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Review

Bioactive Constituents of Marine Sponges of the Genus *Spongosorites*

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Abstract This report reviews the literatures on chemical constituents of marine sponges of the genus *Spongosorites* and also highlights our own research. Specific biological activities of the metabolites from these sponges include: cytotoxic, antitumor, antibacterial, antifungal, antiviral, anti-inflammatory, and other pharmacological activities.

Key words : Marine sponge, *Spongosorites*, indole alkaloids, biological activities, new drug leads

Introduction

The marine environment covers 70% of the earth's surface, and there inhabit more than 500,000 species of organisms, which cover 80% of living beings on the earth. Living in relatively closed surrounding, marine organisms have developed, in their process of evolution, a distinct metabolism. That is to say, marine organisms are abounded with many structurally unique and biologically active substances. Among marine organisms, sponges appear to be one of the richest phyla, which yield bioactive secondary metabolites [6]. As one of the rapidly growing groups of sponge metabolites, various indole alkaloids, especially bisindole alkaloids reported from marine sponges over the past few years, include topsentins, nortopsentins, dragmacidins, and hamacanthins. Some of these metabolites exhibit potent and diverse bioactivities such as cytotoxic, antitumor, antiviral, antifungal, antiinflammatory, and other pharmacological activities. At least more than 32 species of sponges belong to the genus *Spongosorites* (class:*Demospongiae* order: *Halichondrida* family: *Halichondriidae*) [40], and most of the sponge-derived indole alkaloids were reported from this genus of sponges, along with a few other classes of metabolites. In this report we will review the chemical constituents from marine sponges of the genus *Spongosorites* and their biological activities.

Chemical constituents

Since a bisindole alkaloid topsentin and its analogues were reported from *Topsentia genitrix* (*Spongosorites genitrix*) about three decades ago, numerous compounds, in particular bisindole alkaloids have been isolated from sponges of the genus *Spongosorites*. They include bisindole alkaloids such as topsentins, nortopsentins, dragmacidins and hamacanthins, monoindole alkaloids, and polyketide. Some of these metabolites exhibit potent and diverse bioactivities such as cytotoxic, antitumor, antiviral, antifungal, antiinflammatory activities, and other pharmacological activities. The structures of these compounds and their bioactivities are listed in Fig. 1-2, and Tables 1-4.

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Bisindole alkaloids

Topsentin class

Topsentins

Topsentins are bisindoles with an imidazolyl methanone moiety as a linker between two indole rings. In the course of the search for compounds involved in the defense mechanism of sponges, three bisindole alkaloids, topsentin-A (deoxytopsentin) (**1**), -B1 (= topsentin) (**2**), and -B2 (bromotopsentin) (**3**) had been isolated from the Mediterranean shallow-water sponge *Topsentia genitrix* (*Spongosorites genitrix*) [6] . This is the first report about bisindole alkaloids topsentins. Later, topsentin and bromotopsentin were again isolated from Caribbean deep-sea sponges of the genus *Spongosorites* [38]. Isobromotopsentin (**4**), was isolated from a deep-water marine sponge *Spongosorites* sp. colleted off the southern Australian coast [31]. In 1999, two new brominated compounds bromodeoxytopsentin (**5**) and isobromodeoxytopsentin (**6**) were isolated from the shallow-water sponge *Spongosorites genitrix* collected from Jeju Island, Korea [36]. In our search for cytotoxic metabolites, six bisindole alkaloids of the topsentin class (**1**–**3**, and **5**–**7**), including one new brominated compound dibromodeoxytopsentin (**7**), were isolated from a shallow-water sponge *Spongosorites* sp. [3]. It is noteworthy that broadening or doubling of ^{1}H NMR signals of most reported topsentins (**1**–**3**, and **5**–**7**) were observed in neutral solutions (CD_3OD, acetone-d_6, or DMSO-d_6) due to slow interconversion of imidazolylmethanone tautomers and/or rotamers. This phenomenon is likely a result of a combination of tautomerism and rotational isomerism of the imidazolylmethanone, as there expected to be potential hydrogen bonding between the ketone carbonyl and imidazole NH [3]. Although deoxytopsentin (**1**), topsentin (**2**), and bromotopsentin (**3**) were originally isolated as a mixture of tautomeric forms with the favored tautomer being protonated at N-3, in the absence of suitable spectroscopic evidence, the tautomeric form displayed for isobromotopsentin (**4**) was arbitrarily assigned [31].

Spongotines (3,4-dihydrotopsentins)

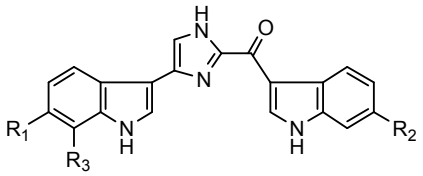
Spongotines (3,4-dihydrotopsentins) are dihydro derivatives of topsentins. In continuation of our search for cytotoxic metabolites from the same sponge which yielded dibromodeoxytopsentin (**7**), three new bisindole alkaloids of 3,4-dihydrotopsentin, spongotines A-C (**8**–**10**), were isolated [4]. Broadening or doubling of ^{1}H NMR signals of these spongotine class of compounds (**8**–**10**) were also observed in neutral solutions (CD_3OD, acetone-d_6, or DMSO-d_6). Compound with the same structure as **8** was previously reported as 4,5-dihydro-6″-deoxybromotopsentin [38]. But the upfield shift of the ketone carbonyl carbon (δ_C 159.12) for the given structure [38], compared to those of topsentin (**2**) (δ_C 173.58) and bromotopsentin (**3**) (δ_C 175.32) in the same literature, could not be properly explained. On the contrary, the ^{13}C NMR data of the carbonyl carbon (δ_C 159.12) and the imino carbon (δ_C 160.49) in the given structure [38] were rather close to those of hamacanthin B (**27**) that were defined thereafter [20] and followed by synthesis [25]. Morris *et al*. also proposed to revise the imidazole/ketone functionality in topsentin C [30] and 4,5-dihydro-6″-deoxybromotopsentin to a dihydropyrazone ring, since their carbonyl resonance (δ_C 157.8 and 159.12, respectively) were close to those of lactam carbonyl carbons of the 2-ketodehydropiperazine ring [41]. As mentioned above, broadening or doubling of ^{1}H NMR signals of compound 8 was observed in neutral solution (DMSO-d_6). Such ^{1}H NMR characteristics of imidazolylmethanone tautomerism was not mentioned for the reported 4,5-dihydro-6″-deoxybromotopsentin [38], while such phenomena were described for other two topsentin derivatives (**2**, **3**) in the same literature[38]. Topsentin-d (4,5-dihydro-deoxytopsentin) (**11**) was isolated from the methanolic extract of *Topsentia genitrix* (*Spongosorites genitrix*), and this derivative was sensitive to air and was transformed into deoxytopsentin (**1**) on standing [7].

