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Review

The understudied phylum Bryozoa as a promising source of anticancer drugs

Blanca Figuerola [1,*], Conxita Avila [2]

[1] Smithsonian Tropical Research Institute, Box 0843-03092, Balboa, Republic of Panama; bfiguerola@gmail.com

[2] Department of Evolutionary Biology, Ecology, and Environmental Sciences, and Biodiversity Research Institute (IrBIO), Faculty of Biology, University of Barcelona, Av. Diagonal 643, 08028 Barcelona, Catalonia; conxita.avila@ub.edu

* Correspondence: bfiguerola@gmail.com

Abstract: Recent advances in sampling and novel techniques in drug synthesis and isolation have promoted the discovery of anticancer agents from marine organisms to combat this major threat to public health worldwide. Bryozoans, filter-feeding, aquatic invertebrates often characterized by a calcified skeleton, are an excellent source of pharmacologically interesting compounds including well-known chemical classes such as alkaloids and polyketides. This review covers the literature for secondary metabolites isolated from marine cheilostome and ctenostome bryozoans that have shown potential as cancer drugs. Moreover, we highlight examples such as bryostatins, the most known class of marine-derived compounds from this animal phylum, which is advancing through anticancer clinical trials due to their low toxicity and antineoplastic activity. The bryozoan antitumour compounds discovered until now show a wide range of chemical diversity and biological activities. Therefore, more research focusing on the isolation of secondary metabolites with potential anticancer properties from bryozoans and other overlooked taxa covering wider geographic areas is needed for an efficient bioprospecting of natural products.

Keywords: antitumour compounds; MNPs; bioactivity; cytotoxicity; marine invertebrates

1. Background

Most bioactive secondary metabolites have been isolated from species inhabiting terrestrial environments, although oceans cover >70% of the Earth's surface and marine natural products (MNPs) generally show higher incidence of significant cytotoxic activity [1,2]. It is well known that most sessile marine invertebrates produce active natural products for a variety of ecological roles such as defence against predators, parasites and infections and/or competition, being thus aquatic environments important potential sources of compounds [3]. A large number of these chemicals have pharmacological activity for their interaction with receptors and enzymes, thus continuously gaining interest in the biomedical field [4]. In particular, there is an increasing demand on the development of new anticancer drugs as cancer is one of the deadliest diseases worldwide.

In the last decades, advances in SCUBA diving, deep-sea sample collection and novel techniques in drug synthesis and aquaculture have promoted the discovery of an important number of compounds derived from marine organisms with potential anticancer properties [5,6]. One good example that the new technologies provide unprecedent access to a previously untapped source of chemical diversity is the recent isolation of a variety of compounds of deep sea taxa, showing cytotoxic properties toward a range of human cancer cell lines, even though most marine compounds have still been isolated from shallow fauna until now [7]. Eight anti-cancer drugs have already been approved for human-use although only a small proportion (one of 5,000-10,000) of the new synthetic molecules becomes a commercial drug due to their toxicity [8]. For example, Eribulin is an analogue of the marine natural product halichondrin-B, which induces apoptosis of cancer cells isolated from

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the *Halichondria* genus Fleming, 1828 of sponges and it is used for the treatment of liposarcoma and breast cancer [8]. Ziconotide is a toxin derived from the mollusc *Conus magus* Linnaeus, 1758 that acts as a painkiller by blocking calcium channels in pain-transmitting nerve cells [8]. Brentuximab ventodin, isolated from the mollusc *Dolabella auricularia* (Lightfoot, 1786), is an antibody-drug conjugate used to treat Hodgkin lymphoma and systemic ALCL [8]. Cytarabine is used to treat acute myeloid leukemia, acute lymphocytic leukemia, chronic myelogenous leukemia, and non-Hodgkin's lymphoma isolated from the sponge *Tectitethya crypta* (de Laubenfels, 1949) [8]. Trabectidin is a drug isolated from the tunicate *Ecteinascidia turbinata* Herdman, 1880 used for the treatment of advanced soft tissue sarcoma [8]. The vast majority of studies on assessment of anticancer properties of marine-invertebrate derived compounds have focused on different groups such as sponges and corals [9,10]. In contrast, few MNPs, and in particular anticancer drugs, have been isolated from bryozoans despite many of them have been shown to be bioactive and/or to have unique chemical structures [11,12].

Bryozoa (sea mats, moss animals, or lace corals), a phylum of aquatic, filter-feeding invertebrates, are abundant, speciose, ubiquitous and important members of many benthic communities from the intertidal to the deep sea in a variety of marine habitats [13]. Over 6000 extant species are known with new taxa being continuously described, particularly in regions previously inaccessible (e.g. deep sea and Antarctica) [14–18]. Species are almost exclusively colonial and their colonies are generally sessile, developing a broad spectrum of forms (ranging from encrusting sheets to erect branching chains), which provide habitats for a wide range of small invertebrates and microorganisms [13]. The individual functional units (modules) of colonies are called zooids. This phylum is traditionally organized into three classes: Phylactolaemata (freshwater), Gymnolaemata (mostly marine), and Stenolaemata (marine). The Gymnolaemata contains two orders: Cheilostomatida and Ctenostomatida. Recent molecular sequence data has shown Phylactolaemata is the sister group to Gymnolaemata + Stenolaemata (e.g. [19]). The Classes Gymnolaemata and Stenolaemata comprise bryozoans with a calcified skeleton, except for ctenostomes.

Bryozoans are excellent sources of pharmacologically interesting substances including alkaloids and polyketides with diverse biological activities (e.g. antimicrobial and antipredation [12,20]). Regarding unexplored regions, our recent studies on chemo-ecological interactions of a range of bryozoan species from different Antarctic locations have reported a variety of ecological roles of their lipophilic and hydrophilic extracts. These activities include defensive strategies against microorganisms [21,22] and against abundant and ubiquitous sympatric predators [22–24], as well as cytotoxicity against a common sea urchin [25,26], reducing its reproductive success. Therefore, more effort is required to isolate and characterize the secondary metabolites involved in these chemical interactions for their potential in pharmacological applications. This phylum has received little attention until now, with most studied species possessing erect, foliose and large colonies. Some of the reasons for these scarce studies may include the usually insufficient biomass of bryozoan samples to allow the isolation of secondary metabolites, related to the fact that many species are heavily calcified, and also the technical difficulties for collecting the specimens due to their often encrusting growth and difficult taxonomy (e.g. lack of taxonomic expertise and laborious and time consuming identification under the microscope) [27]. Therefore, more efforts should be devoted to study encrusting species, which regularly have to compete for available surfaces, and thus could be expected to be a rich source of natural products [12].

