamide G (**52**) and convolutamine H (**53**), possess a fully substituted aromatic ring, and have been isolated from *A. convoluta* in Tasmania [53,54]. Compound **53** (LD_{99} = 0.2 μg/ml) is a more potent nematocide against *Haemonchus contortus* than the commercial anthelmintic, levamisole (LD_{99} = 1.6 μg/ml). In fact, convolutamines A–G (**54–60**) are typical brominated β-phenylethylamine alkaloids, which were first discovered from *A. convoluta* in Florida [55,56]. Convolutamines A (**54**), B (**55**) and D (**56**) exhibit cytotoxic activity against P388 with IC_{50} values of 10.6, 4.8 and 8.6 μg/ml, respectively. Convolutamine F (**59**) exhibits activity against KB, KB/VJ-300 and U937 cells. It also exhibits inhibitory effects for cell division of fertilized sea urchin eggs. Five novel β-phenylethylamine alkaloids, volutamides A–E (**61–65**), were identified from the temperate Atlantic bryozoan *A. convoluta*. The antifeedant bioactive results show that this type of alkaloids may serve as chemical defenses against diverse generalist predators, and against fouling by killing the larvae of competing invertebrates [57]. A new β-phenylethylamine alkaloid, securidine A (**66**), was obtained from the cold water marine bryozoan *Securiflustra securifrons* in west Spitzbergen. However, compound **66** shows no significant cytotoxic, antibacterial and antidiabetic activity. It also shows no inhibition of biofilm formation by *Staphylococcus epidermidis* at 100 μM [58].

Indole alkaloids

Many alkaloids in marine bryozoans could be classified as indole alkaloids. Marine bryozoans *Amathia alternate*, *Zoobotryon verticillatum* and *Cryptosula pallasiana* contain simple indole alkaloids. Secondary metabolites of marine bryozoans *Chartella papyracea*, *Securiflustra securifrons* and *Hincksinoflustra denticulate* provide relatively complex polycyclic indole structures. Moreover, *Flustra foliacea* is a special marine bryozoan, which contains both simple indole alkaloids and complex polycyclic indole alkaloids.

Simple indole alkaloids

Four new bromotryptamine peptides with a simple indole ring, alternatamides A–D (**67–70**) (Supplementary Figure 2), were isolated from the marine bryozoan *A. alternate,* collected along the Atlantic coast of the USA off of northern Carolina [59]. We can conclude from the structure of the four indole alkaloids that they are amides from indole ethylamine and valine, along with methylation and bromination. Compounds **67–69** show modest antibacterial activities against several Gram-positive bacteria with minimum inhibitory concentration (MIC) values ranging from 4 to 32 μg/ml, while they were not active against Gram-negative bacteria. A new brominated indole alkaloid (**71**) and two known brominated indole alkaloids (**72–73**) were identified from bryozoan *Z. verticillatum* in the southern Atlantic coast of Spain. Compounds **72** and **73** were also found in the same bryozoan collected in CA, USA [49]. In the search of antitumor secondary metabolites from marine bryozoan, three known simple indole alkaloids (**74–76**) were found from *C. pallasiana* in Huang Island of China [60].

Polycyclic indole alkaloids

The bryozoan Family Flustridae (Cheilostomata order) is the primary source of polycyclic indole alkaloids. Four of these bryozoans, *Chartella papyracea*, *Securiflustra securifrons*, *Hincksinoflustra denticulate* and *Flustra foliacea*, are the most common source for this type of alkaloids (Supplementary Figure 3). Chartellines A–C (**77–79**) and chartellamides A–B (**80–81**), discovered from *C. papyracea* in the Roscoff region of France, are the first examples of polycyclic indole alkaloids in marine bryozoans. Chartelline A (**77**) was inactive in antimicrobial assays and also inactive against leukemia cells in the NCI's test [49]. Seven halogenated indole-imidazole alkaloids with novel polycyclic skeletons, securamines A–G (**82–88**), were identified from *S. securifrons* in North Sea off the Danish west coast. Interestingly, two macrocyclic alkaloids, securine A (**89**) and securine B (**90**) can be in equilibrium with securamine A (**82**) and B (**83**), respectively, when they are dissolved in DMSO. Further studies indicate that the securines could act as precursors for chartellines as well as for securamines [61,62]. A novel pentacyclic tribromo alkaloid, hinckdentine A (**91**), was identified from *H. denticulate* in Tasmania by single crystal x-ray, and it has subsequently been fully synthesized [63].

In contrast to the above three marine bryozoans, *Flustra foliacea* is a commonly studied bryozoan due to its ease of identification and worldwide distribution. The secondary metabolites of *F. foliacea* vary from the different geographical locations of this species. Sharp *et al.* reviewed the secondary metabolites of *F. foliacea* using an ecological perspective [64]. The typical example is the discovery of flustramines with broad spectrum of antibacterial [65], nonspecific voltage-sensitive potassium channel blocking [66] and subtype-specific nicotinic acetylcholine receptor blocking activities [67]. In the terms of indol alkaloids, both simple ones and polycyclic ones can be found in this species. Recently, a new polycyclic indole alkaloid (**92**) and three new simple bromotryptamine alkaloids (**93–95**), were isolated from *F. foliacea* in the North Sea of Steingrund [66]. Compound **95** shows significant cytotoxic activity against HCT-116 with IC_{50} of 5.8 μM [68]. Eleven new polycyclic indole alkaloids, flustramine F-P (**96–106**), were discovered from *F. foliacea* in the Bay of Fundy of Canada. Flustramine F (**96**), I (**99**) and L (**102**) showed a broad spectrum of antimicrobial activities. A slow interconversion can occur from flustramine L (**102**) to H (**98**) and N (**104**) after years of storage [69].

