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Review

Novel antitumour natural products from the phylum Bryozoa

Boris Pejin[1*], Miloš Mojović[2] and Aleksandar G. Savić[1**]
[1]*University of Belgrade, Institute for Multidisciplinary Research - IMSI, Department of Life Sciences, Kneza Višeslava 1, 11030 Belgrade, Serbia.*
[2]*University of Belgrade, Faculty of Physical Chemistry, Studentski trg 12-16, 11158 Belgrade, Serbia.*
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Summary. This review covers literature published from 2003-2012 for natural products from the phylum Bryozoa. The focus is on new antitumour substances, together with details related to the organism sourced. It describes 19 promising bioactives, originating from 7 marine bryozoans. The chemical structures of all highlighted organic compounds are briefly discussed.

Keywords: alkaloids, biological activity, Invertebrata, moss animals, new leads, secondary metabolites.

INTRODUCTION

The purpose of this review is to consolidate research literature published from 2003 to 2012 in the field of antitumour pharmacology of marine and freshwater bryozoans. Specifically, this review focuses on new bioactive natural products and their relevant source organisms.

Bryozoa constitute a phylum in which there are likely more than 8000 extant species. The bryozoans are a widely--distributed, aquatic, invertebrate group of animals whose members form colonies composed of numerous units known as zooids. Until the mid 18th century, bryozoans, like corals and hydroids, were regarded as plants. This is reflected both in the name of the phylum, which translates as 'moss animals' and in the term 'zoophyte' which was used by Linnaeus to embrace both bryozoans and hydroids. Today bryozoans are separated into three classes: Phylactolaemata (freshwater dwelling), Stenolaemata (exclusively marine) and Gymnolaemata (mostly marine). Being sessile organisms, devoid of any physical capability for defense and found in environmentally different areas, bryozoans needed to develop adaptive responses and specific means for self-protection (Ryland 2005).

Although little research has been undertaken into the secondary metabolites of bryozoans as compared with those of other marine invertebrates (Blunt et al. 2013), these organisms have proven to be an excellent source of novel and/or biologically active compounds. The most well-known of these metabolites are the bryostatins, but other examples include the tambjamines, securamines, euthyroideones and amathaspiramides (Faulkner 1998; Prinsep 2001).

Of all species within the marine animal kingdom, sponges have attracted the most attention to date. Indeed, many research groups working on marine natural products focus on sponges; however very few groups worldwide work on bryozoans. As a consequence, the chemical constituents of sponges, primarily their secondary metabolites, represent a particularly important topic of marine chemistry (Sladić and Gašić 2006; De Rosa et al. 2008; Pejin et al. 2008, 2011; Pejin 2012; Blunt et al. 2013; Pejin et al. 2014).

Some of the reasons why bryozoans are so under studied compared to other marine invertebrate phyla may well involve the problems associated with working on them. These problems can be classified into three general areas associated with accessibility, and the lack of biomass available for extraction and taxonomy (Prinsep et al. 2004).

This review covers 19 new antitumour compounds from the phylum Bryozoa, published from 2003 to 2012 (Table 1). The natural products herein have shown activity against various human (A-2058, CaCo-2, HepG2, HL-60, HT-29, M-14, MALME-3M, MDA-MB-435, MDA-N, NCI-H23 and SK-MEL-5) and murine (IC-2^{WT}, L-1210 and P-388) cancer cell lines.

*Corresponding author: borispejin@imsi.rs & brspjn@gmail.com
**Corresponding author: asavic@imsi.rs & enzo1320@yahoo.com

Table 1. Novel antitumour natural products from marine bryozoans.

2003	1. pterocellins A and B
2004	1. caulibugulones A-F
	2. bryostatin 20
2005	1. none (Blunt et al. 2007)
2006	1. none (Blunt et al. 2008)
2007	1. myriaporones 3 and 4
2008	1. pterocellin D
2009	1. 5-bromo-8-methoxy-1-methyl-*ß*-carboline
2010	1. tambjamine K
2011	1. eusynstyelamide E
	2. 3*ß*,24(*S*)-dihydroxycholesta-5,25-dien-7-one and 3*ß*,25-dihydroxycholesta-5,23-dien-7-one
	3. (23*E*)-25-methoxy-cholesta-5,23-dien-3*ß*-ol and (22*E*)-7*ß*-methoxy-cholesta-5,22-dien-3*ß*-ol

DISCUSSION

The marine bryozoan species *Pterocella vesiculosa* (Catenicellidae), collected by scuba divers from the Hen and Chicken Islands to the north of New Zealand, was the source of the alkaloids pterocellins A to F, which appear to be unique to the *Pterocella* species.

Pterocellins A and **B** were isolated in 2003 (Yao et al. 2003; Blunt et al. 2005), while pterocellins C to F were found in 2008 (Fig. 1) (Prinsep 2008; Blunt et al. 2010). Structural determination of pterocellins A and B relied in part on an X-ray diffraction study of pterocellin A: both compounds possess a unique heterocyclic skeleton (a tricyclic pyrido [4,3-*b*]indolizine ring system). Pterocellins A and B exhibited relatively potent activity against the murine leukaemia cell line P-388, with IC_{50} values of 477 μg/mL and 323 μg/mL, respectively (Yao et al. 2003).

In addition, the cytotoxicity of pterocellins A and B was also evaluated by the National Cancer Institute (NCI), using their 60 cell line panel, which represents a variety of human tumour cell types: leukaemia, non-small cell lung, colon, central nervous system (CNS), melanoma, ovarian, renal, prostate and breast cancer (Table 2) (Prinsep et al. 2004). Evaluation of these compounds at the NCI revealed that both possessed potent cytotoxicity overall, with panel average values of GI_{50} 1.40 μM & 0.70 μM, TGI 4.80 μM & 2.10 μM and LC_{50} 17.00 μM & 6.90 μM for pterocellins A and B, respectively. The leukaemia CCRF-CEM cell line was most sensitive to pterocellin A, with a GI_{50} of 0.05 μM and a TGI of 0.80 μM; however, the high LC_{50} value of >100 μM implies that pterocellin A is cytostatic rather than cytotoxic. The most sensitive cell line to pterocellin B was melanoma MALME-3M, with a GI_{50} of 0.03 μM and a TGI of 0.10 μM. Cell lines that were particularly sensitive to both agents were non-small cell lung NCI-H23, melanoma MALME-3M, M-14 & SK-MEL-5 and breast MDA-MB-435 & MDA-N.

On the basis of these data, both compounds were recommended by the NCI Biological Evaluation Committee for preliminary *in vivo* antitumour evaluation, using a mouse hollow fiber assay: but given their similarity in overall cytotoxicity profiles, and the small amount of each compound available, only pterocellin A was tested. The hollow fiber assay involves cultivation of human tumour cells in polyvinylidene hollow fibers and implantation of these cells into mice, both intraperitoneally (IP) and subcutaneously (SC). The test compound is then administered via an IP route, and its effect on reduction of viable cancer cell mass compared to controls is examined. A value of 2 is assigned for each compound dose that results in a 50% or greater reduction in cell mass, and IP and SC results are scored separately. Compounds with a combined IP + SC score of 20, an SC score of 8, or a total cell kill of one or more cell lines are referred for further study. Pterocellin A had an IP score of 0, an SC score of 4, a combined IP + SC score of 4, and no cell kill. These results show that pterocellin A was not effective *in vivo*. Hence pterocellin A was not chosen to advance to the next stage of testing. Only 2% of compounds in the initial *in vitro* screen advance to the hollow fibre assay and only 10% of compounds tested in the hollow fibre assay advance (0.2% of initially tested compounds), since this assay is crucial in determining if an agent has potential for use *in vivo*.