The origin of the bioactive compounds in marine invertebrates is mostly unknown, although it has often been demonstrated to be originated either from *de novo* biosynthesis, from the diet, or from symbiotic microorganisms [28]. In bryozoans, the origin of bryostatins has been traced to bacterial symbiont *Endobugula sertula* [29,30], but it is still unknown for the rest of compounds.

The purpose of this review is to showcase the secondary metabolites with potential antitumoural properties isolated from 16 marine cheilostome and 2 ctenostome bryozoans (Table 1). In particular, we describe the antitumour activity of different class of compounds including alkaloids, sterols, ceramides and polyketides, namely the bryostatins, the most well-known and promising secondary metabolites in cancer chemotherapy produced by marine organisms.

Table 1. Bryozoan species with anticancer activity by group.

O. Cheilostomatida	**Geographical area**
Fam. Aspidostomatidae	
Aspidostoma giganteum (Busk, 1854)	Patagonia
Fam. Bugulidae	
Bugula neritina (Linnaeus, 1758)	California, China, Gulf of Mexico
Caulibugula intermis Harmer, 1926	Palau
Virididentula (*Bugula*) *dentata* (Lamouroux, 1816)	North Atlantic
Fam. Calloporidae	
Tegella cf. spitzbergensis (Bidenkap, 1897)	North Atlantic
Fam. Catenicellidae	
Paracribricellina (*Cribricellina*) *cribraria* (Busk, 1852)	Australia, New Zealand
Pterocella vesiculosa (Lamarck, 1816)	New Zealand
Fam. Cryptosulidae	
Cryptosula pallasiana (Moll, 1803)	China
Fam. Flustridae	
Chartella papyracea (Ellis & Solander, 1786)	North Atlantic
Flustra foliacea (Linnaeus, 1758)	North Sea
Terminoflustra (Chartella) membranaceotruncata (Smitt, 1868)	White Sea
Fam. Membraniporidae	
Biflustra perfragilis MacGillivray, 1881	South Australia
Fam. Myriaporidae	
Myriapora truncata (Pallas, 1766)	Mediterranean
Fam. *Schizoporellidae*	
Schizoporella unicornis (Johnston in Wood, 1844)	Arabian Gulf and the Gulf of Oman
Fam. Watersiporidae	
Watersipora cucullata (Busk, 1854)	Japan
Watersipora subtorquata (d'Orbigny, 1852)	Japan
O. Ctenostomatida	
Fam. Vesiculariidae	
Amathia convoluta (Lamarck, 1816)	Gulf of Mexico
Amathia wilsoni Kirkpatrick, 1888	New Zealand

2. Antitumoural compounds isolated from marine bryozoans

The MNPs from bryozoans are diverse and display a wide variety of activities, from which here we review the anticancer activity (Table 2).

2.1. Alkaloids

Alkaloids are the most common class of natural products isolated from bryozoans with a unique structural and bioactive diversity (Figure 1; Table 2). Therefore, these secondary metabolites have a huge potential as new drugs [11].

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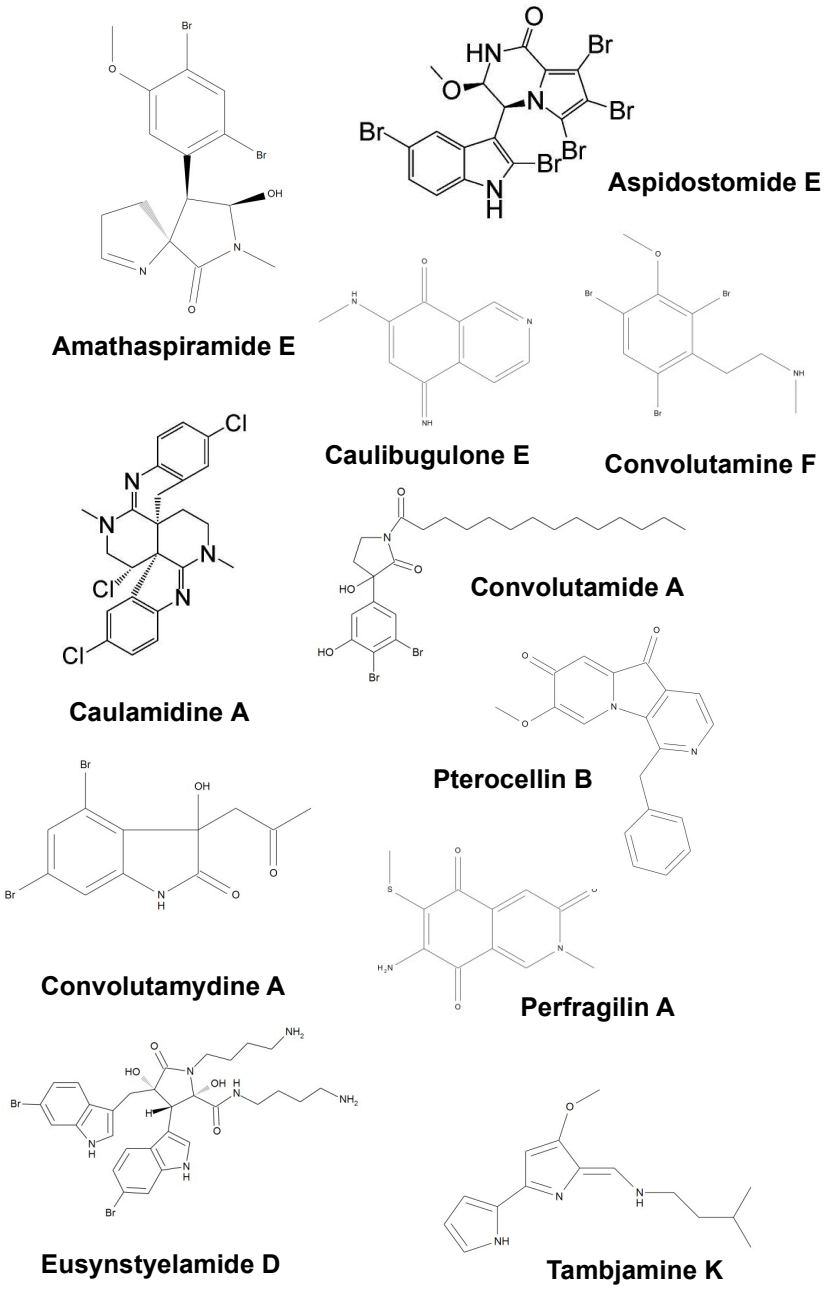

Figure 1. Structures of selected alkaloids from bryozoans.