Other topsentins

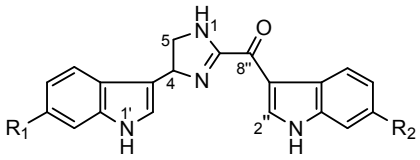
More complex topsentin class of compounds, topsentin D (**12**, incidentally given the same trivial name, but different from topsentin-d, **11**) and topsentin E (**13**), which have 2-aminoimidazole moiety with different side chains as linkers to imidazole ring on the topsentins, were isolated from Bahamas deep-water sponge *Spongosorites* sp. [42].

Nortopsentin class

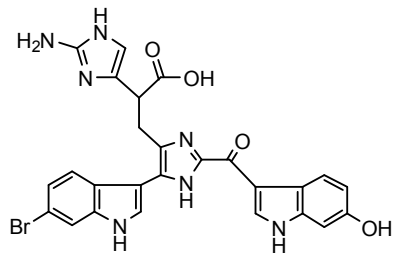
Nortopsentins A-C (**14**–**16**), which lack the central ketone observed in topsentins, have been isolated from the Caribbean deep-sea sponge *Spongosorites ruetzleri*. The unique imidazolediylbis(indole) skeleton of the



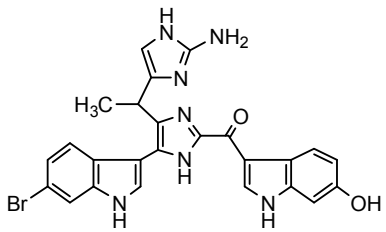
1 R_1 = H, R_2 = H, R_3=H deoxytopsentin
2 R_1 = H, R_2 = OH, R_3=H topsnetin
3 R_1 = Br R_2 = OH, R_3=H bromotopsentin
4 R_1 = H, R_2 = Br, R_3=OH isobromotopsentin
5 R_1 = Br, R_2 = H, R_3=H bromodeoxytopsentin
6 R_1 = H, R_2 = Br, R_3=H isobromodeoxytopsentin
7 R_1 = Br, R_2 = Br, R_3=H dibromodeoxytopsentin



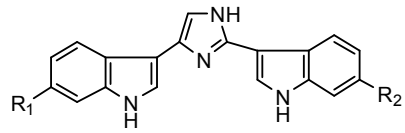
8 R_1 = Br, R_2 = H spongotine A
9 R_1 = H, R_2 = Br spongotine B
10 R_1 = Br, R_2 = Br spongotine C
11 R_1 = H, R_2 = H topsentin-d



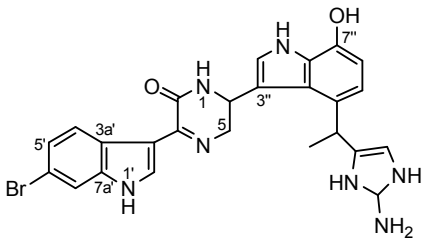
12 topsentine D



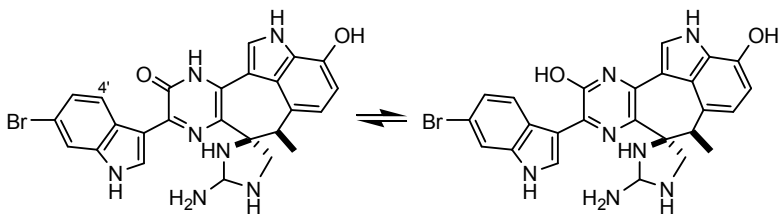
13 topsentine E



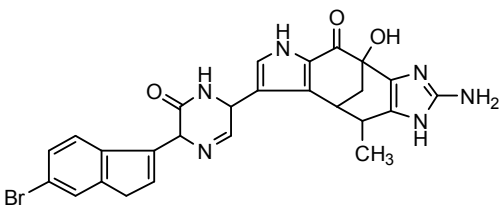
14 R_1 = Br, R_2 = Br nortopsentin A
15 R_1 = Br, R_2 = H nortopsentin B
16 R_1 = H, R_2 = Br nortopsentin C



17 dragmacidin D



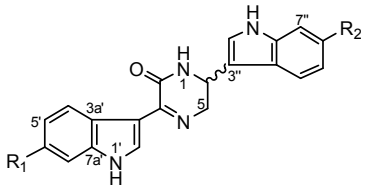
18 dragmacidin E



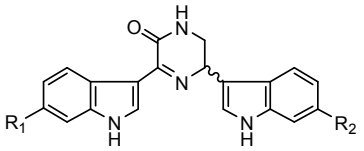
19 dragmacidin F

Fig. 1. Isolated compounds of marine sponges of the genus *Spongosorites*.