γ-Lactam alkaloids

Except for β-phenylethylamine alkaloids from the genus *Amathia*, γ-Lactam alkaloids are also discovered commonly in this genus (Supplementary Figure 4). Convolutamides A–F (**107–112**) were identified from the Floridian bryozoan *A. convoluta*. They were characterized as an *N*-acyl-γ-lactam moiety with a dibromophenol group. Convolutamides A (**107**) and B (**108**) show cytotoxic activity against L1210 with IC_{50} values of 4.8 and 2.8 μg/ml, respectively [70]. In addition, six new γ-lactam alkaloids, amathaspiramides A–F (**113–118**) were identified from *A. wilsoni* in New Zealand. Compounds **113–115** and **117** were assessed for their bioactivities [71]. The result shows that **117** exhibits strong antiviral activity against *Polio* virus Type 1. Compounds **113** and **117** show moderate cytotoxicity against BSC-1 cells, and mild antibacterial activity against the Gram-positive bacterium *Bacillus subtilis* and the fungus *Trichophyton mentagrophytes*, while compounds **114** and **115** were inactive constituents based on these bioactivities. This indicates the pyrrole ring is important for the biological activity of this type of alkaloid. The replacement of it with a γ-lactam may reduce their bioactivities.

Pyrrole alkaloids

Pyrrole alkaloids from marine bryozoans can be classified as single pyrrole, dipyrroles and tetrapyrroles based on the number of pyrrole rings (Supplementary Figure 5). Most of the amathamides, flustramines and amathaspiramides mentioned above contain just a single pyrrole ring. Two single pyrrole alkaloids (**119–120**) were isolated from *C. pallasiana* in Huang Island of China. Compounds **119–120** were isolated from marine bryozoans for the first time, while they have been discovered frequently in marine sponges [60]. A dipyrrole, tambjamine A (**121**), previously reported from the tropical bryozoan *Sessibugula translucens*, has been found in the Antarctic bryozoan *Bugula longissima* [72]. Four new dipyrroles, tambjamines G–J (**122–125**), were identified from *Bugula dentate* in Tasmania of Australia. It has been reported that *B. dentate* can protect themselves through delivering those toxic dipyrroles [73]. An antimicrobial blue pigment (**126**) possessing the tetrapyrroles skeleton has been isolated from *B. dentate* in the Gulf of Sagami of Japan. Compound **126** is ascribed to the dark blue color for *B. dentate* [74].

Other types of alkaloids

In addition to the above-mentioned alkaloids, other types of alkaloids include purine, pyridine, indolizine, quinoline, isoquinoline, quinolinone, quinone methide, β-carboline and 2,6-naphthyridine. Blackman *et al.* reviewed the purine alkaloids from marine bryozoans before 1996, while no new ones are found later. Herein, we mainly summarize the new pyridine, indolizine, quinoline, isoquinoline, quinolinone, quinone methide, β-carboline and 2,6-naphthyridine alkaloids (Figure 4), as well as their bioactivities from marine bryozoans after 1996.

Six novel alkaloids with a pyridine ring in parallel with an indolizine skeleton, pterocellins A–F (**127–132**), were identified from *Pterocella vesiculosa* in the Hen and Chicken Islands to the north of New Zealand [75,76]. Pterocellins A (**127**) and B (**128**) show strong cytotoxicity against P388 cells with IC_{50} values of 477 and 323 ng/ml, respectively, while pterocellin D (**130**) displays modest activity with an IC_{50} value of 4773 ng/ml, and pterocellins C (**129**), E (**131**) and F (**132**) were essentially inactive with IC_{50} values greater than 6250 ng/ml. Similarly, **127** shows strong antiviral activity and cytotoxicity against BSC-1 cells, while **129**, **131** and **132** were inactive. Besides, **127** and **128** inhibit two bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, and three fungi, *Trichophyton mentagrophytesm*, *Candida albicans* and *Cladisporum resinae*, while **129–132** are inactive. The SAR of those pterocellins demonstrates that the H-8 may be crucial to the bioactivity of this type of alkaloids. Furthermore, pterocellin A exhibits strong