Table 2. Compounds and anticancer activity described for bryozoans.

Compounds	Activity against cell lines
Alkaloids	
1. *Amathaspiramides*	HCT-116, PC-3, MV4-11, MiaPaCa-2
2. *Aspidostomides*	768-O
3. *Bromated alkaloids*	HL-60
4. *β-Carboline alkaloids*	NCI-60, P-388
5. *Caulamidines*	NCI-60
6. *Caulibugulones*	IC-2

7. *Convolutamides, convolutamydines and convolutamines*	L-1210, KB, KB/VJ-300, P-388, U937, HL-60, MH-60, and other resistant lines
8. *Eusynstyelamides*	A-2058
9. *Perfragilins*	P-388
10. *Polycyclic indole alkaloids*	HCT-116
11. *Pterocellins*	P-388, CCRF-CEM, MALME-3M, NCI-H23, M-14, SK-MEL-5, MDA-MB-435, MDA-N, Hela, and others
12. *Tambjamines*	CaCo-2, HCT-116, MB-231, H9c2
13. *Terminoflustrindole*	3T3, 3T3-SV40, SK-N-SH, C6, B-16, U937
Lactones	
1. *Bryostatins*	U-937, HL-60, P-388, B-16, K1735-M2, LNcaP, M-5076
2. *Neristatins*	P-388
3. *Myriaporones*	L-1210
4. *Other lactones*	HL-60, Hep-G2, SGC-7901
Ceramides	DNA topoisomerase I enzyme, HepG2, SGC7901, NCI-H460, HL-60
Sterols	HepG2, HT-29, NCI-H460, HL-60, SGC-7901
Other compounds	BAEC
Active extracts	MCF-7, A2058

2.1.1. Amathaspiramides

Amathaspiramides A–F, a series of six dibrominated alkaloids, were isolated from *Amathia wilsoni* Kirkpatrick, 1888 (Vesiculariidae) [31]. This specimen was collected from Barrett Reef in Wellington Harbor (off the North Island of New Zealand). Amathaspiramides were assayed for P388 murine but none of the compounds were active (IC_{50} values > 12.5 μg/mL). These compounds, together with four analogues, were tested for antiproliferative activity against four human cancer cell lines (HCT116 (colon cancer), PC-3 (prostate cancer), MV4-11 (acute myeloid leukemia), MiaPaCa-2 (pancreas cancer)). Amathaspiramides A, C, and E exhibited moderate antiproliferative activity in all four cell lines [32]. This study demonstrated the importance of the amine or imine substructure on the pyrrolidine moiety and the 8*R* stereochemistry on the *N*-acyl hemiaminal moiety for the antiproliferative activity of amathaspiramides.

2.1.2. Aspidostomides

Aspidostomides A-H, are a series of bromopyrrole alkaloids derived~~,~~ from either bromotryptophan or bromotyrosine, isolated from the Patagonian bryozoan *Aspidostoma giganteum* (Busk, 1854) (Aspidostomatidae). Remarkably, there were not previous reports of secondary metabolites from this family and this study was the first report on the chemistry of a bryozoan species from the Patagonian region [33]. Two cheilostome specimens were collected by trawling (60–100 m) in the Gulf of San Jorge (Argentina) having a wide distribution along South America and Antarctic regions. Aspidostomide E exhibited moderate inhibitory activity towards the 768-O renal carcinoma cell line.

2.1.3. Bromated alkaloids

A bromated alkaloid (7-bromo-2,4(1H,3H)-quinazolinedione) from the cheilostome *Cryptosula pallasiana* (Moll, 1803) (Cryptosulidae) collected off Huang Island (Qingdao, China) showed strong cytotoxicity against HL-60 cells with IC_{50} value of 11.87 μg/mL [34].

2.1.4. β-Carboline alkaloids

The crude extracts of the cheilostomes *Paracribricellina* (*Catenicella*) *cribraria*, collected at 14 m depth on Cape Vlamingh (Rottmest Island, Western Australia), and *Paracribricellina* (*Cribricellina*) *cribraria* (Busk, 1852) (Catenicellidae), collected at 15 m depth at Poor Knights Islands (The Tunnel, North Wall, New Zealand), exhibited relatively potent cytotoxicity against the NCI 60-cell tumour assay. The compound 1-vinyl-8-hydroxy-*β*-carboline was the responsible to the activity against the NCI 60-cell tumour in both species [35]. This compound was previously isolated as the major cytotoxic component from the latter species by scuba diving from Sugar Loaf, Kaikoura (off the South Island of New Zealand) [36], showing cytotoxicity against P-388 (IC_{50} value of 100 ng/ml). Other *β*-carboline alkaloids were also isolated from the same species, showing different degrees of biological activity in the P-388 cytotoxicity assay: the IC_{50} value of 1-vinyl-8-methoxy-*β*-carboline and pavettine were determined to be 100 ng/ml, while that of compound 1-vinyl-8-acetoxy-*β*-carboline was 670 ng/ml [36]. The IC_{50} values of 1-ethyl-4-methylsulfone-*β*-carboline, 1-ethyl-8-hydroxy-*β*-carboline and l-ethyl-8-methoxy-*β*-carboline were both greater than 12,500 ng/ml. The authors suggested the vinyl group might be important for P-388 cytotoxicity, as the alkaloids with a 1-vinyl substituent exhibited better activity than those with a 1-alkyl substituent. A previously described, 6-hydroxyharman, and a new *β*-carboline alkaloid, 8-hydroxyharman, from *P. cribraria*, collected from Lighthouse Reef Point (Moeraki, East coast of the South Island, New Zealand), exhibited relatively weak cytotoxicity against P388 cells with an IC_{50} more than 12,500 ng/ml [37]. Moreover, another *β*-carboline alkaloid, 5-bromo-8-methoxy-1-methyl-*β*-carboline, was isolated for the first time from the cheilostome *Pterocella vesiculosa* (Lamarck, 1816) (Catenicellidae) collected from the Alderman Islands (off the North Island, New Zealand). The alkaloid displayed relatively moderate cytotoxicity against P388 cells with an IC_{50} of 5089 ng/ml and also displayed inhibitory action against the Gram-positive bacterium *Bacillus subtilis* and the fungi *Candida albicans* and *Trichophyton mentagrophytes* with MID ranges of 2–4, 4–5, and 4–5 μg/mL [38]. It has been demonstrated that the vinyl substituent at C-1 or bromine at C-5 is important for the cytotoxicity against P388 [38].

