Full Papers

Bisindole Alkaloids of the Topsentin and Hamacanthin Classes from a Marine Sponge *Spongosorites* sp.

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Seven new (**1** and **3**−**8**) and seven known (**2** and **9**−**14**) bisindole alkaloids of the topsentin and hamacanthin classes were isolated from the MeOH extract of a marine sponge *Spongosorites* sp. by bioactivity-guided fractionation. The structure of compound **7** is a revision from our previous report. The planar structures were established on the basis of NMR and MS spectroscopic analyses. Configurations of these compounds were defined by NMR spectroscopy and optical rotation. It is noteworthy that both *R* and *S* isomers were isolated for the hamacanthins (**1**−**4**, **9**, **10**, **15**, and **16**), while a single stereoisomer was isolated for dihydrohamacanthins (**5**, **11**−**14**, **17**, and **18**). Compounds **1**−**4**, **6**, and **8**−**14** showed marginal cytotoxicity against five human solid tumor cell lines, and compound **2** showed weak antibacterial activity against clinically isolated methicillin-resistant strains.

Unique bisindole alkaloids such as topsentins,[1,2] nortopsentins,[3] dragmacidins,[4,5] and hamacanthins[6−8] have been reported from marine sponges. Rhopaladins are another class of related bisindole alkaloids from tunicates.[9] Some of these metabolites exhibit potent and diverse bioactivities such as cytotoxic,[3,8,10,11] antitumor,[4] antiviral,[4] antifungal,[3,6] antibacterial,[8,9] and anti-inflammatory activities.[12] A specific inhibition of ligand binding to α_{1a} and α_{1b} adrenergic receptors was reported for bromotopsentin, topsentin, nortopsentins A−C, and dragmacidin.[13a] Some of the bisindole alkaloids of the topsentin and hamacanthin classes were reported as sortase A inhibitors in a recent research paper.[13b]

In our previous study on cytotoxic compounds from the marine sponge *Spongosorites* sp., a series of bisindole alkaloids were isolated.[8] In continuation of our search for cytotoxic metabolites from the same sponge, seven new [**1** and **3**−**8**, the structure of (*S*)-6″-debromohamacanthin B, which was erroneously reported in our previous paper,[8] was revised to **7** and given the trivial name spongotine B] and seven known (**2** and **9**−**14**) bisindole alkaloids of the topsentin and hamacanthin classes were isolated. Herein we describe the structure elucidation and the biological evaluation of these compounds.

Results and Discussion

(*S*)-6′,6″-Didebromohamacanthin A (**1**) was isolated as a yellow, amorphous powder. The molecular formula was established as $C_{20}H_{16}ON_4$ on the basis of the HRFABMS data. The exact mass of the $[M + H]^+$ ion at *m*/*z* 329.1393 matched well with the expected molecular formula of $C_{20}H_{17}ON_4$ (Δ −1.0 mmu). The NMR spectra of **1** showed the presence of two indole residues, which were reminiscent of those of hamacanthin A (**9**),[6] (*R*)-6″-debromohamacanthin A (**15**),[8] and (*R*)-6′-debromohamacanthin A (**16**).[8] The main difference from those hamacanthin A derivatives was the lack of one (compared to **15**, **16**) or two (compared to **9**) bromine atoms in the molecule. The 3,6-disubstituted 5,6-dihydro-1*H*-pyrazin-2-one moiety was deduced from a conjugated amide carbonyl carbon (δ_C 157.9, C-2), a tetrasubstituted vinylic carbon (δ_C 157.7, C-3), a nitrogen-bearing methine carbon (δ_C 46.5, C-6), and another nitrogen-bearing methylene carbon (δ_C 53.7, C-5) (Table 2). The chemical shift of the carbonyl carbon (δ_C 157.9, C-2) was inconsistent with a ketone functionality as observed in topsentins (**19**−**21**)[8] but would fit that of a lactam, as observed in hamacanthins (**9**, **10**, **15**, and **16**). In the COSY spectrum, the NH proton signal at δ_H 8.74 (H-1) was coupled to the methine proton signal at δ_H 4.99 (H-6), which in turn was coupled to the methylene proton signals at δ_H 4.14 (H-5a) and 4.08 (H-5b). Analysis of the ^{1}H, ^{13}C, COSY, and HMBC data, along with comparison of chemical shift values with those of known bisindole alkaloids,[2] allowed us to establish two indol-3-yl residues as partial structures of **1**. Two singlets at δ_H 11.51 (H-1′) and 8.40 (H-2′) and a spin system comprised of signals at δ_H 8.37 (H-4′), 7.07 (H-5′), 7.15 (H-6′), and 7.43 (H-7′) indicated the presence of an indol-3-yl moiety (Table 1). The long-range correlation between H-2′ (δ_H 8.40) and C-3 (δ_C 157.7) established the connectivity between the dihydropyrazinone ring and the indole moiety. The proton signals at δ_H 11.03 (H-1″), 7.28 (H-2″), 7.70 (H-4″), 7.02 (H-5″), 7.11 (H-6″), and 7.38 (H-7″) indicated the presence of another indole moiety. Long-range correlation between H-2″ (δ_H 7.28) and C-6 (δ_C 46.5) established the connectivity between the dihydropyrazinone ring and the second indole residue. Therefore, compound **1** was defined as a 6′,6″-debrominated derivative of hamacanthin A (**9**). The absolute configuration of compound **1** was defined on the basis of optical rotation. The optical rotation of **1** (+59, *c* 0.72, MeOH) was opposite in sign of that of the synthetic antipode of hamacanthin A, (*R*)-3,6-bis(bromoindol-3-yl)-5,6-dihydro-1*H*-pyrazin-2-one (−79, *c* 0.1, MeOH),[14] indicating that compound **1** has an 6*S* configuration.