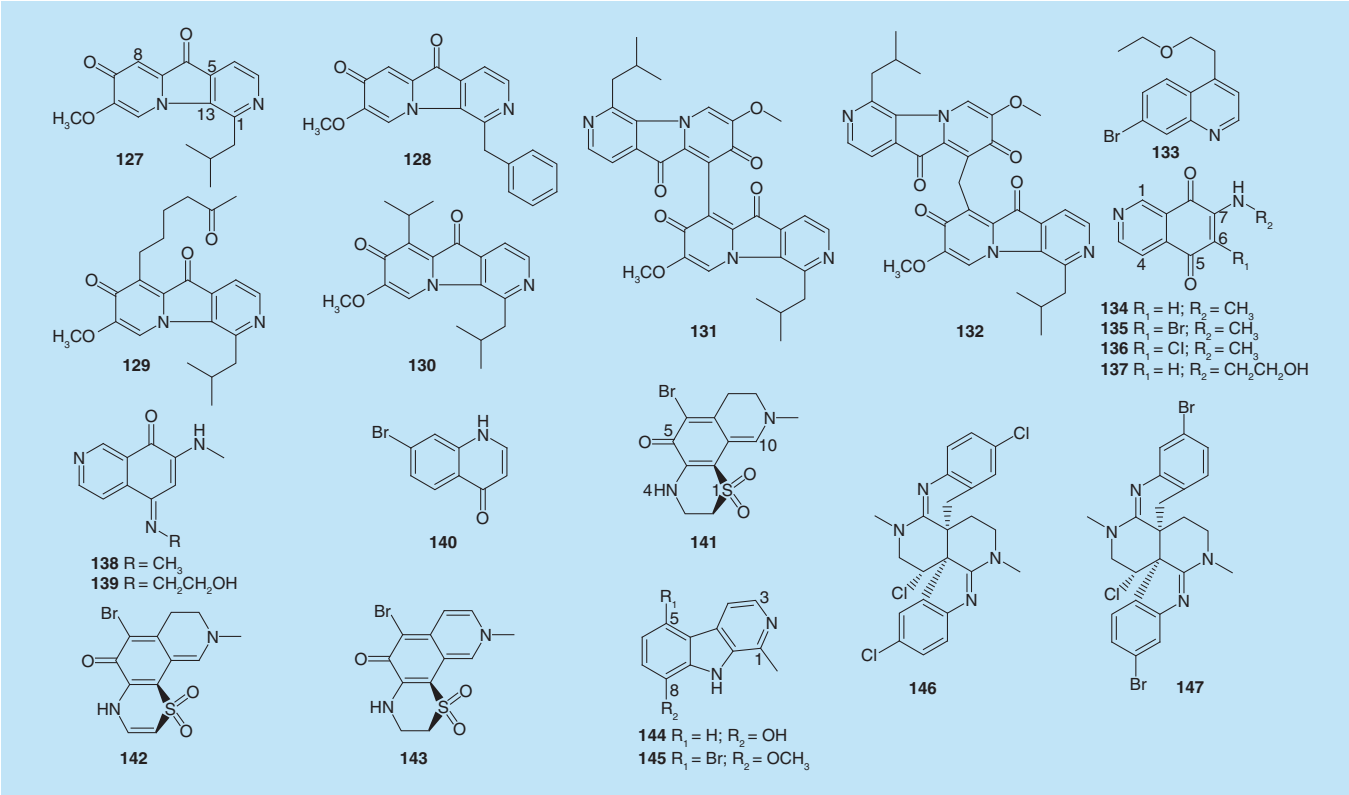

Figure 4. Indolizine, quinoline, isoquinoline, quinone methide, β-carboline and 2,6-naphthyridine alkaloids (127–147) from marine bryozoans.

cytotoxicity against Hela cells with an IC_{50} of 886 ng/ml. The possible mechanism is that pterocellin A is an inducer of apoptosis in Hela cells via mitochondria related processes [77].

Wulff *et al.* first discovered a new naturally occurring bromo-substituted quinoline alkaloid (**133**) from *F. foliacea* [78]. Subsequently, three isoquinolintriones were isolated from *Biflustra perfragilis*, which have been reviewed by Blackman *et al.* [49]. Recently, six new alkaloids with an isoquinoline 5,8-dione skeleton, calibugulones A–F (**134–139**), were identified from *Caulibugula intermis* in the South Pacific off Palau. All of the isolates displayed significant inhibition against the IC-2^{WT} murine tumor cells *in vitro* with IC_{50} from 0.03 to 1.67 μg/ml. The SAR of those caulibugulones demonstrates that the halogen substitution at C-6 is not an important determinant of cytotoxicity, while substitution of an ethyl alcohol at either the C-7 or C-5 nitrogen resulted in a five- to tenfold reduction in cytotoxicity [79]. Besides, a new natural product, 7-bromoquinolin-4(1H)-one (**140**), was isolated from bryozoan *C. pallasiana*, and it was inactive to HL-60 cells [80]. Furthermore, euthyroideones A–C (**141–143**), which possess a unique heterocyclic ring system that contains brominated quinone methide, sulfone and amine groups, were discovered from the bryozoan *Euthyroides episcopalis* in Fiordland of New Zealand. Euthyroideone B (**142**) shows modest cytotoxicity against BSC-1 cells, while **141** and **143** were inactive [81].