2.1.5. Caulamidines

Caulamidine A and B, heterocyclic alkaloids with a 2,6-naphthyridine core and fused by a dihydroindole-derived and tetrahydroquinoline-derived systems, were isolated from the cheilostome *Caulibugula intermis* Harmer, 1926 (Bugulidae). Caulamidine A displayed modest cytotoxicity in NCI-60 cell screen with a single dose (40 μM). Both compounds exhibited also antimalarial activity towards *Plasmodium falciparum* with IC_{50} values from 8.3–12.9 μM [39].

2.1.6. Caulibugulones

Caulibugulones A-F are alkaloids isolated from specimens of the *C. inermis* collected at a depth of 33 m in the south Pacific off Palau [40]. Caulibugulones A-D possess an isoquinoline-5,8-dione carrying a substituted amino group at position C-7 and substitution at C-6 by hydrogen, bromine or chlorine. Caulibugulones E and F are analogues of caulibugulone A carrying an imine group at position C-5 and were the first compounds with an isoquinoline iminoquinone skeleton to be isolated from a natural source [40]. All these alkaloids showed cytotoxicity against the murine IC-2^{wt} tumour cell line *in vitro* with IC_{50} from 0.03 to 1.67 μg/ml although caulibugulone E was the most potent. A series of isoquinoline quinones were isolated from marine sponges. Similar compounds were isolated from bacterial sources, suggesting a bacterial origin. These compounds displayed antitumour activities as well ([40] and references therein).

2.1.7. Convolutamides, convolutamydines and convolutamines

Convolutamides A-F are alkaloids, possessing an *N*-acyl-γ-lactam moiety with a dibromophenol group, isolated from the ctenostome *Amathia convoluta* (Lamarck, 1816) (Vesiculariidae). The specimens were collected off Northeastern Gulf of Mexico in Florida (US). The mixture of

convolutamides A and B displayed cytotoxicity against L1210 murine leukaemia cells and KB human epidermoid carcinoma cells [41].

Convolutamydines A-D belong to a class of alkaloids isolated from *A. convoluta* collected in the same region. Convolutamydine A was the first example of a compound with oxindole for marine bryozoans. This compound (1,4,6-dibromo-3-hydroxy-3-(2-oxopropyl)-2-indolinone) showed potent activity in the differentiation of HL-60 human plomyelocytic leukaemia cells [42]. Further investigation of the research team led to the isolation of three new dibromohydroxyoxindole derivatives, convolutamydines B-D. Convolutamydine B also exhibited bioactivity in the differentiation of HL-60 cells. Given the small amounts of convolutamydines C and D, their biological evaluation could not be achieved [43].

Convolutamines A-G (brominated *β*-phenylethylamine alkaloids) and lutamides A and C (2,4,6-tribromo-3-methoxyphenethylamine alkaloids) were also isolated from the Floridian *A. convoluta*. Convolutamines A, C and F showed inhibition of cell growth of both drug-sensitive and -resistant P388 cell lines and U937. Convolutamine C only displayed weak growth inhibition against IL-6-dependent MH-60 cells [44]. Convolutamines B and D also exhibited cell growth inhibitory activity against P388 with IC_{50} values of 4.8 and 8.6 μg/ml, respectively [45] and convolutamine F against human epidermoid carcinoma KB cells and its vincristine-resistant KB/VJ-300 cells [46]. Convolutamine F also displayed inhibitory effect for cell division of fertilized sea urchin eggs [46]. Convolutamines I-J, isolated from the Southern Ocean bryozoan *A. tortuosa* Tenison-Woods, 1880, were recently validated as potential ATP competitive inhibitors [47]. Lutamides A and C exhibited inhibition against adriamycin (ADM)-resistant P388/ADM, vincristine (VCR)-resistant P388/VCR and KB/VJ300 cells in the presence of ADM or VCR whose concentration did not affect growth of the cells examined [44].

2.1.8. Eusynstyelamides

Eusynstyelamides are alkaloids isolated from different marine organisms such as bryozoans and ascidians [48]. The brominated tryptophan-derived ent-eusynstyelamide B and three new derivatives, eusynstyelamides D, E, and F, were isolated from the Arctic cheilostome *Tegella cf. spitzbergensis* (Bidenkap, 1897) (Calloporidae), being the first report of bioactive metabolites from this genus. Although differences in configuration exist between ent-eusynstyelamide B and eusynstyelamide B, the structures are identical, suggesting these compounds are of bacterial origin. The bryozoan specimen was collected off the Bear Islands (North Atlantic) at 59 m depth. Two compounds, eusynstyelamide D and E, exhibited weakly activity against the human melanoma A-2058 cell line [48]. Eusynstyelamide B, together with its two isomers eusynstyelamide A and C, were previously isolated from the Australian ascidian *Eusynstyela latericius* (Sluiter, 1904) collected using scuba from the waters around Hixson Island and Rib Reef. These compounds were found to be nontoxic toward the three human tumour cell lines MCF-7 (breast), SF-268 (CNS), and H-460 (lung) at concentrations up to 32 mM despite exhibiting inhibitory activity against neuronal nitric oxide synthase and modest antibacterial activity [49]. Eusynstyelamide (4), almost identical to that reported for eusynstyelamide A and isolated from another ascidian species *E. misakiensis* (Watanabe & Tokioka, 1972), was nontoxic towards human colon tumour cell line HCT-116, although its extract displayed weak cytotoxic activity against this tumour cell line (IC_{50} 100 μM) [50].