Unlike pterocellins A-D, which are monomeric, pterocellins E and F are dimeric. Pterocellins C, E and F were essentially inactive against P-388 cells, with IC_{50} values of >6250 ng/mL, while **pterocellin D** displayed modest activity with an IC_{50} value of 4773 ng/mL. This is in marked contrast to pterocellins A and B, which possess relatively potent activity against the same cell line. The fact that pterocellins A and B exhibit much stronger cytotoxicity than pterocellins C-F suggests that H-8 may be crucial for this observed bioactivity.

Table 2. Antitumour activity of pterocellin A (**1**) and pterocellin B (**2**).

	GI_{50} (μM)		TGI (μM)		LC_{50} (μM)	
Cell line	**1**	**2**	**1**	**2**	**1**	**2**
Leukaemia						
CCRF-CEM	0.05	1.7	0.8	5.2	>100	>100
HL-60(TB)	3	1	>100	5.1	>100	43.5
K-562	3	1.2	>100	2.4	>100	4.9
MOLT-4	3.5	1.4	>100	2.9	>100	5.6
RPMI-8226	1.7	1.5	4.2	4.9	>100	>100
SR	2	0.2	>100	1.3	>100	3.7
Non-small cell lung cancer						
A-549/ATCC	1.3	0.2	5.1	0.5	>100	1.8
EKVX	2.1	1.4	5.8	3.8	23.6	10.7
HOP-62	1.7	1	3.1	2.1	5.6	4.6
HOP-92	2.2	1.7	4.9	3.8	14.1	8.8
NCI-H226	1.7	1.4	3.5	3.5	7.4	8.4
NCI-H23	0.3	0.1	1	0.3	6.1	0.7
NCI-H322M	1.6	1.1	3	2.2	5.6	4.7
NCI-H460	0.8	0.2	2.6	0.8	7.1	5.5
NCI-H522	1.7	1.1	3.3	2.4	6.5	5.2
Colon cancer						
COLO-205	1.6	0.7	3.7	2.3	8.4	6.1
HCC-2998	1.6	0.4	3.1	1	6	3.2
HCT-116	1.1	0.5	2.5	1.6	11.2	4
HCT-15	1.6	0.5	4.2	1.8	17.3	4.9
HT-29	1.3	0.5	7.2	3.2	>100	27.3
KM-12	1.6	0.6	3.4	1.9	7.3	4.5
SW-620	1.5	0.7	3.1	2	6.2	4.9
CNS cancer						
SF-268	2.1	1.7	6.7	5.5	26.7	21.5
SF-295	1.3	0.3	2.6	1.6	5.2	4
SF-539	1.8	1.1	3.6	2.7	7.1	6.4
SNB-19	2.9	1.6	11.4	3.3	60	7
SNB-75	1.4	0.2	3.9	0.9	56.8	5.3
U-251	2.2	1	7.5	2.2	42.5	4.7

	GI_{50} (μM)		TGI (μM)		LC_{50} (μM)	
Cell line	**1**	**2**	**1**	**2**	**1**	**2**
Melanoma						
LOX IMVI	1.3	0.4	7.4	2.4	>100	13.2
MALME-3M	0.1	0.03	0.3	0.1	0.8	0.3
M-14	0.2	0.1	0.8	0.2	4.6	0.5
SK-MEL-2	1.9	0.7	3.4	2.2	6.3	5.3
SK-MEL-28	1.9	1	4.2	2.6	9.3	6.7
SK-MEL-5	0.2	0.1	0.3	0.3	0.6	0.5
UACC-257	1.1	0.2	3	0.7	7.9	2.9
UACC-62	1.4	0.2	2.9	0.6	6	4.1
Ovarian cancer						
IGROV1	2.1	1	5.4	2.5	18.4	6
OVCAR-3	1.5	0.2	4.6	0.6	19.3	3.2
OVCAR-4	1.7	1.3	3.6	2.7	7.6	5.6
OVCAR-5	2	1.2	3.4	2.4	5.6	4.9
OVCAR-8	2.2	0.7	17.6	7.6	>100	84.8
SK-OV-3	6.1	2.7	21.9	13.8	59.2	47.1
Renal cancer						
786-0	1.3	1.8	4.7	5	24.4	17.9
A-498	0.9	1.9	2.2	4	5.1	8.5
ACHN	2.3	1.1	9.1	2.9	>100	7.8
CAKI-1	2.1	1.5	4.6	3.3	11.1	7.4
RXF393	3.2	2.4	12.3	6.9	46.6	45.2
SN12C	1.7	0.5	3.3	1.8	6.5	5.5
TK-10	3.1	1.5	6.8	2.9	23.1	5.6
UO-31	2.2	1.3	6.1	2.8	22.2	5.7
Prostate cancer						
DU-145	0.7	0.2	2	0.3	4.7	0.6
PC-3	2.9	1.6	8.4	5.9	45.1	26.6
Breast cancer						
MCF-7	1.5	1	4.9	3.3	>100	>100
NCI/ADR-RES	2.1	1.4	4.9	3.9	>100	15.3
MDA-MB-231/	2.6	3.3	14.1	29.6	51.7	>100
ATCC						
BT-549	1.7	1.5	4	2.9	9.3	5.6
HS-578T	6.3	5.1	>100	>100	>100	>100
MDA-MB-435	0.2	0.2	0.3	0.3	0.6	0.6
MDA-N	0.2	0.2	0.4	0.3	0.6	0.6
MID	1.4	0.7	4.8	2.1	17	6.9
T-47D	1.8	1.3	6.5	3.2	>100	>100