β-Carboline alkaloids have been reported in the marine bryozoans *Costaticella hastata*, *Orthoscuticella ventricosa* and *Cribricellina cribraria* [49]. Recently, two new β-carboline alkaloids (**144–145**) were identified from bryozoans *C. cribraria* and *Pterocella vesiculosa*, respectively [82,83]. Compound **144**, named 8-hydroxyharman, shows relatively weak cytotoxicity against P388 cells with an IC_{50} more than 12,500 ng/ml. Compound **145** is a 5-bromo-8-methoxy-1-methyl-β-carboline, which shows relatively moderate cytotoxicity against P388 cells with an IC_{50} of 5089 ng/ml. It also shows inhibition against the Gram-positive bacteria *C. albicans*, *B. subtilis* and *T. mentagrophytes* with minimum inhibitory doses of 2–5 μg/ml. The SAR indicates that the substituent of a vinyl group at C-1 or bromine at C-5 plays an important role for the cytotoxicity against P388. Furthermore, the antimicrobial activity can be increased by a bromine substituent at C-5, but decreased by an additional 8-methoxy substituent. Interestingly, two novel heterocyclic alkaloids, caulamidine A (**146**) and B (**147**), possessed the 2,6-naphthyridine core and fused by a dihydroindole-derived and tetrahydroquinoline-derived systems, were isolated from bryozoan

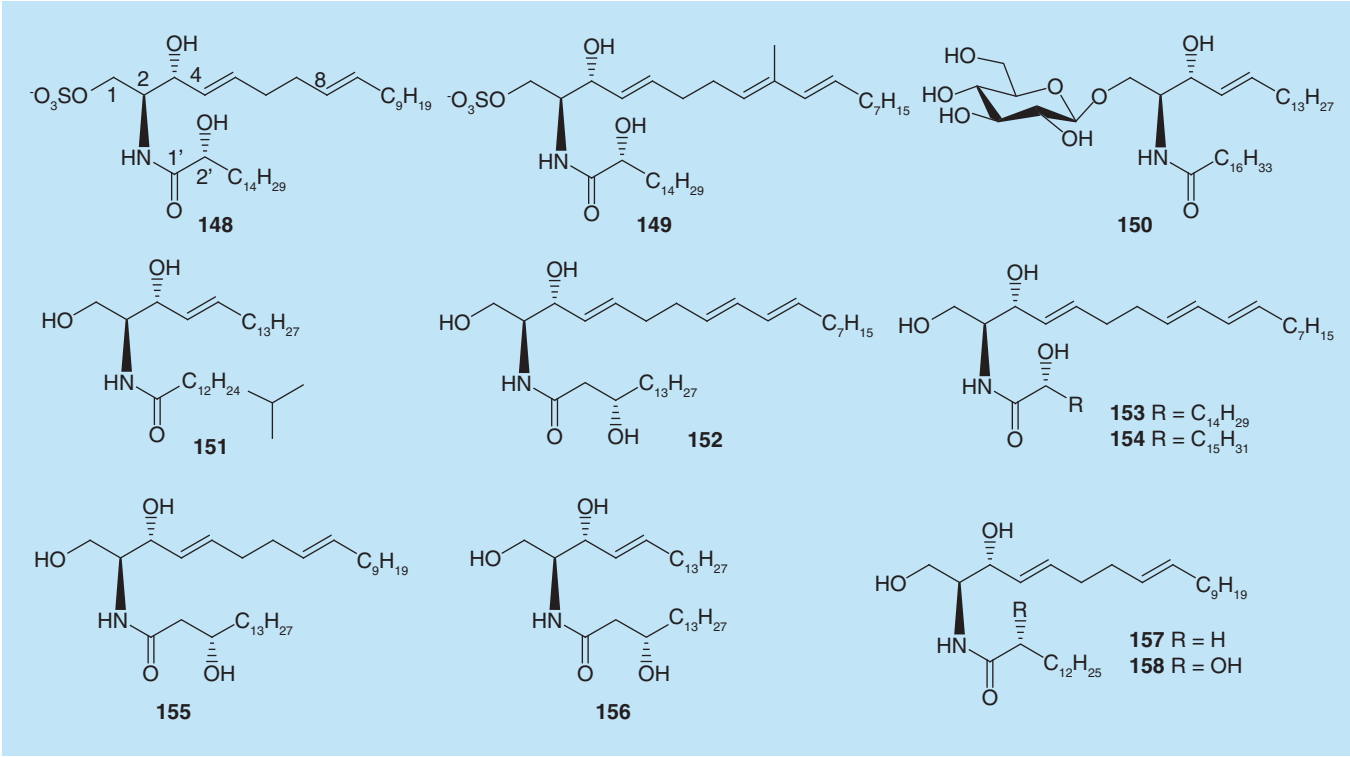

Figure 5. Sphingolipids (148–158) from marine bryozoans.

Caulibugula intermis and elucidated by the new computer-assisted structural tools. Compounds **146** and **147** appear antimalarial activity against *Plasmodium falciparum* with IC_{50} values from 8.3–12.9 μM. Besides, they also show modest cytotoxicity in NCI-60 cell screen with a single dose of 40 μM [84].