2.1.9. Perfragilins

Perfragilins A and B are isoquinoline quinones isolated from the cheilostome *Biflustra perfragilis* MacGillivray, 1881 (Membraniporidae) collected using scuba at Rapid Bay (South Australia). Both perfragilins displayed cytotoxic activity to murine leukaemia cells (P388) lines [51]. Their structural relationship to the alkaloid mimosamycin, isolated from the terrestrial actinomycete *Streptomyces lavendulae* [52], the sponges *Haliclona* (*Reniera*) sp., collected in a marine lake in Palau, Western Caroline Islands [53], and *Niphates* (*Xestospongia*) *caycedoi* (Zea & van Soest, 1986) collected from Sand Island (Suva Harbor, Fiji), at 2 m depth, suggests perfragilins are also of bacterial origin [51].

2.1.10. Polycyclic indole alkaloids

The primary source of these alkaloids are four bryozoan species belonging to the Family Flustridae: *Chartella papyracea* (Ellis & Solander, 1786), *Securiflustra securifrons* (Pallas, 1766), *Hincksinoflustra denticulata* (Busk, 1852) and *Flustra foliacea* (Linnaeus, 1758). However, only some alkaloids have shown to be active against tumour cell lines until now. Chartellines A-C and chartellamides A-B were isolated from *C. papyracea* in the Roscoff region of France, being the first examples of polycyclic indole alkaloids in marine bryozoans. Chartelline A, the first compound isolated from this species with a penta-halogenated indole containing a *β*-lactam ring, was inactive against leukemia cells in the NCI's test [54]. Three new halogenated, hexacyclic indole-imidazole alkaloids, securamines H-J, together with the previously reported compounds securamines C and E were isolated from the Arctic bryozoan *S. securifrons* collected off the coast of Hjelmsøya (Norway). [55]. Securamines C, E and H-J were evaluated for their cytotoxic activity against human cancer cell lines A2058 (skin), HT-29 (colon), and MCF-7 (breast), as well as against non-malignant human MRC-5 lung fibroblasts. Securamines C, E, H and I were found to affect cell viability, being H, I and E the most potent, with IC_{50} values ranging from 1.4 ± 0.1 to 10 ± 1 μM [55]. While a crude extract of *F. foliacea* collected in the southeastern North Sea at a water depth of 33–45 m was not cytotoxic, their purified compounds displayed activity against human colon cancer cell line HCT-116. In particular, a new deformylflustrabromine showed the strongest cytotoxicity and flustramine A, D and dihydroflustramine C displayed weak cytotoxicity [56].

2.1.11. Pterocellins

Pterocellins A-F are a series of alkaloids based on a 4-pyridone group and a pyridine group bound together through a five membered ring which appear to be unique to the cheilostome *Pterocella vesiculosa* [57,58]. Specifically, pterocellins A and B were isolated from an organic extract of this bryozoan species from the Hen and Chicken Islands (off the North Island of New Zealand). Both compounds displayed relatively potent antitumor activity against the murine leukaemia cell line P-388 *in vitro* with IC_{50} values of 477 and 323 ng/ml, respectively, apart from antibacterial and antifungal activities [57]. Moreover, the National Cancer Institute (NCI) tested pterocellins A and B against a variety of human tumour cell types (leukaemia, non-small cell lung, colon, central nervous system (CNS), melanoma, ovarian, renal, prostate and breast cancers), exhibiting potent cytotoxicity overall. The most sensitive cell lines to pterocellins A and B were leukaemia (CCRF-CEM) and melanoma (MALME-3M), respectively. Non-small cell lung (NCI-H23), melanoma (MALME-3M, M-14, SK-MEL-5) and breast (MDA-MB-435 and MDA-N) were especially sensitive to both compounds. Only pterocellin A was tested in preliminary *in vivo* antitumour evaluation in the mouse hollow fibre assay given the other compound had similar cytotoxicity profile. The results showed it was not effective, being discarded for the next stage of testing [27]. Also, this alkaloid was cytotoxic to Hela human cervical cancer cells, with an IC_{50} of 886 ng/ml [59]. Four new pterocellins (C-F) were isolated from another specimen posteriorly collected from the Alderman Islands (off the North Island of New Zealand). Pterocellins C-F displayed variable levels of activity against the Gram-positive bacterium *Bacillus subtilis* but only pterocellin D exhibited moderate activity against P388 cell line with an IC_{50} value of 4773 ng/ml and against the dermatophyte *Trichophyton mentagrophytes*. Overall, pterocellins A and B displayed much stronger cytotoxicity than pterocellins C-F [58].

2.1.12. Tambjamines

The tambjamines A-J are a 2,20-bipyrrolic class of cytotoxic alkaloids isolated from bacteria and several marine invertebrate groups such as bryozoans, nudibranch molluscs and ascidians with a wide range of bioactive activities (e.g. antitumour and immunosuppressive activities) [60–63]. These compounds belong to the group of 4-methoxypyrrolic natural products and their structure is characterized by a pyrrole ring displaying a second pyrrole system at C-2, an enamine moiety at C-5, and a methoxy group at C-4. A new tambjamine K together with the known tambjamines A and B were isolated from the cheilostome *Viridididentula* (*Bugula*) *dentata* (Lamouroux, 1816) (Bugulidae). The

bryozoan specimens were collected from the port of Horta at Faial island (Azores, Atlantic) [64]. Tambjamine K, the isopentenyl derivative of the co-occurring tambjamine A, displayed moderate to potent concentration-dependent cytotoxicity against a spectrum of tumour cells: cytotoxicity towards human epithelial colorectal adenocarcinoma CaCo-2 cells, human colorectal carcinoma HCT-116 and breast carcinoma MB-231 cell lines and rat cardiac myoblast H9c2 cells [65].

2.1.13. Terminoflustrindole

Terminoflustrindoles A-C are a group of brominated akaloids isolated from the White Sea cheilostome *Terminoflustra (Chartella) membranaceotruncata* (Smitt, 1868) (Flustridae). Terminoflustrindole A was recently found to display potent cytotoxic activity against tumour cell lines, like normal mice fibroblasts 3T3, transformed mice fibroblasts 3T3-SV40, human neuroblastome SK-N-SH, rat histioblastome C6, mice melanoma B-16, and histiotypic leukemia U937 cells). Terminoflustrindole A also displayed modest antibacterial and fungicidal activities [67, 68].