(*R*)-6′-Debromohamacanthin B (**2**) was also isolated as a yellow, amorphous powder. In the FABMS data of **2**, the $[M + H]^+$ ion cluster was observed at *m*/*z* 407/409 in the ratio of 1:1, which is

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Chart 1

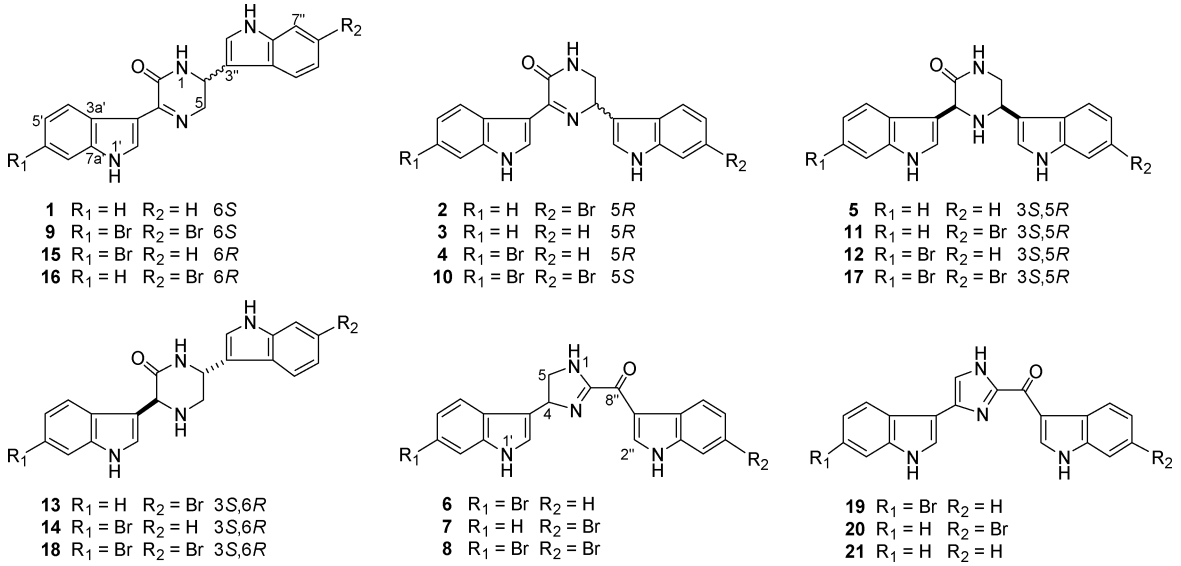

Table 1. ^{1}H NMR Data of Compounds **1**−**5**[a]