In summary, the features of affluent alkaloids types, polythalogen substitutions, unique heterocyclic ring systems, along with accidental sulfone substitutions outline the chemiodiversity for alkaloids from marine bryozoans. The frequent substitutions of one or more bromines in alkaloids increase the possibility to discover novel compounds from marine bryozoans. Especially, the unique heterocyclic ring systems in polycyclic indole alkaloids, such as hinckdentine A (**91**), attract chemists to synthesize it and its derivatives to find pharmacological leads. As for their bioactivities, β-phenylethylamine alkaloids **50** and **53** show significant insecticidal activity against *T. brucei* (LC_{50} = 1.1 μM) and *H. contortus* (LD_{99} = 0.2 μg/ml), respectively. Pyridine paralleled indolizine alkaloids of pterocellins A (**127**) and B (**128**) show significant cytotoxicity against P388 cells (IC_{50} is 477 and 323 ng/ml respectively). Isoquinoline 5,8-dione alkaloids of calibugulones A-F (**134–139**) display significant inhibition (IC_{50} = 0.03–1.67 μg/ml) against IC-2^{WT} murine tumor cells. These indicate the potential to develop those alkaloids as pharmacological leads in the discovery of new drugs.

Sphingolipids from marine bryozoans

Sphingolipids, comprised by a sphingoid base (LCB) with a long fatty acid base (FAB) through an amide bond, are divided into two groups of ceramides and cerebrosides based on whether glycosylated. Sphingolipids are common secondary metabolites for terrestrial plants and marine invertebrates. The bioactivities of sphingolipids include cytotoxicity, antibacteria, immune modulation – among others. Similar to moderate cytotoxic sterols form bryozoans, researches on sphingolipids from marine bryozoans are also limited in discovery of new compounds and evaluation of their cytotoxicity (Figure 5). However, two sulfates of ceramides (**148–149**), identified from bryozoan *Watersipora cucullata*, show significant inhibition against human topoisomerase I with IC_{50} values of 0.4 and 0.2 μM, respectively. This indicates those ceramide sulfates can be developed as inhibitors for topoisomerase I to cure antitumor [85]. Marine bryozoan *B. neritina* is affluent in sphingolipids [86]. Seven new sphingolipids, compounds (**150** and **151**) and neritinaceramides A–E (**152–156**), were isolated from this species in the South China Sea [87,88]. Compound **151** possessed a rare branched methyl fragment of [-$CH(CH_3)_2$] in its FAB.

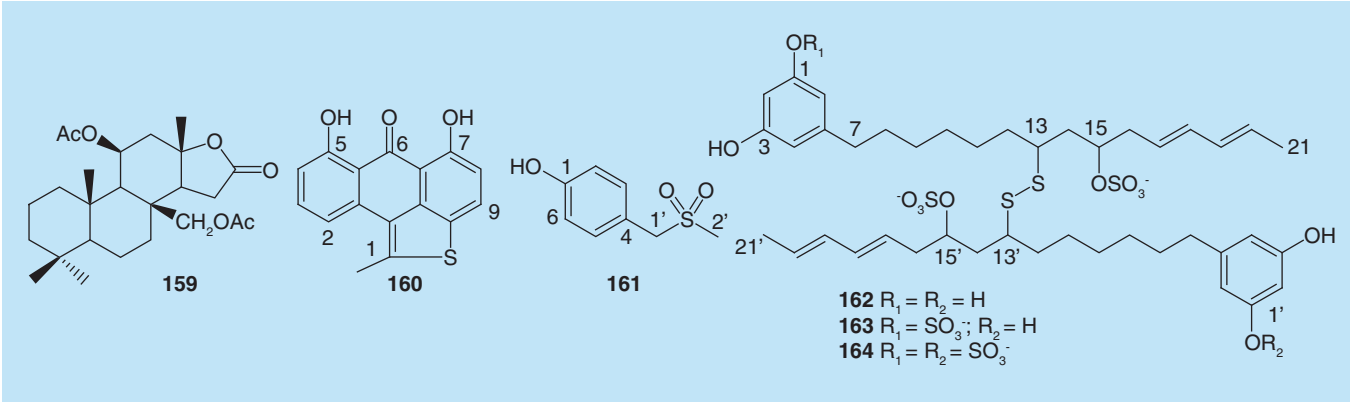

Figure 6. Triterpenoid (159) and sulfur-containing aromatic compounds (160–164) from marine bryozoans.

Compounds **152**, **155** and **156**, characterized at a C-3′*S* hydroxyl group in their FAB fragment, were novel structural feature in sphingolipids. Besides, the rare structural feature of 4*E*,8*E*,10*E*-triene skeleton in the LCB for compounds **152–154**, was first discovered from marine bryozoans. Neritinaceramides A-E (**152–156**) exhibit moderate cytotoxicity against HepG2 and SGC7901 cells with a range of IC_{50} values from 47.3 to 58.1 μM, while they show inactive to NCI-H460 cells. Besides, two new ceramides (**157–158**), possessing 14 carbons in the FAB, were identified from *C. pallasiana* [38]. They are also displayed moderate cytotoxicity against HL-60, Hep-G2 and SGC-7901 with the IC_{50} values from 21.13 to 32.36 μM. The SAR analysis demonstrates that the presence of the *trans* double bond between C-4 and C-5 in the vicinity of their polar head, the category of the sugar moieties at C-1 in the LCB and the additional hydroxyl group at position C-2′ or C-4, are important for their cytotoxicity.