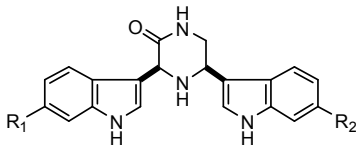
nortopsentins demonstrates a new condensation process in tryptophane metabolism [35]. It is noteworthy that the doubling of NMR signals in neutral solutions, due to slow interconversion of imidazolylmethanone tautomers in the topsentins, was not observed for the imidazole system of the nortopsentins, indicative of rapid proton transfer equilibrium between N-1 and N-3 [35].



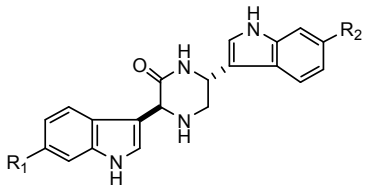
20 R_1 = H, R_2 = H (*S*)-6',6"-didebromohamacanthin A
21 R_1 = Br, R_2 = H (*R*)-6"-debromohamacanthin A
22 R_1 = H, R_2 = Br (*R*)-6'-debromohamacanthin A
23 R_1 = Br, R_2 = Br (*S*)-hamacanthin A



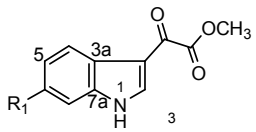
24 R_1 = H, R_2 = H (*S*)-6',6"-didebromohamacanthin B
25 R_1 = Br, R_2 = H (*R*)-6"-debromohamacanthin B
26 R_1 = H, R_2 = Br (*R*)-6'-debromohamacanthin B
27 R_1 = Br, R_2 = Br (*S*)-hamacanthin B



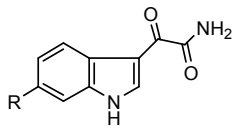
28 R_1 = H, R_2 = H (3*S*,5*R*)-6',6"-didebromo-3,4-dihydrohamacanthin B
29 R_1 = Br, R_2 = H (3*S*,5*R*)-6"-debromo-3,4-dihydrohamacanthin B
30 R_1 = H, R_2 = Br (3*S*,5*R*)-6'-debromo-3,4-dihydrohamacanthin B
31 R_1 = Br, R_2 = Br (3*S*,5*R*)-3,4-dihydrohamacanthin B



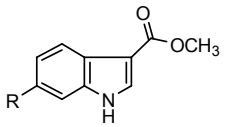
32 R_1 = Br, R_2 = H (3*S*,6*R*)-6"-debromo-3,4-dihydrohamacanthin A
33 R_1 = H, R_2 = Br (3*S*,6*R*)-6'-debromo-3,4-dihydrohamacanthin A
34 R_1 = H, R_2 = Br (3*S*,6*R*)-3,4-dihydrohamacanthin A



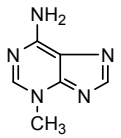
35 R = H
36 R =Br
37 R=OH



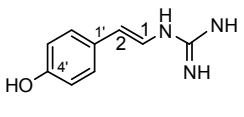
38 R = H
39 R = Br



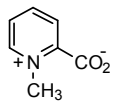
40 R = OH
41 R = H



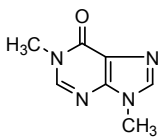
42 3-methyladenine



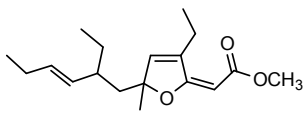
43



44 homarine



45 1,9-dimethylhypoxanthine



46 spongosoritin A

Fig. 2. Isolated compounds of marine sponges of the genus *Spongosorites* (continued).

Dragmacidin class

Dragmacidins incorporate piperazine moiety as a linker between two indole rings. Dragmacidin D (**17**) has been isolated from a deep-sea sponge *Spongosorites* sp. [9,41]. Dragmacidin E (**18**) has been isolated from a southern Australian deep-water sponge *Spongosorites* sp.[9]. Dragmacidin F (**19**), which has an unprecedented carbon skeleton that is very likely derived from cyclization of a partially oxidized form of dragmacidin D, was isolated from a *Spongosorites* sp. [42]. Dragmacidin F was also isolated from the Mediterranean sponge *Halicortex* sp. [13]. Although dragmacedin D is pre-

sented in the literature exclusively in the pyrazinone tautomeric form, treatment of dragmacedin D with acid (HCl or TFA) results in the formation of a deep red color with the expected bathochromic shift in the UV spectrum. This may be due to the formation of a more planar fully conjugated pyrazine. In the same solvents (acetonitrile and DMSO without acid) the compound appears to be greenish in color, suggesting a different nonplanar conformation. In the case of dragmacidin E, a ROESY correlation between H-4′ and an exchangeable proton is only possible for the pyrazine tautomer (the exchangeable proton corresponding to the pyrazine OH). Whether dragmacidin E exists exclusively in the pyrazine form or in tautomeric equilibrium with the pyrazinone form remains unresolved [9]. It is speculated that the addition of acid to the NMR sample diminished the broadening effect of pyrazinone/ pyrazine tautomeric equilibrium, thereby facilitating measurement of the carbon resonances. This conclusion was further supported by a hypsochromic shift in the UV spectrum (427–410 nm) on addition of NaOH [9].

Hamacanthin class

Hamacanthins are derivatives with a pyrazinone or a piperazinone moiety as a linker between two indole rings.

Hamacanthins

Hamacanthins A (**23**) and B (**27**) were isolated from Madeira deep-water sponge *Hamacantha* sp. [20] without stereochemical determination. The stereochemistries of these compounds were defined by synthesis [23-25]. We isolated five new (**20**–**22**, **24**, and **26**), and three known (**23**, **25**, **27**) hamacanthin class of compounds[3,4]. Meanwhile, another Korean research group reported one new debromohamacanthin B (**25**), and three known hamacanthins (**23**, **26** and **27**) from a shallow water sponge *Spongosorites* sp. [32].