position	1	2	3	4	5
1	8.74 (br s)	8.49 (br s)	8.45 (br s)	8.50 (t)	7.83 (d, 4.0)
3					4.88 (d, 4.0)
5	4.14 (dd, 16.0, 5.0)	5.24 (dd, 9.5, 5.0)	5.26 (dd, 9.5, 5.0)	5.29 (dd, 9.5, 5.0)	4.53 (dd, 11.0, 4.0)
	4.08 (dd, 16.0, 8.5)				
6	4.99 (ddd, 8.5, 5.0, 2.0)[b]	3.62 (dt, 12.5, 5.0)[b]	3.66 (dt, 13.0, 5.0)[b]	3.66 (dt, 13.0, 4.5)[b]	3.59 (t, 11.0)
		3.45 (ddd, 12.5, 9.5, 2.5)[b]	3.48 (ddd, 13.0, 9.5, 2.5)[b]	3.50 (td, 9.0, 2.5)[b]	3.40 (dt, 11.0, 4.0)[b]
1′	11.51 (br s)	11.54 (br s)	11.54 (br s)	11.74 (s)	10.85 (br d, 2.0)
2′	8.40 (s)	8.39 (br s)	8.40 (s)	8.43 (s)	7.31 (d, 2.0)
4′	8.37 (d, 8.0)	8.37 (d, 8.0)	8.42 (d, 8.0)	8.35 (d, 8.5)	7.76 (d, 8.0)
5′	7.07 (t, 8.0)	7.04 (t, 8.0)	7.04 (t, 8.0)	7.19 (dd, 9.0, 2.0)	6.99 (t, 8.0)[d]
6′	7.15 (t, 8.0)	7.14 (t, 8.0)	7.14 (t, 8.0)		7.07 (t, 8.0)[c]
7′	7.43 (d, 8.0)	7.43 (d, 8.0)	7.44 (d, 8.0)	7.65(d, 1.5)	7.36 (d, 8.0)
1″	11.03 (br s)	11.16 (br s)	10.99 (br s)	11.03 (s)	10.98 (br d, 2.0)
2″	7.28 (d, 2.0)[b]	7.30 (br s)	7.25 (d, 1.5)[b]	7.24(d, 1.0)[b]	7.34 (d, 2.0)[b]
4″	7.70 (d, 8.0)	7.68 (d, 8.5)	7.71 (d, 8.0)	7.70 (d, 8.0)	7.71 (d, 8.0)
5″	7.02 (t, 8.0)	7.13 (dd, 8.5, 2.0)	7.01(t, 8.0)	7.01 (t, 7.5, 7.0)	6.98 (t, 8.0)[d]
6″	7.11 (t, 8.0)		7.11(t, 8.0)	7.12 (t, 7.5, 8.5)	7.06 (t, 8.0)[c]
7″	7.38 (d, 8.0)	7.59 (d, 2.0)	7.41 (d, 8.0)	7.41 (d, 8.0)	7.35 (d, 8.0)

[a] ^{1}H NMR data of **1**−**5** were measured at 500 MHz in DMSO-d_6. [b]Extra splitting may be due to couplings with vicinal amino protons. Significant couplings with corresponding amino protons were observed in COSY spectra, though the amino proton exhibited broadening rather than clear splitting possibly due to the quadrupole moment of the nitrogen atom. [c,d]Assignments with the same superscript in the same column may be interchanged.