Other secondary metabolites from marine bryozoans

The changeable marine climate and the different geographical location of bryozoans provide the possible complexity and novelty of secondary metabolites for marine bryozoans. Although the novel structures found from bryozoans are relatively little compared with other marine organisms, the strong antitumor activity of bryostatins and the novelty of alkaloids from bryozoans still attract researchers' attention. In addition to the above introduced structural types, the secondary metabolites from marine bryozoans are also including triterpenoids and sulfur-containing aromatic compounds, and so forth (Figure 6). Some of them show drug development value due to their significant bioactivities.

A novel tetracyclic terpenoid lactone, murrayanolide **(159)**, possessing an unusual C_{21} skeleton was identified from bryozoan *Dendrobeania murrayana* in the east coast of Canada. Compound **159**, the first C_{21} tetracyclic terpenoid lactone from bryozoans, exhibits 54% inhibition against metalloprotease collagenase IV at a dose of 25 μg/ml [89]. A sulfur-containing aromatic compound, bryoabthrathiophene (**160**) was isolated from bryozoan *Wateripora subtorquata* in Tsutsumi Island of Japan. Compound **160** exhibits significant antiangiogenic activity with IC_{50} of 0.005 μM on bFGF-induced proliferation of BAEC (Bovine aorta endothelial cell), indicating it can be useful for the development of novel antiangiogenic agent [90]. Besides, a new natural compound, *p*-methylsulfonylmethyl-phenol (**161**), was found from bryozoan *C. pallasiana*, while it was inactive to HL-60 cells [80]. Furthermore, three new disulfides, Pentaporins A–C (**162–164**), were discovered from Mediterranean bryozoan *Pentapora fascialis*. Compounds **162–164** show anthelmintic activity against *Trichinella spiralis*. The sulfate ester groups are responsible for the anthelmintic activity [91].

Strategies to solve source & resupply for bioactive bryozoan metabolites

Based on the above analysis, the structural diversity and bioactive diversity of secondary metabolites from marine bryozoans pour the desires to discover pharmacological leads from this marine organism. Its needed to mention that bryostatin 1 (**1**) and bryostatin 4 (**4**) have entered Phase II clinical trials in the US for the treatment of cancer, AD, effects of stroke and HIV [25,29,35]. However, the yield of this type of macrocyclic lactones from bryozoans is low. It depends on the geographical site, time of year and depths of the collection. For example, 1000 kg of damp *B. neritina* collected from gulf in Mexico gave 306 mg of bryostatin 4, while an approximately equal amount

Table 1. Summary of secondary metabolites from marine bryozoans.

Bryozoans	Order	Structures	Compound type	Ref.
Amathia wilsoni	Ctenostomata	**44–49, 113–118**	Alkaloids	[50,51,71]
Amathia tortusa†	Ctenostomata	**50, 51**	Alkaloids	[52]
Amathia convoluta	Ctenostomata	**52–65, 107–112**	Alkaloids	[53–57,70]
Amathia alternate	Ctenostomata	**67–70**	Alkaloids	[59]
Biflustra grandicella†	Cheilostomata	**36–38, 41**	Sterols	[46]
Bugula dentate	Cheilostomata	**122–126**	Alkaloids	[73,74]
Bugula longissima†	Cheilostomata	**121**	Alkaloids	[72]
Bugula neritina	Cheilostomata	**1–22, 27, 28, 35, 42, 43, 150–156**	Macrocyclic lactones, sterols, sphingolipids	[12–17,36,39,40,44,45,48,87,88]
Caulibugula intermis†	Cheilostomata	**134–139, 146,147**	Alkaloids	[79]
Chartella papyracea	Cheilostomata	**77–81**	Alkaloids	[49]
Cribricellina cribraria	Cheilostomata	**144**	Alkaloids	[82]
Cryptosula pallasiana†	Cheilostomata	**22–26, 29–31, 39, 40, 74–76, 119,120, 140, 157,158, 161**	Sterols, alkaloids, sphingolipids	[37,38,60,80]
Dendrobeania murrayana	Cheilostomata	**159**	Terpenoids	[89]
Euthyroides episcopalis†	Cheilostomata	**141–143**	Alkaloids	[81]
Flustra foliacea	Cheilostomata	**92–104, 133**	Alkaloids	[66–69,78]
Hincksinoflustra denticulate	Cheilostomata	**91**	Alkaloids	[63]
Myriapora truncate	Cheilostomata	**32–34**	Sterols	[43]
Pentapora fascialis†	Cheilostomata	**160–162**	Aromatic compounds	[91]
Pterocella vesiculosa†	Cheilostomata	**127–132, 145**	Alkaloids	[75–77,83]
Securiflustra securifrons†	Cheilostomata	**66, 82–90**	Alkaloids	[58,61,62]
Sessibugula translucens	Cheilostomata	**121**	Alkaloids	[72]
Watersipora cucullata†	Cheilostomata	**148–149**	Alkaloids	[85]
Wateripora subtorquata†	Cheilostomata	**160**	Aromatic compounds	[90]
Zoobotryon verticillatum	Ctenostomata	**71–73**	Alkaloids	[49]

†Secondary metabolites for marine bryozoans studied for the first time since 1996.