Dihydrohamacanthins

Dihydrohamacanthins are dihydro derivatives of hamacanthins. The first report on the isolation of dihydrohamacanthins with only relative configuration was from a Mediterranean shallow water sponge *Rhaphisia lacazei* [12]. The absolute configurations of these compounds were determined by synthesis [18,26]. We isolated one new (**28**), and six known (**29**–**34**) dihydrohamacanthins from the same sponge specimen mentioned above [3,4].

Monoindole alkaloids

In continuation of our search for cytotoxic metabolites from the same *Spongosorites* sponge, six new (**35**–**40**), and one known (**41**) monoindole alkaloids were isolated [5]. Compound **35** was previously known as an intermediate in the synthesis of natural products, such as didemnimides A and B [21], rebeccamycin, and 11-dechlororebeccamycin [16]. Compound **36** was known as an intermediate in the synthesis of some marine natural products, such as didemnimides A and B [21]. Compound **38** was known as an intermediate in the synthesis of some marine natural products, such as arborescidines and dihydrohamacanthins [29]. Compound **39** was also reported as an intermediate in the synthesis of some natural products, such as arborescidines and dihydrohamacanthins [29]. Compound **40** was known as an intermediate in the organic synthesis of a 5-HT_4 receptor antagonist [17]. Compounds **35**, **36**, **38**–**40** have not been previously reported from a natural source. Compound **41** was previously reported from marine-derived bacteria [44] and fungi [22] and red alga [2].

Other alkaloids

In addition to indole alkaloids, there are some other alkaloids isolated from *Spongosorites* sp. A purine derivative, 3-methyladenine (**42**), has been reported from the Mediterranean shallow-water sponge *Topsentia genitrix* (*Spongosorites genitrix*) [37]. An alkaloid with guanidine functionality (**43**) has been isolated from an Australian shallow-water sponge *Spongosorites* sp, along with a known marine metabolite homarine (**44**) [39]. A methylated purine, 1,9-dimethylhypoxanthine (**45**), was isolated from an Australian deep-sea sponge *Spongosorites* sp. [10].

Polyketide

A new polyketide, spongosoritin A (**46**) was isolated from a Fijian shallow-water marine sponge *Spongosorites* sp. [11]. This is the only non-alkaloid compound reported from *Spongosorites*.

Biological activities

Cytotoxicity

The methanolic extract of the sponge *Topsentia genitrix (Spongosorites genitrix)* was weakly toxic to the

fish *Lebistes reticulatus* (LD_{50}<50 mg/L) and to mice (LD_{50}: 10 mg/kg), kills dissociated cells of the freshwater sponge *Ephydatia fluviatilis* before early aggregation (<100 mg/L), and shows antibacterial activities [38]. Three bisindole alkaloids, topsentin-A (deoxytopsentin) (**1**), -B1 (topsentin) (**2**), and −B2 (bromotopsentin) (**3**), isolated from this sponge might be partially responsible for these activities [6]. Topsentin (**2**) had *in vitro* activity against P388 (Murine leukemia cell line) (IC_{50} 3.0 μg/mL) and human tumor cells (HCT-8, A-549, T47D, 20μg/mL) and *in vivo* activity against P388 leukemia (%T/C=137, 150 mg/kg) and B16 melanoma (%T/C=144, 37.5 mg/kg) [38]. Effects of 30 μM topsentin (1-h exposures) on incorporation of radiolabeled precursors by P388 cells indicated inhibition of DNA synthesis (91%), and to a lesser extent RNA synthesis (57%), whereas synthesis of protein was unaffected (0%). Topsentin also interacted with DNA and bound to DNA in the minor groove [8]. Bromodeoxytopsentin (**5**) and isobromodeoxytopsentin (**6**) exhibited moderate cytotoxicity against the human leukemia cell line K-562 (LC_{50} 0.6 and 2.1 μg/mL, for 5 and 6, respectively) [36]. Topsentin (**2**) and bromotopsentin (**3**) showed *in vitro*

Table 1. Cytotoxicity ($IC_{50:}$ μg/mL)

Compound		NSCLC-N6	AGS	L1210	BC	HepG2	P388	K-562	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
Deoxytopsentin	(**1**)		1.30	7.40	10.70	3.30			>30	>30	>30	>30	26.18
Topsentin	(**2**)	12.00					3.00		>30	>30	>30	>30	13.33
Bromotopsentin	(**3**)	6.30	1.40	6.90	>20	>20	7.00		>30	28.14	7.02	14.99	>30
Bromodexoytopsentin	(**5**)		3.30	1.10	>20	>20		0.60	>30	>30	>30	>30	11.48
Isobromodeoxytopsentin	(**6**)							2.10	12.30	8.70	4.54	5.51	6.38
Spongotine A	(**8**)								6.82	3.71	5.04	7.22	9.80
Spongotine C	(**10**)								5.22	4.81	4.82	5.16	4.88
Nortopsentin A	(**14**)						7.60						
Nortopsentin B	(**15**)						7.80						
Nortopsentin C	(**16**)						1.70						
Dragmacidin D	(**17**)						1.40		4.40				
(*S*)-6′,6″-Didebromo-hamacanthin A	(**20**)								8.30	11.50	5.00	>10.00	4.10
(*R*)-6″-Debromo-hamacanthin A	(**21**)								5.61	4.20	4.73	4.12	3.58
(*R*)-6′-Debromo-hamacanthin A	(**22**)		7.50	9.00	>20	>20			>30	>30	>30	>30	26.91
Hamacanthin A	(**23**)		3.90	3.00	12.50	12.50			4.49	5.24	5.44	5.60	4.66
(*R*)-6′,6″-Didebromohamacanthin B	(**24**)								11.70	12.60	13.70	>10.00	4.79
(*R*)-6″-Debromohamacanthin B	(**25**)								7.86	7.85	7.71	9.21	6.31
(*R*)-6′-Debromohamacanthin B	(**26**)		7.50	7.70	>20	>20			3.71	8.50	7.60	8.30	4.20
Hamacanthin B	(**27**)		5.10	6.70	>20	>20			2.14	2.61	1.59	2.93	1.52
(3*S*,5*R*)-6′,6″-Didebromo-3,4-Dihydrohamacanthin B	(**28**)								>10.00	9.64	>10.00	>10.00	>10.00
(3*S*,5*R*)-6″-Debromo-3,4-dihydrohamacanthin B	(**29**)								9.67	5.67	>10.00	9.74	>10.00
(3*S*,5*R*)-6′-Debromo-3,4-dihydrohamacanthin B	(**30**)								4.20	6.00	7.10	6.80	6.30
(3*S*,5*R*)-3,4-Dihydrohamacanthin B	(**31**)								3.41	3.62	3.85	3.22	2.83
(3*S*,6*R*)-6″-Debromo-3,4-dihydrohamacanthin A	(**32**)								>10.00	4.92	>10.00	>10.00	>10.00
(3*S*,6*R*)-3,4-Dihydrohamacanthin A	(**33**)		6.30	5.30	>20	>20			8.28	8.03	9.14	6.88	5.35
(3*S*,6*R*)-6′-debromo-3,4-Dihydrohamacanthin A	(**34**)								7.50	12.10	13.10	19.10	6.30
Methyl indole-3-carboxylate	(**41**)								24.10	13.40	15.20	26.20	4.85