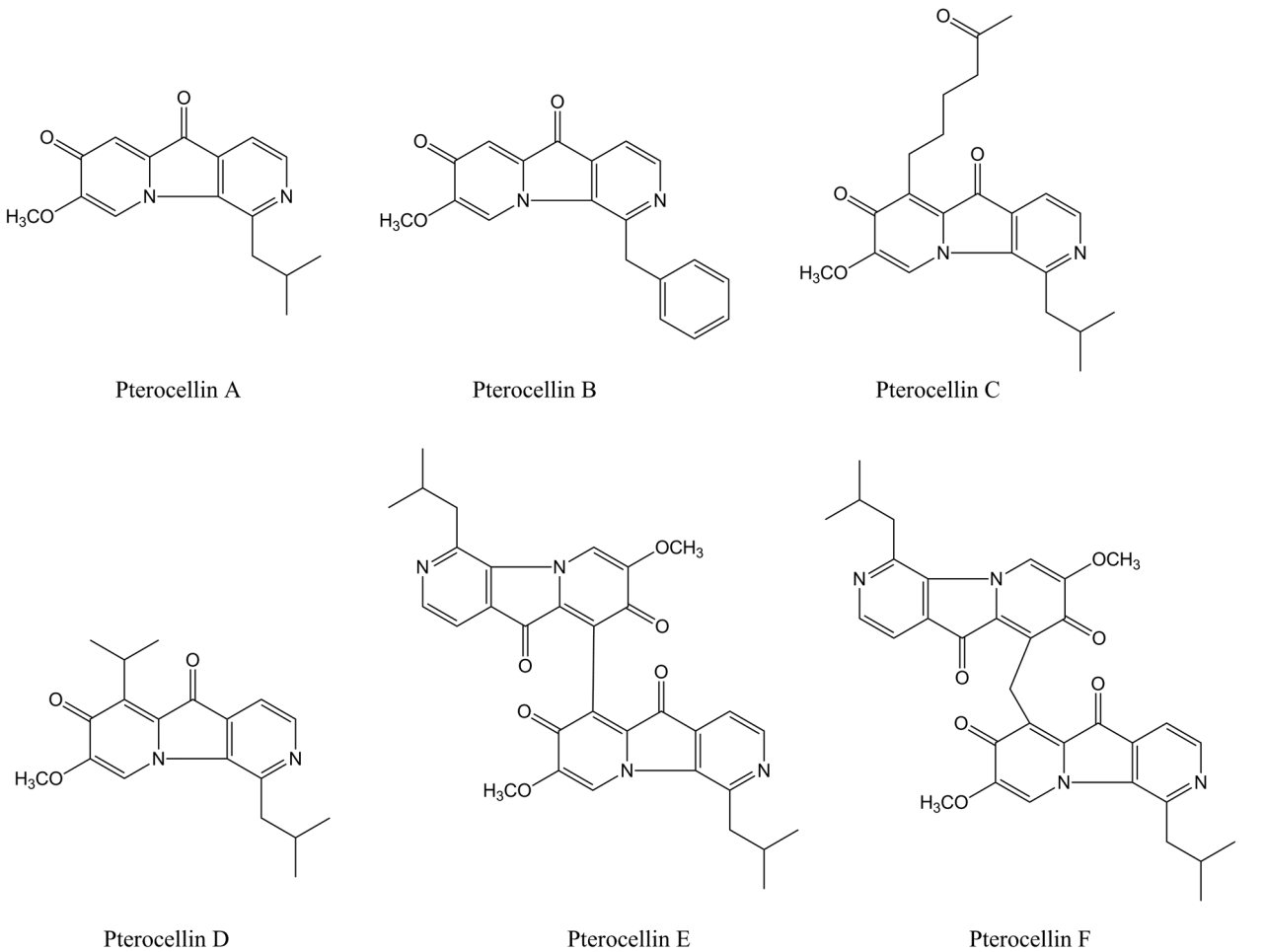


Figure 1. Pterocellins A–F.

The first total synthesis of pterocellin A was recently achieved in 10 linear steps from commercially available kojic acid and 2-bromo-3-pyridinol in a convergent sequence (yield 57%). The key constructive steps are a directed lithiation to couple two pyridines and an intramolecular nucleophilic aromatic substitution to form pterocellin A (O'Malley et al. 2006).

Six alkaloids, **caulibugulones A-F**, were isolated from *Calibugula intermis* (Bugulidae) collected at a depth of 33 m in the south Pacific off Palau (Fig. 2) (Milanowski et al. 2004; Blunt et al. 2006). The main structural characteristic of caulibugulones is 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 A–D); caulibugulones E and F are analogues of caulibugulone A carrying an imine group at position C-5 (Alagille et al. 2004).

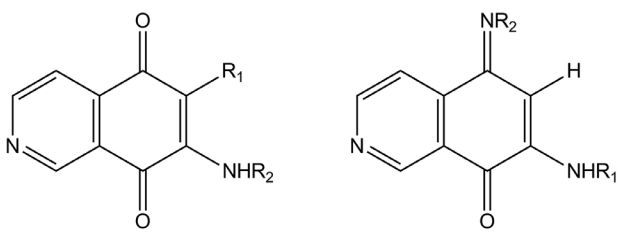


Figure 2. Caulibugulones A–F.

All caulibugulones displayed cytotoxicity against the murine IC-2^{WT} tumour cell line *in vitro* (0.03–1.67 μg/mL), with caulibugulone E being the most potent (Table 3). Caulibugulones A, B and C displayed similar IC_{50} values, indicating that halogen substitution at C-6 is not an important determinant of cytotoxicity. The iminoquinone caulibugulo-

nes E was approximately an order of magnitude more potent than the corresponding quinone caulibugulones A. Substitution of an ethyl alcohol at either the C-7 or C-5 nitrogen resulted in a 5 to 10-fold reduction in cytotoxicity. The caulibugulones are of similar potency to the cribrostatins and related isoquinoline quinines, as well as to the iminoquinone isobatzellines and secobatzelline A.

Table 3. IC_{50} values of caulibugulones A–F for the murine IC-2^{WT} cell line.

Compound	IC_{50} (μg/mL)
caulibugulone A	0.34
caulibugulone B	0.22
caulibugulone C	0.28
caulibugulone D	1.67
caulibugulone E	0.03
caulibugulone F	0.10

One proposed molecular mechanism involves inhibition of full-length human dual specificity protein phosphatase (Cdc25B); the IC_{50} values ranging from 2.70 μM to 32.50 μM, with caulibugulones B and E being the most and least potent, respectively (Table 4) (Wipf et al. 2004). All caulibugulones exhibited a minimum 30-fold preference as inhibitors against Cdc25B compared to the dual specificity protein phosphatase (VHR) or the protein tyrosine phosphatase (PTP1B). In addition, the cell growth inhibitory activity of human tumour cells was consistent with the Cdc25B inhibition.

Table 4. IC_{50} values of synthesised caulibugulones A-E for inhibition of recombinant human protein phosphatases Cdc25B, VHR and PTP1B.

Compound	Cdc25B (μM)	VHR (μM)	PTP1B (μM)
caulibugulone A	6.7	>500	>1000
caulibugulone B	2.7	130	183
caulibugulone C	5.4	175	322
caulibugulone D	19.1	>1000	>1000
caulibugulone E	32.5	>1000	>1000

The total synthesis of naturally occurring cytotoxic caulibugulones A–E proceeded efficiently, with high overall yields, starting from readily available isoquinolin-5-ol *via* hypervalent iodine oxidation, regioselective halogenations and amination reactions (Wipf et al. 2004). The other total synthesis of caulibugulones A-D, employing similar methods starting from 5-aminoisoquinoline, was reported concurrently (Alagille et al. 2004).

Larvae of *Bugula neritina* (Bugulidae) collected from a depth of 4 m to 10 m along the Radio Island Jetty near Morehead (North Carolina, USA) were the source of the macrolide lactone **bryostatin 20**, along with the known bryostatin 10 and an as yet uncharacterised bryostatin (Fig. 3) (Lopanik et al. 2004; Blunt et al. 2006). In 2009 it was reported that bryostatin 1 enhanced the efficacy of cytotoxic agents through modulation of the protein kinase C pathway and was active in combination with vincristine for diffuse large B-cell lymphoma (Blunt et al. 2011). In 2010 bryostatin 16 (Fig. 3) was synthesised in 39 steps from 2,2-dimethyl-3-(*tert*-butyldimethylsiloxy)propanal, using a palladium-catalysed alkyne-alkyne coupling as a macrocyclisation reaction (Blunt et al. 2012). Bryostatin 1, the lead member of the bryostatin family, is currently in Phase I and II clinical trials for cancer, and appears to be exceptionally potent, with only ~1.2 mg required for a full multi-week treatment cycle (DeChristopher 2010).

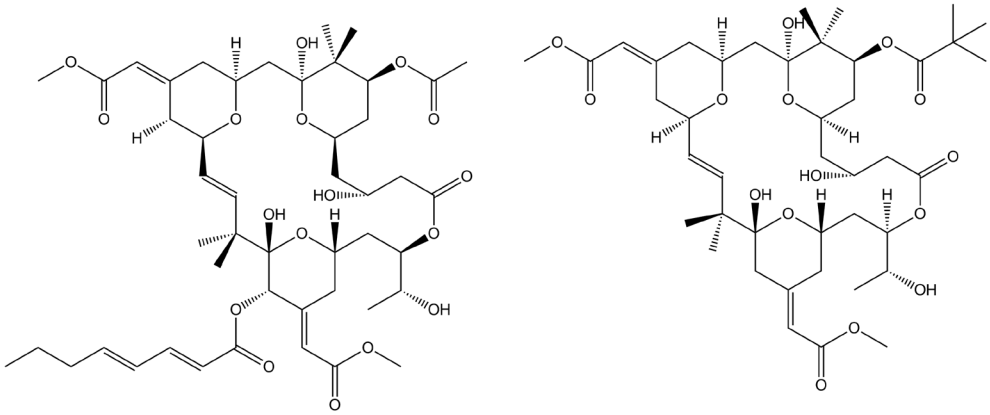
Myriaporones 1–4, four novel polyketide-derived metabolites, have been isolated from specimens of *Myriapora truncata* (Myriozoidae) collected in the Western Mediterranean Sea (Fig. 4) (Cheng et al. 2007; Blunt et al. 2009). Preliminary tests indicate that methanol extracts of these specimens were active against murine leukaemia L1210 cells (99% inhibition at 50 μg/mL, 87% inhibition at 25 μg/mL). The inseparable equilibrium mixture of **myriaporones 3** and **4** showed 88% inhibition of these cells at 0.20 μg/mL.