collection from the South China Sea gave 970 mg of bryostatin 4. The low and uncertain yield of bryostatins made them hard to be used in clinic. Fortunately, cultivation of bryozoans is an effective way to solve the source and resupply for bioactive bryozoan metabolites. Compared with collection bryozoans from nature, cultivation of bryozoans can provide a controlled environment to avoid regional difference about the yield of bryostatins. *Bugula neritina* can commonly be cultured in the laboratory since its abundance and the larvae ease to be collected and induced to adhere to a surface [92]. The company of CalBioMarine Technologies from California has grown *B. neritina* into the sea after their initial colonization by the larvae on plastic plates. The way to cultivate bryozoans solved the problem of the changeable marine climate, while it not solves the fact that the low yield of bryostatins. In fact, bryostatins were first isolated from *B. neritina*, and later found in marine bryozoan *Amathia convolute* and other marine organisms *Lissodendoryx isodictyalis* and *Aplidium californium* [13]. Recent studies have proved that bryostatins were actually produced by an uncultured symbiotic bacterium *Endobugula sertula* from *B. neritina*. Thus, heterologous expression of the putative bryostatin polyketide synthase gene cluster from *E. sertula*, with the help of cultured *B. neritina* in laboratory, provided new eyesight to produce the bioactive bryozoan metabolites in large enough quantities for further development of them into pharmaceutical [93].

Conclusion

In this review, the bioactivities of 164 compounds isolated from 24 marine bryozoans including two orders of Cheilostomata and Ctenostomata are discussed. The bioactive secondary metabolites of 11 bryozoan species are studied for the first time since 1996 (Table 1). Bryozoan *B. neritina* is still a focus to produce new bioactive compounds, followed by *A. wilsoni*, *A. convolute*, *C. pallasiana*, *F. foliacea*, *P. vesiculosa* and *S. securifron*. Alkaloids including β-phenylethylamine, indole, γ-lactam, pyrrole and other types of pyridine, indolizine, quinoline, isoquinoline, quinolinone, quinone methide, β-carboline and 2,6-naphthyridine, make up the major types of

Table 2. Bioactivities of the typical compounds from marine bryozoans.

Compound types	Typical compounds	Biological activities	Ref.
Macrocyclic lactones	**1–18**	Antitumor activity Enhancing memory and learning Immune modulation	[12,13,17,25–35]
	20	Antipredator defense	[15]
	19, 21	Antitumor activity	[14,16]
Sterols	**22, 23, 26–30, 39, 40, 42, 43**	Antitumor activity	[37,38,40,44]
	40	Inhibitory effects for cholesterol acyltransferase	[47]
Alkaloids	**50, 51**	Antitrypanosomal activity	[52]
	50, 51, 54–56, 59, 95, 107,108, 113, 117, 127,128, 130, 134–139, 142, 144–147	Antitumor activity	[52,55,56,68,70,71,75–77,79,81–84]
	53	Nematicidal activity	[54]
	59	Inhibitory effects for cell division	[55,56]
	61–65	Antifeedant activity	[57]
	67–69, 96, 99, 102, 113, 117, 126–128, 145	Antibacterial activity	[59,69,71,74–76,82,83]
	117, 127	Antiviral activity	[71,75,76]
	122	Antipredator defense	[73]
	146,147	Antimalarial activity	[84]
Sphingolipids	**148,149**	Inhibitory effects for human DNA topoisomerase I	[86]
	152–158	Antitumor activity	[38]
Tetracyclic terpenoid lactone	**159**	Inhibitory effects for metalloprotease collagenase IV	[89]
Sulfur-containing aromatic compounds	**160**	Antiangiogenic activity	[90]
	162–164	Anthelmintic activity	[91]

chemical structures for bryozoans. The rests are the sterols, sphingolipids, macrocyclic lactones, triterpenoids and sulfur-containing aromatic compounds – among others. Antitumor activity is the most important bioactivity for these discovered secondary metabolites from bryozoans (Table 2). Macrocyclic lactones and some types of alkaloids appear excellent antitumor activity, while most sterols and sphingolipids exhibit moderate cytotoxicity. Interestingly, alkaloids show variety of biological activities including antitrypanosomal, antitumor, nematicidal, antifeedant, antibacterial, antiviral, antipredator and antimalarial activities, as well as the inhibitory effects for cell division. Besides, the inhibitory effect for human DNA topoisomerase I of sphingolipids, inhibition for metalloprotease of tetracyclic terpenoid lactone, along with the antiangiogenic and anthelmintic activities of sulfur-containing aromatic compounds, demonstrate the diversity of bioactivities for secondary metabolites of marine bryozoans. Finally, we use the LC_{50}, IC_{50} or LD_{99} value near to 1 or below 1 μg/ml (μM) for a certain biological target samples or cells as the evaluation standard, to summarize the potential pharmacologic leads or drug candidates for the 164 secondary metabolites from marine bryozoans. The results show macrocyclic lactones, such as bryostatin 1 (**1**) and bryostatin analog (**BA1**), can be developed as antitumor, enhancing memory and learning, and immune modulating related agents. β-Phenylethylamine alkaloids (**50** and **53**) can be developed as insecticidal agents. Pyridine paralleled indolizine alkaloids of pterocellins A (**127**) and B (**128**), isoquinoline 5,8-dione alkaloids of calibugulones A–F (**134–139**) and sphingolipids sulfates (**148** and **149**) can be developed as antitumor agents. Sulfur-containing aromatic compound, bryoabthrathiophene (**160**), can be developed as antiangiogenic agent. Although various pharmacological leads have been discovered from marine bryozoans, no one has been successfully approved as new drugs. The most promising bryostatin 1 is still hard to progress to Phase III clinical trials as antitumor agent. Except the drawbacks of low productivity for bryostatin 1, its toxic side effects of myalgia, fatigue, local phlebitis, thrombocytopenia, nausea and vomiting during clinical studies need to pay attention [25,94]. Compared with successful development of bretuximab vedotin as antibody–drug conjugate in its safe treatment of Hodgkin lymphoma [95,96], the strategies to selective deliver cancer drugs to tumor cells, while avoid to influence the nontarget cells can be considered to reduce the toxic side effects in the development of these pharmacological leads.