$NSCLC\text{-}N_{6:}$ human broncopulmonary cancer cells; AGS: human gastric adenocarcinoma; L1210: mouse lymphocytic leukemia; BC: human breast cancer; HepG2: hepatoma; P388: Murine leukemia cell line; K-562: human leukemia cell line; A549: human lung cancer; SK-OV-3: human ovarian cancer; SK-MEL-2: human skin cancer; XF498: human CNS cancer; HCT: human colon cancer.

antiproliferative activity against human broncopulmonary cancer cells (NSCLC- N6) with an IC_{50} of 12 and 6.3 μg/mL [12]. Nortopsentins A (**14**), B (**15**), and C (**16**) exhibited *in vitro* cytotoxicity against P388 cells with an IC_{50} of 7.6, 7.8, and 1.7 μg/mL, respectively [35]. Dragmacidin D (**17**) inhibits the *in vitro* growth of the P388 murine and A549 human lung tumor cell lines with IC_{50} of 1.4 and 4.4 μg/mL, respectively [42]. Deoxytopsentin (**1**), bromotopsentin (**3**), bromodeoxytopsentin (**5**), 6′-debromohamacanthin A (**22**), hamacanthin A (**23**), (*R*)-6′-debromohamacanthin B (**26**), and *trans*-3,4-dihydrohamacanthin A (*3S, 6R*-3,4-dihydrohamacanthin A)(**34**) exhibited moderate cytotoxicity against AGS (human gastric adenocarcinoma), L1210 (mouse lymphocytic leukemia), BC (human breast cancer), and HepG2 (hepatoma) cancer cell lines at concentrations between 1.1 and >20 μg/mL [33]. Deoxytopsentin (**1**), topsentin (**2**), bromotopsentin (**3**), bromodeoxytopsentin (**5**), isobromodeoxytopsentin (**6**), spongotine A (**8**), spongotine C (**10**), (*S*)-6′,6″-didebromohamacanthin A (**20**), (*R*)-6″-debromohamacanthin A (**21**), (*S*)-hamacanthin A (**23**), (*R*)-6′,6″-didebromohamacanthin B (**24**), (*R*)-6′-debromohamacanthin B (**26**), (*S*)-hamacanthin B (**27**), (3*S*,*5R*)-6″-debromo-3,4-dihydrohamacanthin B (**29**), (3*S*,*5R*)-6′-debromo-3,4-dihydrohamacanthin B (**30**), (*3S*,*6R*)-6″-debromo-3,4-dihydrohamacanthin A (**32**), (*3S*,*6R*)-6′-debromo-3,4-dihydrohamacanthin A (**33**), and indole-3-carboxylic acid methyl ester (**41**) showed moderate to significant cytotoxicity against five human tumor cell lines, A549 (human lung cancer); SK-OV-3 (human ovarian cancer); SK-MEL-2 (human skin cancer); XF498 (human CNS cancer) and HCT 15 (human colon cancer) (Table 1) [3-5].

Antimicrobial activity

Antibacterial: Dragmacidin D (**17**) has antibacterial activity against *Escherichia coli* with a MIC of 15.6 μg/mL; *Bacillus subtilis*, 3.1 μg/mL; *Pseudomonas aeruginosa*, 62.5 μg/mL; *Candida albicans*, 15.6 μg/mL; *Cryptococcus neoformans*, 3.9 μg/mL[41]. Dragmacidin D (**17**) and dragmacidin E (**18**) displayed MICs against *Escherichia coli* at 16 and 22 ppm and against *C. albicans* of 20 and 36 ppm, respectively [9]. Deoxytopsentin (**1**), bromotopsentin (**3**), bromodeoxytopsentin (**5**), 6′-debromohamacanthin A (**22**), hamacanthin A (**23**), (*R*)-6′-debromohamacanthin B (**26**), and *trans*-3,4-dihydrohamacanthin A (*3S*,*6R*-3,4-dihydrohamacanthin A) (**34**) exhibited antibacterial activity against three gram-negative bacteria and eight gram-positive bacteria[33]. 6′-Debromohamacanthin A (**22**), (*S*)-6′-debromohamacanthin B (**26**), *cis*-3,4-dihydrohamacanthin B (3*S*,*5R* -3,4-dihydrohamacanthin B) (**31**), and *3S*,*6R*-6″-debromo-3,4-dihydrohamacanthin A (**32**) showed weak antibacterial activity (MIC values <12.5 μg/mL) against seven of the twenty clinically isolated methicillin-resistant strains: *Streptococcus pyogenes* 308A, *S. pyogenes* 77A, *S. aureus* SG 511, *S. aureus* 285, *S. aureus* 503, *Escherichia coli* DC 2 and *Klebsiella oxytoca* 1082 E (Tables 2 and 3) [3,4].