2.2. Lactones

Macrocyclic lactones are a relevant class of secondary metabolites with antitumour activity (Figure 2). Bryostatin-1 was the first macrocyclic lactone identified from *Bugula neritina* (Linnaeus, 1758) (Bugulidae).

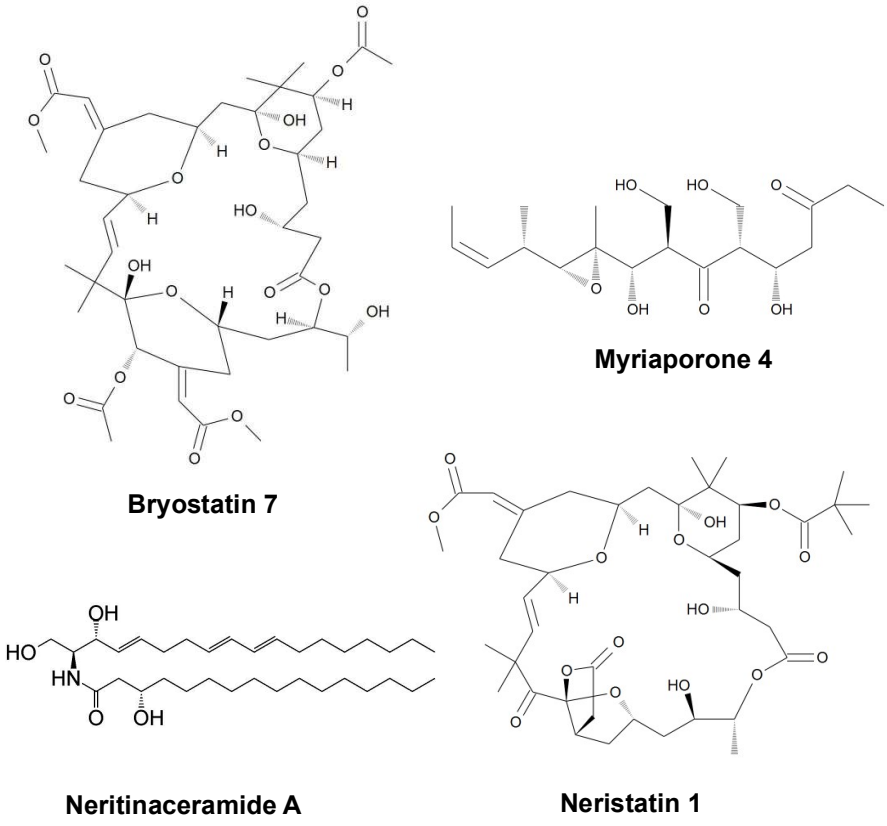

Figure 2. Structures of selected lactones and a ceramide from bryozoans.

2.2.1. Bryostatins

Bryostatins are one the most known class of marine-derived compounds from bryozoans and the most promising compound candidates as anticancer agents due to their low toxicity and antineoplastic activity. Since their discovery, a wide range of studies have been published focusing on their potential use in cancer chemotherapy. This class of highly oxygenated macrolides are

complex polyketides based on the bryopyran ring system [67]. It has been demonstrated that the structures with α methyl at C-28 and α hydroxyl at C-9 play a significant role for their potent cytotoxicities [11]. Bryostatins 1-18 were identified and tested for antitumour activity [67]. Among *in vitro* and *in vivo* anticancer effects against a range of tumour lines, bryostatins have been shown to display activity against histiocytic lymphoma cell U937, human leukaemia HL-60, lymphocytic leukaemia P388, melanoma B-16, murine melanoma K1735-M2, prostate cancer cells LNcaP and M5076 reticulum cell sarcoma [68,69]. Apart from their antitumour properties, these compounds can be used to treat other diseases, as bryostatins improve memory and learning (e.g. Alzheimer's disease, depression and traumatic brain injury) [70].

Bryostatins are able to selectively modulate the function of several protein kinase C (PKC) enzymes, which possess an important role in the regulation of cell growth and death. In particular, their anti-tumour effects are the consequence of binding to the PKC, whose activation by phorbol esters promotes the growth of tumour cells but its interaction with bryostatins produce antineoplastic activity. In particular, bryostatin-7 exhibits the most potent binding affinity to PKC and Tian et al. [11] suggested that this compound could therefore be an effective substitute for bryostatin-1. Bryostatins 1, 5, and 8 promote the activation of PKC for a short period followed by its deregulation, leading to growth inhibition, cell differentiation and programmed cell death [67,71]. It has been demonstrated that structures with a 20-membered macrolactone ring are essential for good PKC binding activity, a C-(3)-hydroxyl with (R)-stereochemistry and a free hydroxyl at C-26 are significant for a high enzyme affinity and the structure of the A-ring at the C7-C9 region is needed for displaying antitumour activity [68].

Bryostatin-1, a highly oxygenated macrolide with a unique polyacetate backbone, is the most studied compound first isolated and characterised by Pettit in 1982 [72] from the cheilostome *B. neritina*. This temperate intertidal bryozoan species forms chitinous, branching colonies that are often found attached to vessel hulls and docks across the globe. This compound has been isolated from different colonies in a range of locations such as California and the Gulf of Mexico. Apart from being a PKC inhibitor, this bioactive compound has been shown to induce differentiation and promote apoptosis in various tumour cell lines [71], immunomodulatory properties (e.g. stimulation of cytokine production and activation of cytotoxic T lymphocytes) [73,74] and antitumour activity in preclinical models [75]. In addition, synergistic effects with a number of established oncolytic agents, including vincristine, paclitaxel, gemcitibine, and flavopyridol have been shown [68]. Therefore, bryostatin-1 is a promising compound against several tumour cell types although it is still under investigation in Phase II clinical trials for cancer [69]. In addition, several analogues have been shown to have growth *in vitro* inhibitory activity against human cancer cell lines [5].

Bryostatin-5 showed a strong differentiation-inducing ability in human myeloid blast cells [76] and inhibited the growth of murine melanoma K1735-M [77] and HL60 leukemic cells [78]. Bryostatin-19 isolated from *B. neritina* in the South China Sea displayed strong cytotoxic activity against the U937 cell line [79].