Future perspective

Summarily, in the last two decades, although relatively little research has been undertaken into the secondary metabolites of bryozoans comparing with other marine invertebrates, bryozoans have proven to be an excellent source for novel and bioactive compounds. However, the low productivity with complicated structural feature for bryozoans' metabolites is a bottleneck to discover pharmacologic leads from marine bryozoans. Fortunately, there are evidences to verify that some of bioactive secondary metabolites are actually produced by co-epiphyte microorganisms of marine bryozoans. It provides possibility to engineering produce bioactive secondary metabolites for further systematic evaluation of their bioactivities. In our view, like the success to develop bryostatin 1 as new drug candidate, more and more novel lead compounds can be discovered from marine bryozoans and their co-epiphyte microorganisms in the progress of new drug discovery. Furthermore, the success of antibody–drug conjugate in reducing drug's toxic side-effects provides potential for the development of these lead compounds as new drugs.

Executive summary

Background

- Natural products serve as an inspiration to discovery of new drug candidates. Marine bryozoans are well known producers of bioactive secondary metabolites and important marine drug sources.

Macrocyclic lactones from marine bryozoans

- The structure features, bioactivities, structure–activity relationship (SAR) and mechanisms are summarized for 21 bryostatins (**1–21**) and three derivatives from marine bryozoans.
- The 26-membered macrolactone ring, hydroxyl at C-3 with (*R*)-stereochemistry, free hydroxyl at C-26 and the structure in the C7-C9 region of the A-ring are critical to bryostatins for their potent inhibition of tumor cells.
- The significant bioactivity including antitumor, enhancing memory and learning, and immune modulatory properties for bryostatins inspire researcher focused on total synthesis and synthesis bryostatin analogs for new drug candidates.

Sterols from marine bryozoans

- Sterols with moderate cytotoxicity from bryozoans can be divided into four types of 3β-hydroxy Δ^5-steroid, 3β-hydroxy Δ^5–7-one-steroid, 3β,5α,6β-trihydroxy Δ^7-steroid and 3β,7ζ-dihydroxy Δ^5-steroid, and other types involving 3α-hydroxy Δ^5-steroid and 3β,6β-dihydroxy Δ^4-steroid.
- The SAR for sterols indicates the double bond at C-7 and missing *trans* double bond at C-22 can decrease their cytotoxicity.

Alkaloids from marine bryozoans

- Alkaloids are the major components for bryozoans. They can be divided into four main types of β-phenylethylamine alkaloids, indole alkaloids, γ-lactam alkaloids and pyrrole alkaloids. Besides, other types of alkaloids including pyridine, indolizine, quinoline, isoquinoline, quinolinone, quinone methide, β-carboline and 2,6-naphthyridine are also summarized.
- Alkaloids from marine bryozoans show variety of biological activities including antitrypanosomal, antitumor, nematicidal, antifeedant, antibacterial, antiviral, antipredator and antimalarial activities, as well as inhibitory effects for cell division.
- Alkaloids (**50** and **53**) can be developed as insecticidal agents. Alkaloids (**127**, **128** and **134–139**) have potential to be developed as antitumor agents.

Sphingolipids from marine bryozoans

- Sphingolipids from bryozoans with moderate cytotoxicity are discussed. Sphingolipids sulfates (**148** and **149**) can be developed as antitumor agents.
- The SAR analysis demonstrates the *trans* double bond between C-4 and C-5, the category of the sugar moieties at C-1 and the hydroxyl at C-2′ or C-4, are important for their cytotoxicity.

Other secondary metabolites from marine bryozoans

- Inhibitory effects for metalloprotease of tetracyclic terpenoid lactone, as well as the antiangiogenic and anthelmintic activities of sulfur-containing aromatic compounds are reviewed from bryozoans. Bryoabthrathiophene (**160**) can be developed as antiangiogenic agent.

Strategies to solve the source & resupply for bioactive bryozoan metabolites

- Cultivation of bryozoans is an effective way to solve the source and resupply for bioactive bryozoan metabolites.

Supplementary data

FS: See online at: https://www.future-science.com/doi/10.4155/fmc-2018-0012

Author contributions

As first author and corresponding author, XR Tian organized and wrote the whole manuscript. Co-first author, HF Tang gave the idea, financial support and careful revision of the manuscript. XL Tian, JJ Hu and LL Huang searched the related references, drew the related figures and tables and gave suggestions to improve the manuscript. KR Gustafson edited and corrected the final version of the manuscript.

Financial and competing interests disclosure

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