Antiviral: Topsentin (**2**) and bromotopsentin (**3**) showed in vitro activity against HSV-1, *Vesicular stomatitis* virus (VSV), and the corona virus A-59 [38].

Table 2. Antibacterial activity (MIC: μg/mL)

Compound		B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8	B_9	B_{10}	B_{11}	B_{12}	B_{13}	B_{14}	B_{15}	B_{16}
Deoxytopsentin	(**1**)	6.25	6.25	3.12	3.12	12.5	12.5	12.5	6.25	>100	6.25	12.5					
Bromotopsentin	(**3**)	12.5	12.5	25	12.5	25	25	25	25	>100	25	50					
Bromodexoytopsentin	(**5**)	12.5	50	50	50	50	100	50	50	>100	25	100					
Dragmacidin D	(**17**)									>100			15.6	3.1	62.5	15.6	3.9
Dragmacidin E	(**18**)												22.0			36.0	
(*R*)-6'-Debromo-hamacanthin A	(**22**)	6.25	6.25	6.25	6.25	6.25	6.25	12.5	12.5		3.12	12.5					
Hamacanthin A	(**23**)	1.56	1.56	3.12	3.12	3.12	3.12	3.12	3.12	>100	0.78	3.12					
(*R*)-6'-Debromo-hamacanthin B	(**26**)	50	50	50	50					>100	50	100					
Hamacanthin B	(**27**)	6.25	3.12	6.25	3.12	6.25	6.25	3.12	6.25	>100	3.12	6.25					
(*3S,6R*)-3,4-Dihydrohamacanthin A	(**28**)	6.25	6.25	12.5	12.5	12.5	12.5	25	25	>100	12.5	25					

B_1: *Bacillus subtilis* ATCC 6633; B_2: *Micrococcus leuteus* IFO 12708, B_3: *Staphylococcus aureus* ATCC 6538p; B_4: *S. aureus* 13709; B_5: *S. aureus* 29213; B_6: Methicillin-resistant (MRSA) ATCC 43300; B_7: MRSA ATCC 700787; B_8: MRSA ATCC 700788; B_9: *Escherichia coli* ATCC 35218; B_{10}: *Proteus vulgaris* ATCC 3851; B_{11}: *Salmonella typhimurium* ATCC 14028; B_{12}: *Escherichia coli*; B_{13}: *Bacillus subtilis*; B_{14}: *Pseudomonas aeruginosa*; B_{15}: *Candida albicans*; B_{16}: *Cryptococcus neoformans*.

Table 3. Antibacterial activity (continued) (MIC: μg/mL)

Compound		B_{17}	B_{18}	B_{19}	B_{20}	B_{21}	B_{22}	B_{23}	B_{24}	B_{25}	B_{26}	B_{27}
Spongotine B	**(9)**	25.0	25.0	>25.0	25.0	25.0	25.0	25.0	>25.0	>25.0	25.0	25.0
(*S*)-6′,6″-Didebromo-hamacanthin A	**(20)**	25.0	25.0	>25.0	>25.0	>25.0	>25.0	>25.0	25.0	25.0	>25.0	>25.0
(*R*)-6″-Debromo-hamacanthin A	**(21)**	25.0	>25.0	>25.0	>25.0	25.0	>25.0	>25.0	>25.0	25.0	>25.0	>25.0
(*R*)-6′-Debromo-hamacanthin A	**(22)**	12.5	12.5	25.0	12.5	12.5	12.5	>25.0	>25.0	12.5	25.0	12.5
(*R*)-6′,6″-Didebromo-hamacanthin B	**(24)**	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	25.0	25.0	>25.0	>25.0
(*R*)-6′-Debromohamacanthin B	**(26)**	6.3	12.5	>25.0	>25.0	12.5	12.5	25.0	>25.0	25.0	25.0	25.0
(3*S*,5*R*)-6″-Debromo-3,4-dihydrohamacanthin B	**(29)**	25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0
(3*S*,5*R*)-6′-Debromo-3,4-dihydrohamacanthin B	**(30)**	25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	25.0	25.0	>25.0	>25.0
(3*S*,5*R*)-3,4-Dihydro-hamacanthin B	**(31)**	12.5	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0
(3*S*,6*R*)-6″-Debromo-3,4-dihydrohamacanthin A	**(32)**	12.5	25.0	>25.0	12.5	25.0	25.0	>25.0	>25.0	25.0	25.0	25.0
(3*S*,6*R*)-6′-Debromo-3,4-dihydrohamacanthin A	**(34)**	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	25.0	25.0	>25.0	>25.0

B_{17}, *Streptococcus pyogenes* 308A; B_{18}, *Streptococcus pyogenes* 77A; B_{19}, *Streptococcus faecium* MD 8b; B_{20}, *Streptococcus aureus* SG 511; B_{21}, *Streptococcus aureus* 285; B_{22}, *Streptococcus aureus* 503; B_{23}, *Escherichia coli* DC 2; B_{24}, *Pseudomonas aeruginosa* 1592E; B_{25}, *Pseudomonas aeruginosa* 1771; B_{26}, *Pseudomonas aeruginosa* 1771M; B_{27}, *Klebsiella oxytoca* 1082 E. MIC values>25 μg/mL were determined for *Escherichia coli* 078; *Escherichia coli* DC O; *Escherichia coli* TEM; *Escherichia coli* 1507 E; *Pseudomonas aeruginosa* 9027; *Salmonella typhimurium; Klebsiella aerogenes* 1522 E; *Enterobcter cloacae* P 99; *Enterobcter cloacae* 1321 E.