The bryozoan *Pterocella vesiculosa* (Catenicellidae), collected by scuba divers from the Alderman Islands off the North Island of New Zealand, was the source of a new alkaloid, **5-bromo-8-methoxy-1-methyl-*β*-carboline** (Fig. 5), which displayed moderate inhibition of P-388 cells (IC_{50} 5089 ng/mL); the bromine substituent at C-5 may be of significance for the activity observed (Till and Prinsep 2009; Blunt et al. 2011). *β*-Carboline alkaloids have been found previously from the family Catenicellidae, to which *P. vesiculosa* belongs, but this is the first example of a *β*-carboline alkaloid reported from the genus *Pterocella*.

A new member of the tambjamine family of alkaloids A–J (Fig. 6), the isopentyl-containing **tambjamine K** (Fig. 7), was isolated from the bryozoan *Bugula dentata* (Bugulidae) collected in the port of Horta at Faial island (Azores, Atlantic) by scuba diving (Carbone et al. 2010; Blunt et al. 2012).

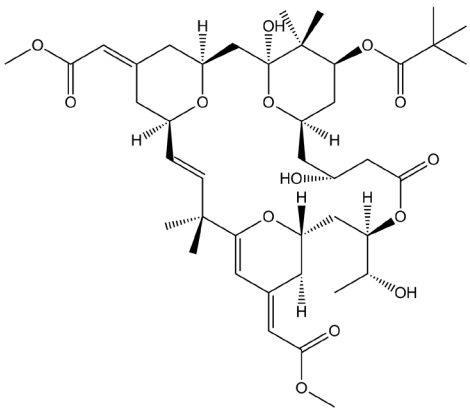
Tambjamine K exhibited moderate to potent concentration-dependent cytotoxicity towards a panel of tumour and non-tumour cells (Table 5). In particular, this compound was demonstrated to be cytotoxic against human epithelial colorectal adenocarcinoma CaCo-2 cells (nanomolar range) and rat cardiac myoblast H9c2 cells (micromolar range). In addition, tambjamine K displayed cytotoxicity against human colorectal carcinoma HCT-116 and breast carcinoma MB-231 cell lines (Aldrich et al. 2010).

The syntheses of tambjamine K and a library of unnatural analogues were reported in the same year. The biological evaluation of this library against a number of tumour and non-tumour cells identified several examples with potent antiproliferative and anti-invasive properties. Indeed, unnatural analogs were shown to be more potent in viability, proliferation, and invasion assays than the corresponding natural product in multiple cancer cell lines, with minimal

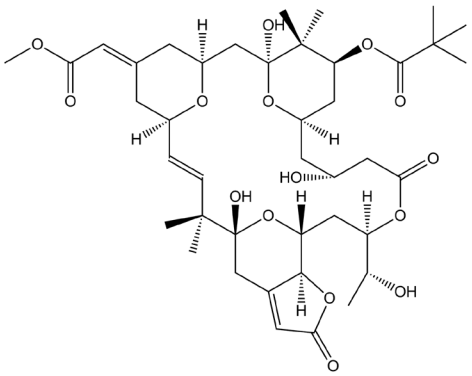


Bryostatin 1

Bryostatin 10

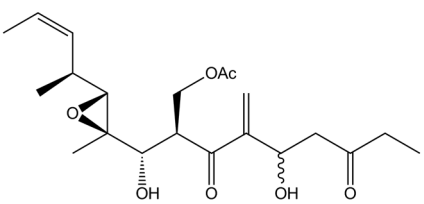


Bryostatin 16

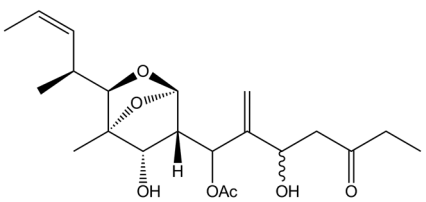


Bryostatin 20

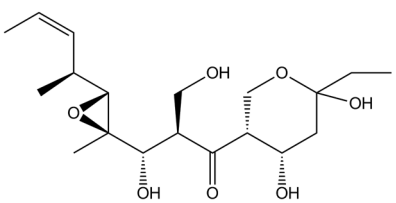
Figure 3. Bryostatins 1, 10, 16 and 20.



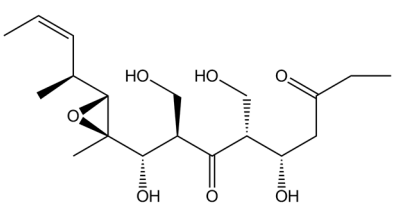
Myriaporone 1



Myriaporone 2



Myriaporone 3



Myriaporone 4

Figure 4. Myriaporones 1–4.

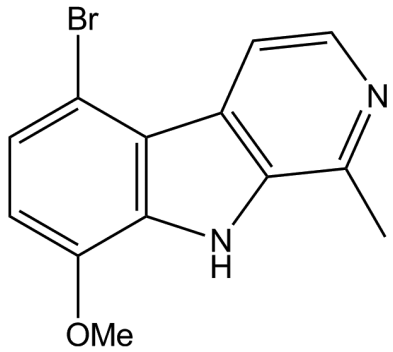
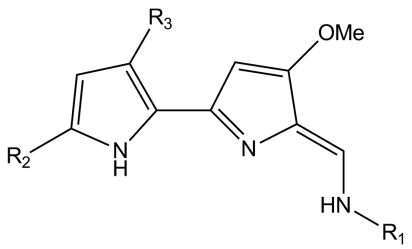


Figure 5. 5-bromo-8-methoxy-1-methyl-ß-carboline.



Tambjamine A	R_1=H	R_2=H	R_3=H
Tambjamine B	R_1=H	R_2=Br	R_3=H
Tambjamine C	R_1=CH_2CHMe_2	R_2=H	R_3=H
Tambjamine D	R_1=CH_2CHMe_2	R_2=H	R_3=Br
Tambjamine E	R_1=CH_2CH_3	R_2=H	R_3=H
Tambjamine F	R_1=$(CH_2)_2Ph$	R_2=H	R_3=H
Tambjamine G	R_1=CH_2CH_3	R_2=H	R_3=H
Tambjamine H	R_1=$CH_2CH_2CH_3$	R_2=Br	R_3=H
Tambjamine I	R_1=$CH_2CH(CH_3)_2$	R_2=Br	R_3=H
Tambjamine J	R_1=$CH_2CH(CH_3)CH_2CH_3$	R_2=Br	R_3=H

Figure 6. Tambjamines A–J.