These natural products, with a broad range of biological activities, have mainly been isolated from cheilostome and ctenostome bryozoans (e.g. *B. neritina* and *Amathia convoluta* (Lamarck, 1816) (Vesiculariidae), respectively), although other substances with different variations of the basic bryostatin structure have been reported in other marine organisms such as the sponge *Lissodendoryx isodictyalis* (Carter, 1882) and the ascidian *Aplidium californicum* (Ritter & Forsyth, 1917) [67,80,81]. Several studies have demonstrated that the bryostatins are more likely to be produced by the uncultured symbiotic bacterium *Endobugula sertula* [29,30] rather than being diet-derived compounds, or biosynthesized by the bryozoan itself. For example, some populations which do not harbour this symbiont, do not have bryostatins either [82]. In laboratory experiments, *B. neritina* with the symbiont produced less bryostatins when the colonies were treated with antibiotics [29]. This bacterium is transmitted vertically to the larvae of *B. neritina* prior to their release from the adult [83]. It its documented that bryostatins cause the *B. neritina* larvae to be unpalatable to predators with a thousand-fold higher concentration found in the larvae than the adult colonies [30].

Until now, six bryostatins (1, 2, 3, 7, 9 and 16) have been synthesized. A spectrum of bryostatin analogues have also been synthesized to replace bryostatins due to their scarcity from natural sources and the complexity of synthesis, modification and extraction methods for their isolation. Importantly, these analogues retain biological activity despite their simplified structure [68]. Until now, several analogues have shown PKC inhibition activity with strong *in vitro* antitumour effects. For example, the analogue 1 was a successful compound with stronger growth inhibition of MYC-induced lymphoma *in vitro* compared with bryostatin-1 ([84]; also see the review by Ruan et al. [68]).

2.2.2. Neristatins

Neristatin 1, a macrocyclic lactone, was isolated from the cheilostome *B. neritina*. Specimens were collected from the Gulf of Mexico coast of Florida (US). This compound is similar to the bryostatins, exhibiting weakly activity against the P-388 leukaemia cell line [85].

2.2.3. Myriaporones

Myriaporones are polyketide-derived metabolites isolated from the Mediterranean cheilostome *Myriapora truncata* (Pallas, 1766) (Myriozoidae). Isolation and structural determination of the Myriaporones 1-4 were achieved from a specimen collected from the Western Mediterranean Sea. Given that myriaporones 3 and 4 are isomers in equilibrium, their structures were determined by analysis of a mixture which showed 88% inhibition against murine leukaemia L1210 cells at 0.2 μg/mL [86].

2.2.4. Other lactones

A lactone was isolated from *Cryptosula pallasiana* collected from Huang Island (Qingdao, China) [87]. The compound showed stronger cytotoxicity against human tumour cell lines HL-60, Hep-G2 and SGC-7901, with IC_{50} values from 4.12 to 7.32μM, than sterols and ceramides.

2.3. Ceramides

Two sulfates of ceramides were isolated from the cheilostome *Watersipora cucullata* (Busk, 1854) (Watersiporidae). The specimen was collected in Aichi Prefecture (Japan). Both compounds were inhibitors of the principal target of anticancer drugs DNA topoisomerase I enzyme with IC_{50} values of 0.4 and 0.2 μM, respectively [88].

Five ceramides, neritinaceramides A–E (2S,3R,3′S,4E,8E,10E)-2-(hexadecanoylamino)-4,8,10-octadecatriene-l,3,3′-triol, (2S,3R,2′R,4E,8E,10E)-2-(hexadecanoylamino)-4,8,10-octadecatriene-l,3,2′-triol,(2S,3R,2′R,4E,8E,10E)-2-(octadecanoylamino)-4,8,10-octadecatriene-l,3,2′-triol, (2S,3R,3′S,4E,8E)-2-(hexadecanoylamino)-4,8-octadecadiene-l,3,3′-triol and (2S,3R,3′S,4E)-2-(hexadecanoylamino)-4-octadecene-l,3,3′-triol, were isolated from *B. neritina*. This specimen was collected in Daya Bay (Shenzhen, China). While all the compounds exhibited moderated cytotoxicity against HepG2 and SGC7901 cells with a range of IC_{50} values from 47.3 to 58.1 μM and relative weak activity to NCI-H460 cell line, neritinaceramides did not display activity against NCI-H460 cells [89].

Two new ceramides, (2S,3R,4E,8E)-2-(tetradecanoylamino)-4,8-octadecadien-l,3-diol and (2S,3R,20R,4E,8E)-2-(tetradecanoylamino)-4,8-octadecadien-l,3,20-triol, together with two known ceramides were isolated from *C. pallasiana* [87]. The specimen was collected at Huang Island (China). These compounds displayed weak cytotoxicity against human myeloid leukaemia cell line HL-60, human hepatocellular carcinoma Hep-G2 and human gastric carcinoma SGC-7901 with the IC_{50} values from 21.13 to 58.15 μM, being stronger against HepG-2 and SGC-7901 than against HL-60. The possession 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 considered to play an important role for their cytotoxicity.

2.4. Sterols

Sterols have been shown to exhibit many bioactivities (e.g. antibacterial, antitumour and anti-inflammatory) in marine taxa such as corals, sponges and echinoderms although the position and stereochemistry of hydroxyl or methoxyl groups between C-23 and C-25 in the side chain are characteristic for marine bryozoans [11]. Tian et al. [11] suggest that more studies on testing their cytotoxicity should be carried out in view of their potential medical applications.

Two oxygenated sterols, namely 3*β*,24(S)-dihydroxycholesta-5,25-dien-7-one and 3*β*,25-dihydroxycholesta-5,23-dien-7-one, were isolated from the cheilostome *B. neritina* (Bugulidae) inhabiting in Daya Bay (Shenzhen, China) [90]. These compounds showed weak cytotoxicity towards three human cancer cell lines: HepG2, HT-29 and NCI-H460 [90]. Other two new sterols ((22E)-cholest-4,22-diene-3*β*,6*β*-diol (1) and (23S,24R)-dimethylcholest-7-ene-3*β*, 5*α*,6*β*-triol (3)), a sterol reported for the first time from natural sources ((22E,24S)-24-methylcholest-4,22-diene-3*β*,6*β*-diol (2)), a known steroid glycoside (4) and six known sterols (5-10) were also isolated from *B. neritina* in the same region. While all compounds were evaluated for their cytotoxicity against human tumour cell lines HepG2, NCI-H460 and SGC7901, only 1, 2 and 5 displayed inhibition against HepG2 cancer cell line [91].