Dragmacidin D (**17**) inhibited *in vitro* replication of feline leukemia virus (FeLV) with a minimum inhibitory concentration (MIC) of 6.25 μg/mL (ELISA assay) [41]. Dragmacidin F (**19**) showed *in vitro* antiviral activity against HSV-1 (EC_{50} = 95.8 M) and HIV-1 (EC_{50} = 0.91 M), thus proving to be responsible for the antiviral property exhibited by *Halicortex* extract (Table 4) [13].

Antifungal: Nortopsentins A-C (**14-16**) exhibited *in vitro* antifungal activity against *Candida albicans* with IC_{50} of 3.1, 6.2, and 12.5 μg/mL, respectively [35]. Dragmecidin D (**17**) and dragmecidin E (**18**) displayed MICs against *Candida albicans* at 20 and 36 ppm, respectively [9]. Hamacanthins A (**23**) and B (**27**) showed significant antifungal activity against *Candida albicans* RPMI with MICs 1.6 and 6.2 μg/mL, respectively; against *C. neoformans* with MICs 3.1 and 6.2 μg/mL, respectively; against *Bacillus subtilis* with MICs 3.1, 1.6 μg/mL, respectively [20]. Hamacanthin A (**23**) also showed potent inhibitory activity against medically important pathogenic fungi: *Candida algicans* ATCC 10231 (MIC 6.25 μg/mL); *C. albicans* IFO 1594 (MIC 6.25 μg/mL); *Trichophyton rubrum* IFO 9185 (MIC 25 μg/mL); *Trichophyton mentagrophytes* IFO 40996 (MIC 12.5 μg/mL); *Aspergillus fumigatus* HIC 6094 (MIC 50 μg/mL) (Table 4)[33].

Table 4. Antiviral and antifungal activity (MIC: μg/mL)

Compound		V_1	V_2	V_3	V_4	V_5	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
Deoxytopsentin	**(1)**							>100	>100	100	>100	>100		
Topsentin	**(2)**	+	+	+										
Bromotopsentin	**(3)**	+	+	+										
Nortopsentin A	**(14)**						3.1							
Nortopsentin B	**(15)**						6.2							
Nortopsentin C	**(16)**						12.5							
Dragmacidin D	**(17)**				6.25		20.0							
Dragmacidin E	**(18)**						36.0							
Dragmacidin F	**(19)**	+				+								
(*R*)-6′-Debromo-hamacanthin A	**(22)**							50	50	50	25	100		
hamacanthin A	**(23)**							6.25	6.25	25	12.5	50	1.6	3.1
(*3S,6R*)-3,4-Dihydro-hamacanthin A	**(34)**							50	50	50	25	100		
hamacanthin B	**(27)**							25	100	>100	50	>100	6.2	6.2

V_1: HSV-1; V_2: Vesicular stomatitis virus (VSV); V_3: corona virus A-59; V_4: feline leukemia virus (FeLV); V_5: HIV-1. F_1: *Candida albicans*; F_2: *C. algicans* ATCC 10231; F_3: *C. albicans* IFO 1594; F_4: *Trichophyton rubrum* IFO 9185; F_5: *T. mentagrophytes* IFO 40996; F_6: *Aspergillus fumigatus* HIC 6094. F_7: *Candida algicans* RPMI; F_8: *C. neoformans*

Antiinflammation

Topsentin (**2**), bromotopsentin (**3**), nortopsentins A (**14**), B (**15**), C (**16**), and hamacanthins A (**23**) and B (**27**) displayed significant antiinflammatory activity in PMA-induced mouse ear edema. Their mechanism of action appears to be a consequence of inactivation of phospholipase A_2. Dragmacidin F (**19**) and topsentin D (**12**) and E (**13**) were isolated from *Spongosorites* and they showed antiinflammatory activity in resiniferatoxin-induced inflammation of the mouse ear [42].

Inhibition of soatase A

Deoxytopsentin (**1**), bromotopsentin (**3**), bromodeoxytopsentin (**5**), 6′-debromohamacanthin A (**22**), hamacanthin A (**23**), (*R*)-6′-debromohamacanthin B (**26**), and *trans*-3,4-dihydrohamacanthin A (*3S,6R*-3,4-dihydrohamacanthin A) (**34**) showed inhibitory activities toward sortase A (SrtA), that play key roles in cell-wall protein anchoring and virulence in gram-positive pathogenic bacteria *Staphylococcus aureus*, with various IC_{50} values [32].

Binding to $\alpha 1_a$ and $\alpha 1_b$ adrenergic receptors

Topsentin (**2**), bromotopsentin (**3**), nortopsentins A (**14**), B (**15**), C (**16**) were shown to display ligand binding to αl_a and αl_b adrenergic receptors with Ki values for the αl_b receptor ranging from 0.08 to 1.15 μM. All these compounds showed selectivity for αl_b relative to αl_a adrenergic receptor [34].