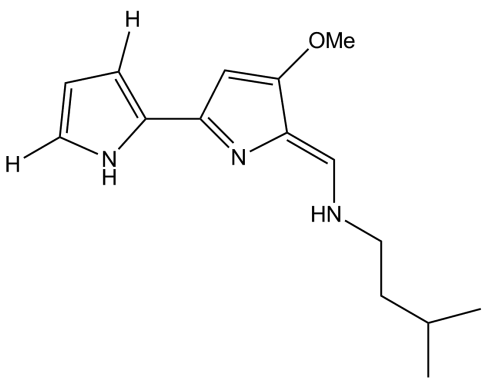


Figure 7. Tambjamine K.

to no cytotoxicity against non-transformed cells (Aldrich et al. 2010).

Table 5. IC_{50} values of tambjamine K for tumour and nontumour cell lines tested.

Cell line	Tambjamine K (μM)
CaCo-2	3.5×10^{-3}
H9c2	2.7
HCT-116	13.7
C-6	14.0
HeLa	14.6
MB-231	15.3
3T3-L1	19.0

The Arctic bryozoan *Tegella cf. spitzbergensis* (Calloporidae), collected off the Bear Islands in the North Atlantic using an Agassiz trawl at 59 m depth, afforded the alkaloid **eusynstyelamide E** (Fig. 8), a brominated tryptophan-derived metabolite which was weakly active against the human melanoma A-2058 cell line (Tadesse et al. 2011; Blunt et al. 2013).

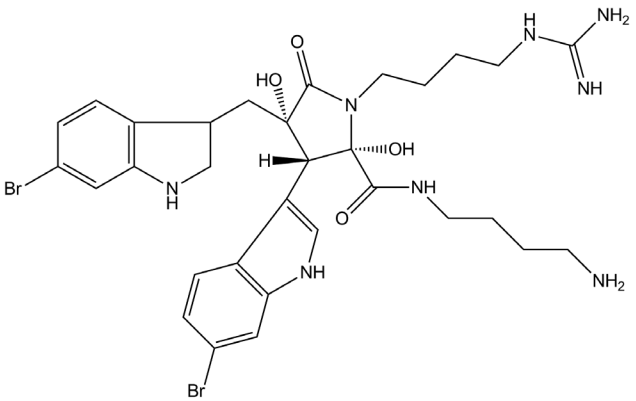
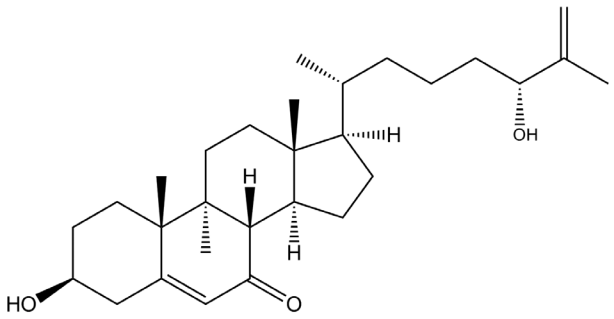


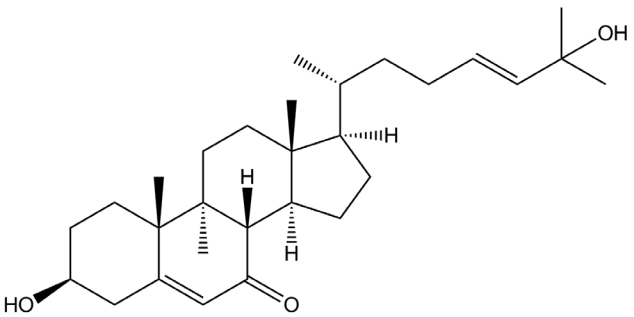
Figure 8. Eusynstyelamide E.

Two new oxygenated sterols, 3*β*,24(*S*)-dihydroxycholesta-5,25-dien-7-one and 3*β*,25-dihydroxycholesta-5,23-dien-7-one (Fig. 9), were isolated from the marine bryozoan *Bugula neritina* (Bugulidae) collected in Daya Bay, Shenzhen, Guangdong Province, PR China. Both compounds exhibited weak cytotoxicity against three human cancer cell lines (HepG2, HT-29 and NCI-H460, respectively) with IC_{50} values between 22.58 μg/mL and 53.41 μg/mL (Yang et al. 2011; Blunt et al. 2013).

Six new sterols (**1**-**6**), namely (23*E*)-25-methoxycholesta-5,23-dien-3*β*-ol (**1**), (22*E*)-7*β*-methoxy-cholesta-5,22-dien-3*β*-ol (**2**), 7*β*-methoxy-cholest-5-en-3*β*-ol (**3**), (23*E*)-3*β*-hydroxy-27-norcholesta-5,23-dien-25-one (**4**), 24(*R*)-Cholesta-5,25-diene-3*β*,24-diol (**5**) and 24(*S*)-Cholesta-5,25-diene-3*β*,24-diol (**6**) (four known only as synthetic **3**-**6**), together with seven known sterols (**7**-**13**), namely (23*Z*)-cholesta-5,23-diene-3*β*,25-diol (**7**), cholest-5-ene-3*β*,7*β*-diol (**8**), cholest-5-ene-3*β*,7*α*-diol (**9**), (22*E*)-3*β*-hydroxy-24-norcholesta-5,22-dien-7-one (**10**), (22*E*)-3*β*-



3-beta,24(S)-dihydroxycholesta-5,25-dien-7-one



3-beta,25-dihydroxycholesta-5,23-dien-7-one

Figure 9. 3*β*,24(*S*)-dihydroxycholesta-5,25-dien-7-one and 3*β*,25-dihydroxycholesta-5,23-dien-7-one.

hydroxycholesta-5,22-dien-7-one (**11**), 3*β*-hydroxycholest-5-en-7-one (**12**) and (4*E*,22*E*)-12*β*-hydroxy-24-norcholesta-1,4,22-trien-3-one (**13**), were isolated from marine bryozoan *Cryptosula pallasiana* (Cryptosulidae) collected off Huang Island, Qingdao City, Shandong Province, PR China (Fig. 10) (Tian et al. 2011; Blunt et al. 2013).

(23*E*)-25-methoxy-cholesta-5,23-dien-3*β*-ol (**1**) is a new sterol with *trans*-double bonds between C-23 and C-24, together with a methoxy group at C-25 in the side chain. **(22*E*)-7*β*-methoxy-cholesta-5,22-dien-3*β*-ol** (**2**) was isolated as a isomer of **1**; the 7*β* methoxy group in the nucleus of **2** and **3** is a rare feature and first encountered among natural sterols. Compound **4** is unique in that it contains carbonylation at C-25, accompanied by a loss of a methyl group in the side chain, while sterols **5** and **6** are stereoisomeric with a C-24 hydroxyl group in the side chain.

Among these steroids, several of them displayed cytotoxic effects against human myeloid leukaemia HL-60 cells: although **12** and **13** did not show any apparent cytotoxicity, sterols **1**–**4**, **7**, **10** and **11** displayed moderate activity (Table 6) (Tian et al. 2011; Blunt et al. 2013).

Table 6. IC_{50} values of compounds **1**–**4**, **7** and **10**–**13** for human myeloid leukaemia HL-60 cells.

Compound	IC_{50} (μg/mL)	Compound	IC_{50} (μg/mL)
1	17.91	10	15.12
2	21.30	11	14.73
3	22.11	12	N.A.*
4	15.05	13	N.A.*
7	18.28	Adriamycin	2.50

*N.A. – no activity.

Computers occupy a special place in modern medicinal chemistry and are important both in drug discovery and development. Rapid advances in computer hardware and software have enabled many of the operations which were once the exclusive province of experts to be carried out on ordinary laboratory computers with little specialist expertise in molecular or quantum mechanics (Graham 2001). For example, topological polar surface area (TPSA) values of pterocellins A & B, caulibugulones A-C, tambjamine K and 5-bromo-8-methoxy-1-methyl-β-carboline are < 60 $Å^2$ and may be considered to be particularly favorable from the point of view of potential applications in pharmacy (Table 7). On the other hand, cLogP (partition coefficient of solubility in *n*-octanol and water) values ranging from 0.13 (caulibugulone F) to 4.15 (bryostatin 20) also suggest that the bioactive compounds reported herein possess high cell permeability.