characteristic of a monobrominated compound. The exact mass of the $[M + H]^+$ ions at *m*/*z* 407.0385 and 409.0462 matched with the expected molecular formula of $C_{20}H_{16}ON_4{}^{79}Br$ (Δ −12.2 mmu) and $C_{20}H_{16}ON_4{}^{81}Br$ (Δ −2.5 mmu), respectively. The NMR data indicated the presence of a 6-substituted indol-3-yl, an indol-3-yl, and a 3,5-disubstituted 5,6-dihydro-1*H*-pyrazin-2-one moiety. In the COSY spectrum, H-1 (δ_H 8.49) showed couplings to the methylene protons at δ_H 3.62 (H-6a) and 3.45 (H-6b), which in turn were coupled to the methine proton at δ_H 5.24 (H-5). Long-range C−H correlations were observed from H-1 (NH, δ_H 8.49) and H-5 (1H, δ_H 5.24) to C-3 (δ_C 157.3) and from H-6 (1H, δ_H 3.62) to C-2 (δ_C 157.5). These observations confirmed the presence of the dihydropyrazinone ring system. Long-range C−H correlations from H-6 (1H, δ_H 3.62) and H-5 (1H, δ_H 5.24) to C-3″ (δ_C 115.1) established the connectivity between the dihydropyrazinone ring and the 6-bromoindole residue. The connectivity between the dihydropyrazinone ring and the unsubstituted indol-3-yl moiety was presumed on comparison of chemical shift values with those of hamacanthin B (**10**). Thus, compound **2** was defined as a 6′-debrominated derivative of hamacanthin B.[6] The specific rotation of compound **2** (−194, *c* 0.25, MeOH) was opposite in sign of that of the natural hamacanthin B (**10**, +176, *c* 0.1, MeOH) and the synthetic (*S*)-hamacanthin B (+183, *c* 0.1, MeOH),[15] indicating

Table 2. ^{13}C NMR Data of Compounds **1**−**5**[a]

position	1	2	3	4	5
2	157.9[b]	157.5[b]	157.6[b]	157.1[b]	169.7
3	157.7[b]	157.3[b]	157.3[b]	157.4[b]	57.8
5	53.7	53.7	53.9	53.9	50.9
6	46.5	43.3	43.4	43.4	48.5
2′	131.9	132.0	132.0	132.9	124.2
3′	112.0[c,d]	111.0	111.1	111.2	114.4[b]
3a′	125.6	126.0	126.0	125.1	126.6
4′	123.5	122.5	122.6[c]	124.2	119.1
5′	121.3	122.1	120.5	123.4	118.4
6′	122.5	121.0	122.1	114.8	121.0
7′	112.2[c,d]	111.6	111.6	114.4[c]	111.5
7a′	136.1	136.2	136.2	137.2	136.3[c]
2″	123.8[d]	123.8	122.7[c]	122.8	122.4
3″	113.5[d]	115.1	114.7	114.5[c]	114.5[b]
3a″	126.0	125.1	126.0	126.0	125.8
4″	119.0	121.3	118.5	118.6	120.0
5″	118.7	122.1	119.1	119.1	118.1
6″	121.8[d]	113.9	121.2	121.2	120.7
7″	112.4[c,d]	114.2	111.6	111.7	111.2
7a″	136.4[d]	137.4	136.6	136.6	136.2[c]

[a] ^{13}C NMR data of **1**−**5** were measured at 75 MHz in DMSO-d_6. [b,c] Assignments with the same superscript in the same column may be interchanged. [d]Signal was assigned by HSQC or HMBC experiment.

that compound **2** has a 5*R* configuration. A literature survey revealed that this compound was reported as a metabolite of the sponge *Spongosorites* sp. during the preparation of the manuscript, but the stereochemistry of hamacanthin B was erroneously presented in this reference.[13b]

(*R*)-6′,6″-Didebromohamacanthin B (**3**) was also isolated as a yellow, amorphous powder. Its molecular formula was established as $C_{20}H_{16}ON_4$ on the basis of the FABMS data. The exact mass of the $[M + H]^+$ ion at *m*/*z* 329.1398 matched well with the expected molecular formula of $C_{20}H_{17}ON_4$ (Δ −0.5 mmu). The main difference from compound **2** was the lack of a bromine atom on the indole ring. Thus, compound **3** was defined as a 6′,6″-didebrominated derivative of hamacanthin B (**10**).[6] The specific rotation of compound **3** (−288 *c* 0.4, MeOH) suggested its 5*R* configuration.

(*R*)-6″-Debromohamacanthin B (**4**) was also isolated as a yellow, amorphous powder. In the EIMS data of **4**, the $[M]^+$ ion cluster was observed at *m*/*z* 406/408 in the ratio of 1:1, which is characteristic of a monobrominated compound. The exact mass of the $[M]^+$ ions at *m*/*z* 406.0429 and 408.0444 matched well with the expected molecular formula of $C_{20}H_{15}ON_4{}^{79}Br$ (Δ 0.0 mmu) and $C_{20}H_{15}ON_4{}^{81}Br$ (Δ +3.3 mmu), respectively. The main difference from compound **2** was that the bromine atom was located on the indole ring of the left half of the compound. Thus, compound **4** was defined as a 6″-debrominated derivative of hamacanthin B (**10**).[6] The specific rotation of compound **4** (−83 *c* 0.5, MeOH) suggested its 5*R* configuration.