Inhibition of bNOS

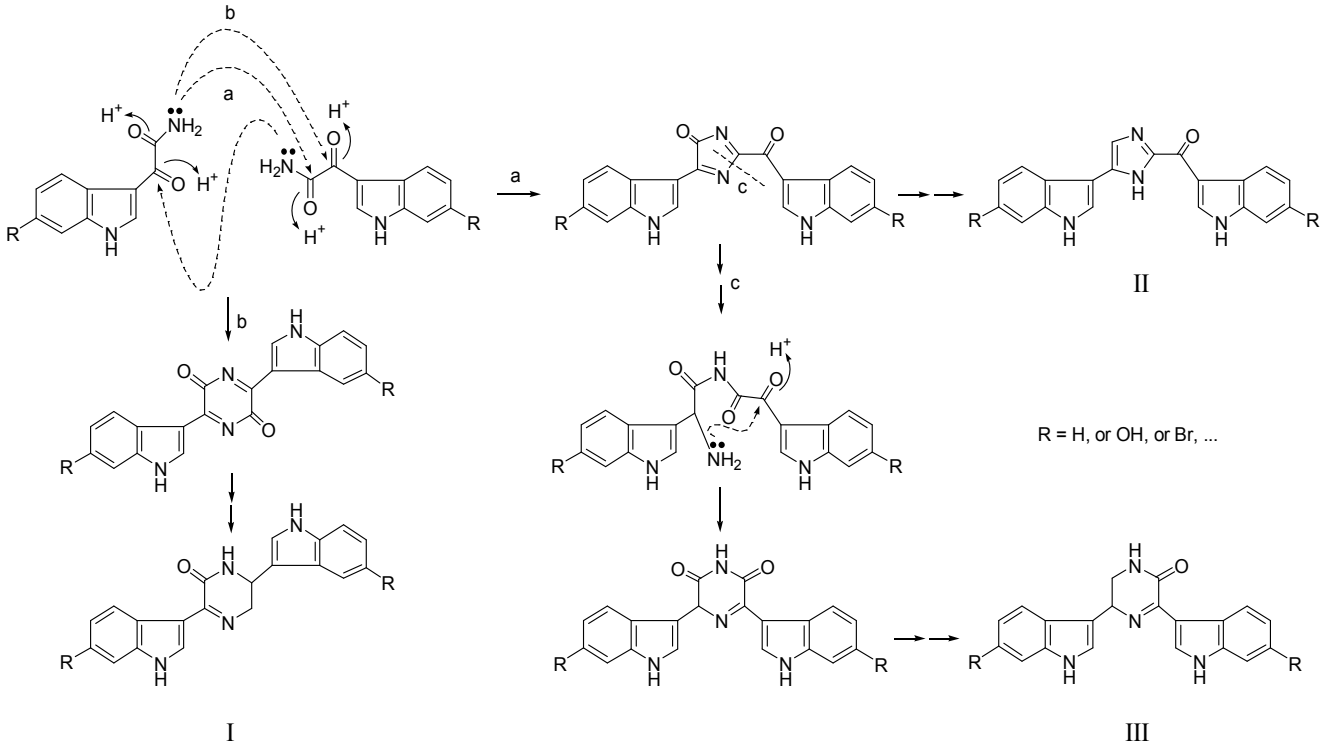
Nortopsentin C (**16**) inhibited neural nitric oxide synthase (bNOS) as well as calcineurin activities suggesting that its actions are directed against calmodulin, a co-factor common to these two enzymes. Two indole compounds, as well as dragmacidin D (**17**), inhibited bNOS, but not calcineurin activity [28].

Inhibition of protein phosphatases

Dragmacidin D (**17**) and E (**18**) are potent inhibitors of serine-threonine protein phosphatases. Dragmacidin E (**18**) inhibits both PP1 and PP2A. Dragmacidin D is a selective inhibitor of PP1 [9].

Biosynthesis

From a biogenetic point of view, the topsentins, nortopsentins, hamacanthins, and dragmacidins most prob-



Scheme 1. Hypothetical biogenesis of topsentins and hamacanthins.

ably derived from the combination of two tryptamine (or tryptophane) units. It is expected that (1*H*-indol-3-yl)oxoacetamide derivatives serve as intermediate for the biogenesis of marine bisindole alkaloids, topsentins and hamacanthins (Scheme 1). Schiff base formation between amino and carbonyl groups may (either via a or b) leads to genesis of hamacanthin A (Ⅰ) and topsentin (Ⅱ) skeletons. Cleavage of the C–N bond (c) in the topsentin skeleton and successive Schiff base formation between newly generated amino group may lead to genesis of hamacanthin B skeleton (Ⅲ)[5].

Up to now, dozens of tryptamine-derived indole compounds have been isolated from marine invertebrates in general, and from sponges in particular. Most of the marine indoles are rather simple compounds, presumably representing small deviations or sidelines in tryptophane metabolism. The metabolites derived from *Spongosorites* sp. fit nicely into this scheme and reinforce the idea that the complex indole alkaloid metabolic pathways that have appeared in higher plants probably have no counterpart in the marine environment [6].

Sponges are widely distributed in the world's oceans, occurring at all depths and latitudes. A large amount of work has shown that sponge secondary metabolism is spectacularly diverse, complex, and novel. It is already clear, however, that chemistry is not restricted to species of sponges collected in shallow waters. What remains to be determined is the extent to which very deep water sponges will yield metabolites that are substantially different from those found in their shallow water relatives [43]. It is interesting to mention that some bisindole alkaloids, such as topsentins D (**12**) and E (**13**), nortopsentins A (**14**), B (**15**), C (**16**), dragmacidins D (**17**) and E (**18**) were reported only from deep-water sponges, while hamacanthins and other topsentins were reported from both shallow water and deep water sponges. It suggests that there may be some unique indole alkaloid metabolic pathways in deep-water sponges.

It is noteworthy that both *R* and *S* isomers (however, they are not enantiomer, they are different derivatives) were isolated for the hamacanthins (**20**–**27**), while a single stereoisomer was isolated for dihydrohamacanthins (**28**–**34**).

It was recently suggested that the 6-bromotryptophan-derived alkaloids might be produced by associated microorganisms [41]. The biological activity of marine indole alkaloids is clearly a product of the unique functionality and elements involved in the biosynthesis of marine natural products. For instance, the bromination of many of the mentioned natural products has the potential to increase the biological activity significantly [19].

Conclusion

Numerous indole alkaloids have been reported from marine environment. Especially, biologically active bisindole alkaloids have been reported from marine sponges of genera *Spongosorites* over the past two decades. A deep interest in this class of compounds is due to both to their new molecular structures and their wide range of biological and pharmacological activities. In the future it is not unlikely that these compounds or parts thereof may form important structures as lead compounds for new drugs.

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