To the best of our knowledge, there are no records of antitumour and/or bioactive secondary metabolites originating from freshwater bryozoans. However, very recently, our research group has begun focusing its attention in this direction (Pejin et al. 2012; Pejin, Stanimirovic et al. 2013; Pejin, Stosic-Grujicic et al. 2013); for example, a methanol extract of *Hyalinella punctata* (Plumatellidae) collected in the river Danube (Belgrade, Serbia) was shown to be cytotoxic against human breast adenocarcinoma MCF-7 cells (IC_{50} 24.13 μg/mL). At the same time, this extract displayed low cytotoxicity against nontumour human fetal lung fibroblasts MRC-5 cells (Pejin, Stosic-Grujicic et al. 2013). According to National Cancer Institute guidelines, crude extracts with an IC_{50} < 30 μg/mL is considered active (Suffiness and Pezzuto, 1990); thus *H. punctata* extracts may be considered to be a good natural source of new anticarcinogens. In addition, the bryozoan species *Plumatella repens* (Plumatellidae) obtained from Dunedin City Council has been recently reported to be a promising source of highly active metabolite/s against P-388 cells (IC_{50} 22443 ng/mL); however, isolation of the metabolite/s responsible for this activity has not yet been achieved (Andersen 2012).

CONCLUSIONS

In this review, the antitumour activity of 19 novel natural products isolated from 7 marine bryozoan species has been discussed. Alkaloids (12) make up the majority

Table 7. TPSA and cLogP values for the majority of antitumour natural products reported.

Compound	TPSA (Å^2)	cLogP
pterocellin A	58.97	0.33
pterocellin B	58.97	0.44
pterocellin C	76.04	0.98
pterocellin D	58.97	1.76
pterocellin E	117.94	-0.18
pterocellin F	117.94	0.18
caulibugulone A	58.53	0.97
caulibugulone B	58.53	1.83
caulibugulone C	58.53	1.74
caulibugulone D	78.76	0.15
caulibugulone E	65.31	0.21
caulibugulone F	74.05	0.13
bryostatin 1	240.14	5.38
bryostatin 10	213.83	4.58
bryostatin 16	193.61	5.71
bryostatin 20	213.84	4.15
myriaporone 1	113.43	1.10
myriaporone 2	102.29	1.51
myriaporone 3	119.75	0.96
myriaporone 4	127.59	1.33
tambjamine A	59.64	0.36
tambjamine B	59.64	1.30
tambjamine C	45.65	2.79
tambjamine D	45.65	3.03
tambjamine E	45.65	1.86
tambjamine F	45.65	3.43
tambjamine G	45.65	1.86
tambjamine H	45.65	3.33
tambjamine I	45.65	3.73
tambjamine J	45.65	4.26
tambjamine K	45.65	3.32
5-bromo-8-methoxy-1-methyl-ß-carboline	33.62	4.00
eusynstyelamide E	201.85	1.95

Table 7. continued

TPSA – topological polar surface area: TPSA < 60 Å^2 indicates that the molecules are likely to pass the blood-brain barrier and penetrate cells, while TPSA < 140 Å^2 indicates that molecules are likely to penetrate cells; cLogP – partition coefficient of solubility in *n*-octanol and water: cLogP > 5 indicates that the molecules are less likely to be permeable; the calculations were performed in ChemDraw Ultra 12.0.

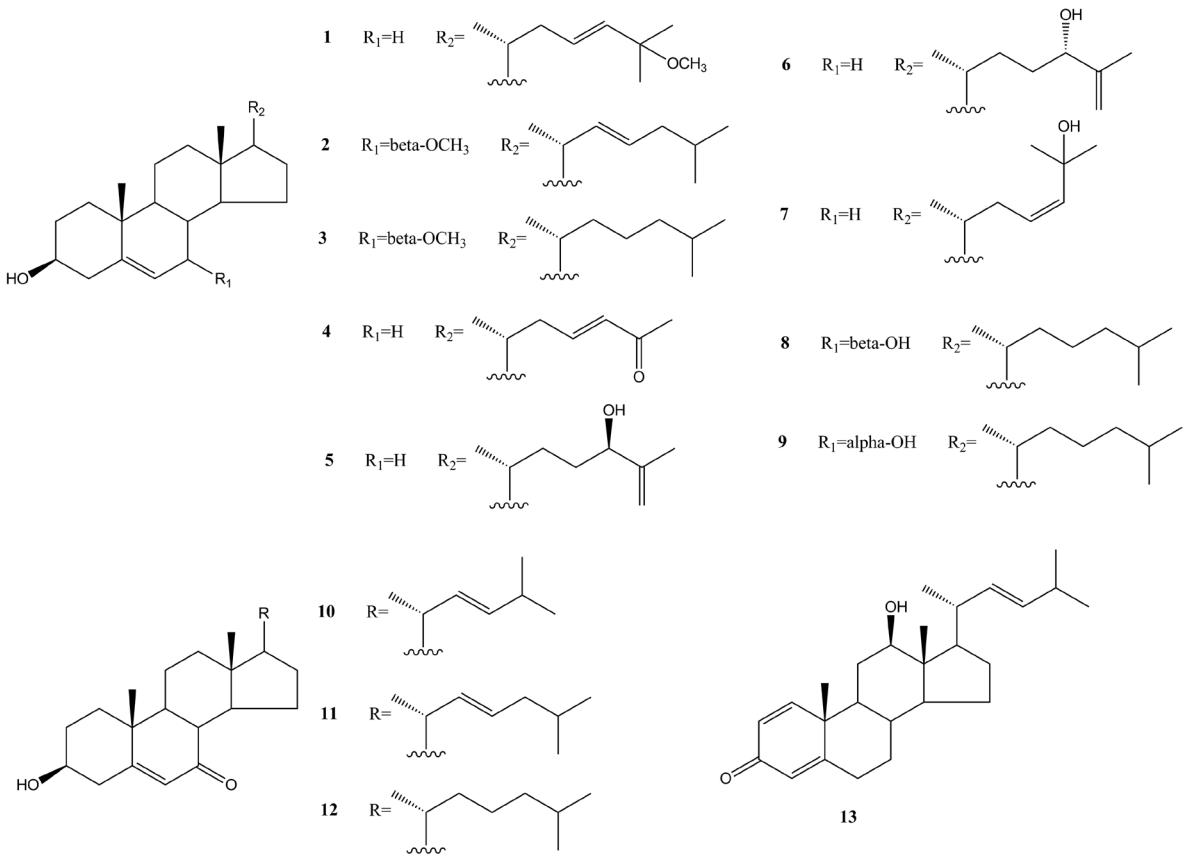


Figure 10. Sterols isolated from the marine bryozoan *Cryptosula pallasiana*: (23*E*)-25-methoxy-cholesta-5,23-dien-3*β*-ol (**1**); (22*E*)-7*β*-methoxy-cholesta-5,22-dien-3*β*-ol (**2**); 7*β*-methoxy-cholest-5-en-3*β*-ol (**3**); (23*E*)-3*β*-hydroxy-27-norcholesta-5,23-dien-25-one (**4**); 24(*R*)-Cholesta-5,25-diene-3*β*,24-diol (**5**); 24(*S*)-Cholesta-5,25-diene-3*β*,24-diol (**6**); (23*Z*)-cholesta-5,23-diene-3*β*,25-diol (**7**); cholest-5-ene-3*β*,7*β*-diol (**8**); cholest-5-ene-3*β*,7*α*-diol (**9**); (22*E*)-3*β*-hydroxy-24-norcholesta-5,22-dien-7-one (**10**); (22*E*)-3*β*-hydroxycholesta-5,22-dien-7-one (**11**); 3*β*-hydroxycholest-5-en-7-one (**12**); (4*E*,22*E*)-12*β*-hydroxy-24-norcholesta-1,4,22-trien-3-one (**13**).

of compounds presented, while the rest are sterols (4) and polyketides (3). Until 2007, only 1% of total marine natural products characterised have been obtained from Bryozoa (also known as sea mats or sea mosses) (Sharp et al. 2007). From the number of new substances published each year covered by this article, it is evident that the field of natural products chemistry of marine bryozoans is slowly developing. It is worth noting that, in a phylum containing over 8000 species, only 7 of these have afforded novel chemicals over the last 9 years. The Bugulidae family (represented with 3 species) have been a source of 10 of the natural products reported here, while Catenicellidae (represented with 1 species) have provided 4.