(3*S*,5*R*)-6′,6″-Didebromo-3,4-dihydrohamacanthin B (**5**) was also isolated as a yellow, amorphous powder. Its molecular formula was established as $C_{20}H_{18}ON_4$ on the basis of NMR data and FABMS data. The exact mass of the $[M + H]^+$ ion at *m*/*z* 331.1577 matched well with the expected molecular formula of $C_{20}H_{19}ON_4$ (Δ +1.8 mmu). Analysis of the ^1H and ^{13}C NMR data (Tables 1 and 2) suggested the presence of two independent indol-3-yl residues. The remaining 3,5-disubstituted piperazin-2-one moiety was deduced by comparison of spectral data with those of dihydrohamacanthin B derivatives (**11**, **12**, and **17**).[7,8] In the COSY spectrum, H-1 (δ_H 7.83) showed couplings to H-6 (δ_H 3.59 and δ_H 3.40), which in turn were coupled to H-5 (δ_H 4.53). Long-range C−H correlations were observed from H-1 (NH, δ_H 7.83), H-3 (1H, δ_H 4.88), and H-6 (1H, δ_H 3.59) to C-2 (δ_C 169.7) and from H-5 (1H, δ_H 4.53) to C-3 (δ_C 57.8). These observations confirmed the presence of the 3,5-disubstituted piperazin-2-one moiety. Long-range correlations from H-3 to C-3′ (δ_C 114.4) and C-3a′ (δ_C 126.6) and from H-5 and H-6 to C-3″ (δ_C 114.5) confirmed the connectivity between the piperazinone ring and the indole moieties. Therefore, compound **5** was defined as 6′,6″-didebromo-3,4-dihydrohamacanthin B. The relative stereochemistry at positions C-3 and C-5 was established as *cis* on the basis of the diaxial coupling (J = 11.0 Hz) between H-6 (δ_H 3.59) and H-5 (δ_H 4.53) and the dipolar correlation between H-3 and H-5 in the ROESY spectrum (Figure 1). The conformer **B** with the bulky indole rings at equatorial positions is predominant, while the contribution of the alternative conformer **A** is negligible. The absolute configuration was determined as (3*S*,5*R*) on the basis of optical rotation. The specific rotation of **5** (+127, *c* 0.08, MeOH) was opposite that of the synthetic enantiomer, (3*R*,5*S*)-3,5-bis(6-bromoindol-3-yl)piperazin-2-one (−92.3, *c* 0.66, acetone).[16] 6′,6″-Didebromo-*cis*-3,4-dihydrohamacanthin B (**5**) was previously reported as a synthetic product without assignment of absolute stereochemistry.[17]