Thirteen sterols were isolated from the cheilostome *Cryptosula pallasiana* (Cryptosulidae) collected off Huang Island (Qingdao, China) [92]. Three of these sterols were also isolated from the sponge *Cliona viridis (copiosa)* (Schmidt, 1862) (Clionaidae), collected in the Bay of Naples (Italy) at a depth of 15 m [93] and the deep-water sponge *Stelodoryx chlorophylla* Lévi, 1993 (Myxillidae), collected south of New Caledonia at a depth of 600-540 m [94]. Among the sterols, seven of them ((23E)-25-methoxycholesta-5,23-dien-3*β*-ol, (22E)-7*β*-methoxy-cholesta-5,22-dien-3*β*-ol, 7*β*-methoxy-cholest-5-en-3*β*-ol, (23E)-3*β*-hydroxy-27-norcholesta-5,23-dien-25-one, (23Z)-cholesta-5,23-diene-3*β*,25-diol, (22E)-3*β*-hydroxycholesta-5,22-dien-7-one and (22E)-3*β*-hydroxy-24-norcholesta-5,22-dien-7-one) exhibited moderate cytotoxic effects against human myeloid leukaemia HL-60 cells.

A new sterol (1), (23R)-methoxycholest-5,24-dien-3*β*-ol, together with three known sterols (2-4), were posteriorly isolated from *C. pallasiana* collected off Huang Island (China) [87]. All compounds were evaluated for their cytotoxicity against human tumour cell lines HL-60, HepG-2 and SGC-7901. While, sterol 1 exhibited moderate cytotoxicity with IC_{50} values ranging from 12.34 μM to 18.37 μM, sterols 2–4 were not active.

3. Other compounds

A new antiangiogenic (inhibitor of the proliferation of endothelial cells) compound, bryoanthrathiophene, 5,7-dihydroxy-1-methyl-6-oxo-6*H*-anthra[1,9-*bc*]thiophene, together with two known compounds, 5,7- dihydroxy-1-methoxycarbonyl-6-oxo-6*H*-anthra[1,9-*bc*]thiophene and 1,8-dihydroxyanthraquinone, were isolated from bryozoan *Watersipora subtorquata* (d'Orbigny, 1852) (Watersiporidae) [95]. The specimen was collected in Tsutsumi Island (Fukuoka, Japan) at depths of 5-10 m. The three compounds were evaluated for antiangiogenic activity on bFGF-induced proliferation of bovine aortic endothelial cell (BAEC). Bryoanthrathiophene was the most active compound with IC_{50} of 0.005 μM, being thus potentially used as treatments for cancer and angiogenesis dependent diseases such as diabetic retinopathy and arthritis.

Three aromatic compounds, *p*-methylsulfonylmethyl-phenol, *p*-hydroxybenzaldehyde and methylparaben were isolated for the first time from the bryozoan species *C. pallasiana* apart from the alkaloids previously mentioned [34]. The new natural product *p*-methylsulfonylmethyl-phenol was evaluated for cytotoxicity against HL-60 cells and it appeared to be inactive.

4. Active extracts

The anti-cancer activity of an extract from the cheilostome *Schizoporella unicornis* (Johnston in Wood, 1844) (Schizoporellidae), inhabiting the Arabian Gulf and the Gulf of Oman, was tested in a Michigan Cancer Foundation (MCF-7) cell line breast adenocarcinoma model. The extract displayed

medium anti-cancer activity. Further studies should be carried out to identify the compound/s involved [96].

An organic extract from the Arctic bryozoan *Alcyonidium gelatinosum* (Linnaeus, 1761), collected at Hopenbanken, off the coast of Edgeøya (Svalbard), was recently shown to inhibit the viability of the human melanoma cancer cell line A2058, leading to the evaluation of the chemical constituents of the organic extract and the further isolation of ponasterones (ecdysteriods) for the first time in a bryozoan (ponasterone F and ponasterone A) [97]. As ecdysteroids are arthropod steroid hormones controlling molting (ecdysis), the authors hypothesized these compounds could be used by the bryozoan to reduce fouling on their colonies. In fact, molting has been reported in the same genus (*A. sanguineum* Cook, 1985) and in the free-living bryozoan species *Cupuladria doma* under conditions of heavy fouling colonies [98]. The compounds were posteriorly assayed for cytotoxic properties against A2058 and the non-malignant human fibroblasts MRC-5, resulting in no affection of the survival of these cell lines at concentrations up to 215 and 223 μM, respectively [97]. Further isolation of compounds is thus necessary to identify the compound(s) responsible for the bioactivity observed.

5. Future research directions

This review discusses natural products of diverse nature isolated from a range of bryozoan species that have shown potential as cancer therapies: alkaloids, lactones, ceramides, sterols and other compounds and active extracts. It is proven that bryozoans like other aquatic invertebrates are a very relevant source for anticancer agents although some limitations exist, such as the low amounts of compounds produced by the organisms, the presence of toxins and inorganic salts in the extracts, and the variability of the chemical compounds (chemotype) produced by an organism on different environmental conditions. However, these limiting factors can be overcome by using chemical synthesis, analytical techniques (e.g. isolation, characterization and separation of active compounds), and controlled aquaculture techniques. The precise role of symbionts in the production of bioactive compounds is also an exciting field for further research.

We previously reported intraspecific variability in a range of biological activities (e.g. feeding repellence and antibacterial) for bryozoans depending on location and/or depth, as an adaptive response to diverse abiotic and biotic factors and/or genetic or symbiotic variability [21,24]. In addition, these bryozoan species tested, together with the discovery of more than 20 new species [14,15], were collected in remote areas (Polar regions and/or deep sea environments) using advanced diving and survey technologies. Therefore, accurate taxonomic and geographic information of organisms producing bioactive compounds and the exploration of untapped geographical locations and overlooked taxa using advanced technologies are critical for further efficient bioprospecting of natural products from bryozoans and other understudied taxa.

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