The most of cited articles were obtained from the *Journal of Natural Products* followed by *Bioorganic & Medicinal Chemistry Letters*. Interestingly, this scientific output is associated with only a few countries, with notable contributions by authors from New Zealand and PR China. The geographical data suggest that the North Atlantic region may be a particularly important location for researchers in the field (Fig. 11).

The number of natural products that have been isolated and characterised from any freshwater animal or plant species, let alone freshwater bryozoans, is relatively small in comparison to marine natural products. The main reason for this large discrepancy is that, of all the water on Earth, less than 3% is freshwater. Furthermore, most of the water that makes up this 3% is either groundwater or locked in ice caps and glaciers. In fact, less than 0.01% of all water on Earth is found in freshwater rivers and lakes, and this has a significant effect on the amount of biodiversity that is found in freshwater compared to marine environments. This lack of biodiversity is reflected in the number of freshwater bryozoan species versus their marine counterparts. To date, only one order of freshwater bryozoans has been described, containing only five extant families, compared to three orders with over 100 families containing mostly marine species (Andersen 2012).

One key questions that has arisen from studies of the phylum Bryozoa relating to the origin of natural products is how many of these are actually produced by Bryozoa and how many are produced by microbial endosymbionts (Sharp et al. 2007). Indeed, there are examples to support the hypothesis that a number of natural products isolated from Bryozoa are not actually bryozoan in origin, such as the bryostatins (Davidson et al. 2001). Although the antitumour activities of bryostatin 1 have made this compound a highly desirable target for clinical applications, it has only been possible to isolate gram amounts from 10000 gallons of field-collected material. Synthesis of bryostatin by a bacterium could potentially be of benefit in the future, as it may facilitate production by microbial fermentation. Problems of raw material supply might therefore be overcome if symbiotic bacteria, or their genes, can be harnessed for the production of specific metabolites. However, there are problems associated with this approach. The inherent difficulties associated with culturing symbionts have resulted in limited progress so far, and the future may lie in developing improved techniques for culturing marine microbes or cloning biosynthetic clusters (Sharp et al. 2007).

Figure 11. Geographical distribution of bryozoan samples reported herein.

Since biochemical and genetic mechanisms involved in the development of tumours differ significantly, the treatment of each type requires specified agents. In the ideal case, isolated metabolites should be tested on a large number of tumours, with the aim of resolving the mechanism of action. However, in practice they are often examined on only several cancer cell lines. As can be seen, much of the research conducted to date on the compounds reported herein has been devoted to leukaemia: indeed, 8 natural products have been shown to be more or less active against the relevant leukaemia cell lines. This type of tumour can occur at any age, but is more frequent in childhood; approximately 24000 deaths has been predicted for the year 2013 (ACS 2013). Taking into account the incidence in lung cancer mortality with over one million deaths per year (Parkin et al. 2005), novel investigations in this direction are needed as well.

A crucial stage in the process of drug discovery and development is drug design, in which the aim is to improve the activity and properties of some lead compound. There are various strategies which can be used to improve the interactions between a drug and its target. Simplification is a strategy which is commonly used on the often complex lead compounds arising from natural sources. The advantages of simpler structures are that they are much easier, quicker and cheaper to synthesise in the laboratory. Usually complex lead compounds obtained from natural sources are impractical to synthesise and have to be extracted from source material, while the problem with available biomass makes this quite a challenging task (Graham 2001).

Over the last 60 years, much effort in natural products chemistry has been focused on marine derived metabolites, the only truly unexplored environment on Earth (Haefner 2003). No marine organisms, including bryozoans, produce final drugs themselves, but make bioactive compounds tightly linked with adaptations to their ecosystem. Natural organic compounds of marine bryozoans and/or their symbiotic microorganisms can inspire researchers (using simplification and computer-aided design) in the search for ever better antitumour agents. In fact, it is reasonable to assume that Neptune chemists might support the well-being of cancer patients in the years to come, regardless of the organisms studied (Pejin, Jovanović et al. 2013).

Abbreviations

GI_{50} – the concentration required to achieve 50% growth inhibition

IC_{50} – the half maximal inhibitory concentration

LC_{50} – the concentration lethal to 50% of the cells

TGI – the concentration required to achieve total growth inhibition

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REFERENCES

Alagille D, Baldwin RM, Tamagnan GD. 2004. Total synthesis of the marine cytotoxic caulibugulones A–D. Tetrahedron Letters. 45(32):6179–6181.

[ACS] American Cancer Society. 2013. Cancer Fact and Figures 2013. Available from: http://www.cancer.org/cancer/leukemia-acute-myeloidaml/detailedguide/leukemia acute-myeloid-myelogenous-key-statistics. 20.11.2013.

Andersen AJC. 2012. Investigations of natural products from Bryozoa inhabiting Aotearoa New Zealand. Master of Science in Chemistry Thesis. The University of Waikato. Hamilton, New Zealand.

Aldrich LN, Stoops SL, Crews BC, Marnett LJ, Lindsley CW. 2010. Total synthesis and biological evaluation of tambjamine K and a library of unatural analogs. Bioorganic & Medicinal Chemistry Letters. 20(17):5207–5211.

Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR. 2005. Marine natural products. Natural Product Reports. 22(1):15–61.

Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR. 2006. Marine natural products. Natural Product Reports. 23(1):26–78.

Blunt JW, Copp BR, Hu W-P, Munro MHG, Northcote PT, Prinsep MR. 2007. Marine natural products. Natural Product Reports. 24(1):31–86.

Blunt JW, Copp BR, Hu W-P, Munro MHG, Northcote PT, Prinsep MR. 2008. Marine natural products. Natural Product Reports. 25(1):35–94.

Blunt JW, Copp BR, Hu W-P, Munro MHG, Northcote PT, Prinsep MR. 2009. Marine natural products. Natural Product Reports. 26(2):170–244.

Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR. 2010. Marine natural products. Natural Product Reports. 27(2):165–237.

Blunt JW, Copp BR, Munro MHG, Northcote PT, Prinsep MR. 2011. Marine natural products. Natural Product Reports. 28(2):196–268.

Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsep MR. 2012. Marine natural products. Natural Product Reports. 29(2):144–222.

Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsep MR. 2013. Marine natural products. Natural Product Reports. 30(2):237–323.

Carbone M, Irace C, Costagliola F, Castelluccio F, Villani G, Calado G, Padula V, Cimino G, Cervera JL, Santamaria R, Gavagnin M. 2010. A new cytotoxic tambjamine alkaloid from the Azorean nudibranch *Tambja ceutae*. Bioorganic & Medicinal Chemistry Letters. 20(8):2668–2670.