Spongotine A (**6**) was isolated as a yellow, amorphous powder. Its molecular formula was established as $C_{20}H_{15}ON_4Br$ on the basis of the ^1H NMR, ^{13}C NMR, and FABMS data. The exact mass of the $[M + H]^+$ ions at *m*/*z* 407.0464 and 409.0508 matched well with the expected molecular formula of $C_{20}H_{16}ON_4{}^{79}Br$ (Δ −4.3 mmu) and $C_{20}H_{16}ON_4{}^{81}Br$ (Δ +2.1 mmu), respectively. As previously reported for topsentins,[1,2,8] broadening or doubling of ^1H NMR signals of compound **6** was observed in neutral solution (DMSO-d_6) due to slow interconversion of imidazolylmethanone tautomers and/or rotamers (52:48 between two forms of the imidazole ring). Protonation of the compound by addition of trifluoroacetic acid (TFA, 1%) yields imidazolium cation and renders its spectrum uncomplicated.[1] Therefore, NMR measurements were performed in 1% solution of TFA in DMSO-d_6. The presence of a 6-substituted indol-3-yl and an indol-3-yl residue was evident from analysis of the ^1H and ^{13}C NMR data (Tables 3 and 4). In the COSY spectrum of **6**, two downfield NH proton signals (H-3, δ_H 11.35 and H-1, δ_H 11.14) showed coupling to a methine proton (H-4, δ_H 5.89) and two methylene protons (H-5, δ_H 4.53 and 4.09), respectively, and the methine proton at δ_H 5.89 (H-4) showed coupling to methylene protons at δ_H 4.53 and 4.09 (H-5). The ^{13}C NMR spectrum of **6** showed a carbonyl carbon at δ_C 172.8 (C-8″), an imino carbon at δ_C 161.6 (C-2), a nitrogen-bearing methylene carbon at δ_C 51.1 (C-5), and another nitrogen-bearing methine carbon at δ_C 54.3 (C-4). Long-range correlations from H-1 (NH, δ_H 11.14), H-3 (NH, δ_H 11.35), H-4 (1H, δ_H 5.89), and H-5 (2H, δ_H 4.53 and 4.09) to C-2 (C═N−, δ_H 161.6) were observed. These data suggested the presence of a 2,4-disubstituted-4,5-dihydro-1*H*-imidazole moiety, which was observed in the form of an imidazolium salt of TFA. Long-range correlations from H-5 to C 3′ and from H-4 to C 3′ and C 3a′ established the connectivity between the dihydroimidazole ring and the 6-bromoindole residue. Although the long-range correlation between the dihydroimidazole/ketone and another indole moiety was not detected, the connectivity between them was determined by comparison of NMR with those of bromodeoxytopsentin (**19**)[10] and isobromodeoxytopsentin (**20**)[10] (Tables 3 and 4) and MS fragmentations of **6** (Figure 2). A compound with the same structure as **6** was previously reported as 4,5-dihydro-6″-deoxybromotopsentin.[2] However, the upfield shift of the ketone carbonyl carbon (δ_C 159.12) for the given structure,[2] compared to those of topsentin (δ_C 173.58) and bromotopsentin (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[2] were rather close to those of hamacanthin B, which were defined thereafter[6] and followed by synthesis.[15] Morris et al. also proposed to revise the imidazole/ketone functionality in topsentin C[11] and 4,5-dihydro-6″-deoxybromotopsentin[2] to a dihydropyrazone ring, since their carbonyl resonances (δ_C 157.8 and 159.12, respectively) were close to those of lactam carbonyl carbons of the 2-ketodehydropiperazine ring.[4] As mentioned above, broadening or doubling of the ^1H NMR signals of compound **6** was observed in neutral solution (DMSO-d_6). Such ^1H NMR characteristics of imidazolylmethanone tautomerism was

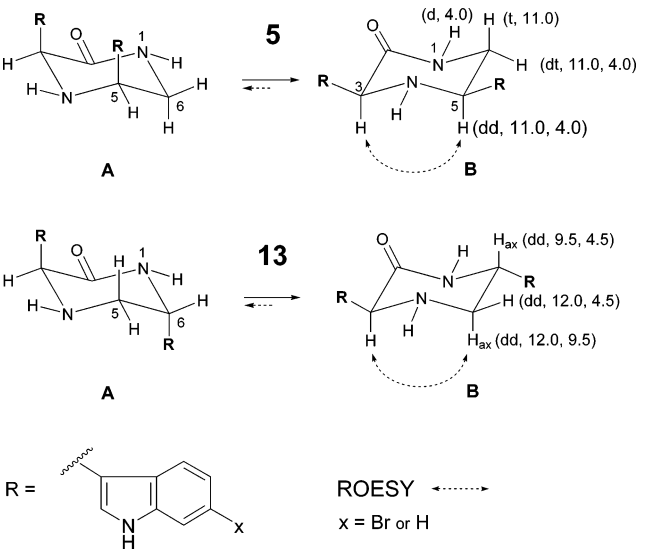

Figure 1. Relative configurations of compounds **5** and **13**.