Cheng J-F, Lee J-S, Sakai R, Jares-Erijman EA, Silva MV, Rinehart KL. 2007. Myriaporones 1–4, cytotoxic metabolites from the Mediterranean bryozoan *Myriapora truncata*. Journal of Natural Products. 70(3):332–336.

Davidson SK, Allen SW, Lim GE, Anderson CM, Haygood MG. 2001. Evidence for the biosynthesis of bryostatins by the bacterial symbiont "*Candidatus* Enobugula sertula" of the bryozoan *Bugula neritina*. Applied and Environmental Microbiology. 67(10):4531–4537.

DeChristopher BA. 2010. The design, synthesis and biological evalua-

tion of novel protein kinase C ligands based on the bryostatin and diacylglyserol scaffolds. Dissertation for the degree of Doctor of Philosophy. Stanford University. Stanford (CA), USA.

De Rosa S, Pejin B, Tommonaro G. 2008. Anti-inflammatory marine natural products with antipsoriatic effects. Book of Abstracts of the 4th International Conference on Oxidative Stress in Skin Medicine and Biology. Andros, Greece. p. 53.

Faulkner DJ. 1998. Marine natural products. Natural Product Reports. 15(2):113–158.

Graham PL. 2001. An Introduction to Medicinal Chemistry. 2nd edition. New York: Oxford University Press Inc.

Haefner B. 2003. Drugs from the deep: marine natural products as drug candidates. Drug Discovery Today. 8(12):536–544.

Lopanik N, Gustafson KR, Lindquist N. 2004. Structure of bryostatin 20: a symbiont produced chemical defense for larvae of the host bryozoan, *Bugula neritina*. Journal of Natural Products. 67(8):1412–1414.

Milanowski DJ, Gustafson KR, Kelley JA, McMahon JB. 2004. Caulibugulones A-F, novel cytotoxic isoquinoline quinones and iminoquinones from the marine bryozoan *Caulibugula intermis*. Journal of Natural Products. 67(1):70–73.

O'Malley MM, Damkaci F, Kelly TR. 2006. Synthesis of pterocellin A. Organic Letters. 8(12):2651–2652.

Parkin DM, Bray F, Ferlay J, Pisani P. 2005. Global cancer statistics. CA: A Cancer Journal for Clinicians. 55(2):74–108.

Pejin B. 2012. Thio-avarol derivatives and Alzheimer's disease. Proceedings of Serbia-Italia Status and Perspectives of the Scientific and Technological Bilateral Cooperation. Belgrade, Serbia. p. 131–134.

Pejin B, Glamoclija J, Ciric A, Radotic K, Vajs V, Tesevic V, Hegedis A, Karaman I, Horvatovic M, Sokovic M. 2012. Antimicrobial activity of the freshwater bryozoan *Hyalinella punctata* (Hanckock, 1850). Digest Journal of Nanomaterials and Biostructures. 7(3):1021–1026.

Pejin B, Iodice C, Tommonaro G, De Rosa S. 2008. Synthesis and biological activities of thio-avarol derivatives. Journal of Natural Products. 71(11):1850–1853.

Pejin B, Iodice C, Tommonaro G, Bogdanovic G, Kojic V, De Rosa S. 2014. Further *in vitro* evaluation of cytotoxicity of the marine natural product 4'-leucine-avarone. Natural Product Research. 28(5):347–350.

Pejin B, Jovanović KK, Mojović M, Savić A. 2013. New and highly potent antitumor natural products from marine-derived fungi: covering the period from 2003 to 2012. Current Topics in Medicinal Chemistry. 13(21):2745–2766.

Pejin B, Stanimirovic B, Djordjevic N, Hegedis A, Karaman I, Horvatovic M, Radotic K. 2013. *In vitro* radioprotective activity of the bryozoan *Hyalinella punctata*. Asian Journal of Chemistry. 25(8):4713–4714.

Pejin B, Stosic-Grujicic S, Bogdanovic G, Hegedis A, Karaman I, Stojanovic I, Nikolic I, Kojic V, Horvatovic M, Radotic K. 2013. *In vitro* evaluation of the immunomodulatory and anticarcinogenic activity of the freshwater bryozoan *Hyalinella punctata* methanolic extract. Digest Journal of Nanomaterials and Biostructures. 8(1):187–195.

Pejin B, Vitale RM, Tommonaro G, Iodice C, De Rosa S, Amodeo P. 2011. Development of new semisynthetic inhibitors of acetylcholinesterase (AChE) as potential anti Alzheimer drugs by an integrated computational-experimental approach. Science and Supercomputing in Europe. p. 93.

Prinsep MR. 2001. Novel Alkaloids from Marine Bryozoans. In: Fingerman M, Nagabhushanam R, editors. Recent Advances in Marine Biotechnology. Vol. 6. Bio–Organic Compounds: Chemistry and Biomedical Applications. New Hampshire: Science Publishers Inc. p. 165–186.

Prinsep MR. 2008. Further pterocellins from the New Zealand marine bryozoan *Pterocella vesiculosa*. Journal of Natural Products. 71(1):134–136.

Prinsep MR, Yao B, Nicholson BK, Gordon DP. 2004. The pterocellins, bioactive alkaloids from the marine bryozoan *Pterocella vesiculosa*. Phytochemistry Reviews. 3(3):325–333.

Ryland JS. 2005. Bryozoa: an introductory overview. In: Wöss E, editor. Moostiere (Bryozoa). Linz, Austria: Biologiezentrum der O.Ö. Landesmuseum. p. 9–20.

Sharp JH, Winson MK, Porter JS. 2007. Bryozoan metabolites: an ecological perspective. Natural Product Reports. 24(4):659–673.

Sladić D, Gašić MJ. 2006. Reactivity and biological activity of the marine sesquiterpene hydroquinone avarol and related compounds from sponges of the order Dictyoceratida. Molecules. 11(1):1–33.

Suffiness M, Pezzuto JM. 1990. Assays related to cancer drug discovery. In: Hostettmamann, K, editor. Methods in plant biochemistry: assays for bioactivity. London, UK: Academic Press. p. 71–133.

Tadesse M, Tabudravu JN, Jaspars M, Strøm MB, Hansen E, Andersen JH, Kristiansen PE, Haug T. 2011. The antibacterial ent-eusynstyelamide B and eusynstyelamides D, E, and F from the Arctic bryozoan *Tegella cf. spitzbergensis*. Journal of Natural Products. 74(4):837–841.

Tian X-R, Tang H-F, Li Y-S, Lin H-W, Chen X-L, Ma N, Yao M-N, Zhang P-H. 2011. New cytotoxic oxygenated sterols from the marine bryozoan *Cryptosula pallasiana*. Marine Drugs. 9(2):162–183.

Till M, Prinsep MR. 2009. 5-bromo-8-methoxy-1-methyl-ß-carboline, an alkaloid from the New Zealand marine bryozoan *Pterocella vesiculosa*. Journal of Natural Products. 72(4):796–798.

Wipf P, Joo B, Nguyen T, Lazo JS. 2004. Synthesis and biological evaluation of caulibugulones A–E. Organic & Biomolecular Chemistry. 2(15):2173–2174.

Yang F, Zhang H-J, Chen J-T, Tang H-F, Piao S-J, Chen W-S, Lin H-W. 2011. New cytotoxic oxygenated sterols from marine bryozoan *Bugula neritina*. Natural Product Reserch. 25(16):1505–1511.

Yao B, Prinsep MR, Nicholson BK, Gordon DP. 2003. The pterocellins, novel bioactive alkaloids from the marine bryozoan *Pterocella vesiculosa*. Journal of Natural Products. 66(8):1074–1077.