Table 3. ^{1}H NMR Data of Compounds **6**−**8**, **19**, and **20**[a]

position	6	7	8	19	20
1	11.14 (br s)	11.16 (s)	11.19 (s)		
3	11.35 (br s)	11.35 (br s)[b]	11.38 (br s)		
4	5.89 (dd, 12.5, 12.0)	5.86 (dd, 11.0, 10.0)	5.88 (t, 12.5)		
5	4.53 (t, 11.5, 12.5)	4.51 (t, 12.0, 11.0)	4.52 (t, 12.5)	8.01 (s)	7.91 (s)
	4.09 (dd, 12.0, 11.5)	4.10 (dd, 12.0, 10.0)	4.08 (t, 12.5)		
1′	11.50 (br s)	11.35 (br s)[b]	11.51 (d, 1.0)	11.81 (d, 2.8)	11.54 (d, 2.6)
2′	7.68 (d, 1.5)	7.62 (s)	7.66 (d, 4.0)	8.06 (d, 2.8)	8.03 (d, 2.6)
4′	7.57 (d, 8.5)	7.58 (d, 7.5)	7.56 (d, 8.5)	7.88 (d, 8.2)	7.98 (br d 7.8)
5′	7.26 (dd, 8.5, 1.5)	7.10 (t, 7.5)	7.25 (d, 8.5, 2.0)	7.29 (dd, 8.2, 1.4)	7.16 (br dd 7.8, 7.8)
6′		7.18 (t, 7.5)			7.20 (br dd 8.2, 7.8)
7′	7.68 (d, 1.5)	7.47 (d, 7.5)	7.67 (d, 2.0)	7.71 (d, 1.4)	7.48 (br d 8.2)
1″	12.85 (br s)	12.96 (br s)	12.99 (br s)	12.58 (d, 2.3)	12.44 (d, 3.4)
2″	8.57 (d, 4.0)	8.60 (s)	8.59 (d, 4.0)	8.57 (d, 2.3)	8.95 (d, 3.4)
4″	8.19 (d, 7.5)	8.11 (d, 8.5)	8.11 (d, 8.5)	8.23 (dd, 7.8, 1.2)	8.24 (d, 8.6)
5″	7.36 (t, 7.5)	7.51 (dd, 8.5, 2.0)	7.51 (dd, 8.5, 2.0)	7.31 (m)	7.42 (dd, 8.6, 1.9)
6″	7.39 (t, 7.5)			7.33 (m)	
7″	7.63 (d, 7.5)	7.84 (d, 2.0)	7.85 (d, 2.0)	7.58 (dd, 7.8, 1.4)	7.78 (d, 1.9)

[a] ^{1}H NMR data of **6**−**8** were measured at 500 MHz in DMSO-d_6+TFA (ca. 1%). The ^{1}H NMR data of **19** and **20** are cited from the literature.[10] [b] Overlapped signals.

Table 4. ^{13}C NMR Data of Compounds **6**−**8**, **19**, and **20**[a]

position	6	7	8	19	20
2	161.6	161.1	161.3	141.7	142.9
4	54.3	54.8	54.4	131.2	133.1
5	51.1	51.1	51.2	116.9	118.7
2′	125.8	124.7[b]	125.9	127.0	124.8
3′	112.5	111.9	112.5	103.6	105.0
3a′	124.7	124.6[b]	124.0	123.8	124.6
4′	120.0	118.1	120.1	121.6	119.7
5′	122.3	119.3	122.3	123.9	120.3
6′	114.7[b]	121.8	114.7	115.7	122.3
7′	114.5[b]	112.1	114.8	115.5	112.3
7a′	137.6	136.8	137.7	137.9	136.6
2″	140.0	140.6	140.8	139.1	138.6
3″	113.3	113.2	113.2	114.2	113.8
3a″	124.8	123.9	123.9	126.3	125.5
4″	121.1	122.7	122.8	121.9	123.3
5″	123.7[c]	126.5	126.7	123.7	125.4
6″	123.9[c]	117.1	117.1	124.8	116.2
7″	113.1	115.9	116.0	113.4	115.5
7a″	137.1	138.0	138.1	137.5	137.6
8″	172.8	173.8	173.2	172.7	174.1

[a] ^{13}C NMR data of **6**−**8** were measured at 75 MHz in DMSO-d_6+TFA (ca. 1%). ^{13}C NMR data of **19** and **20** are cited from the literature.[10] [b,c] Assignments with the same superscript in the same column may be interchanged.

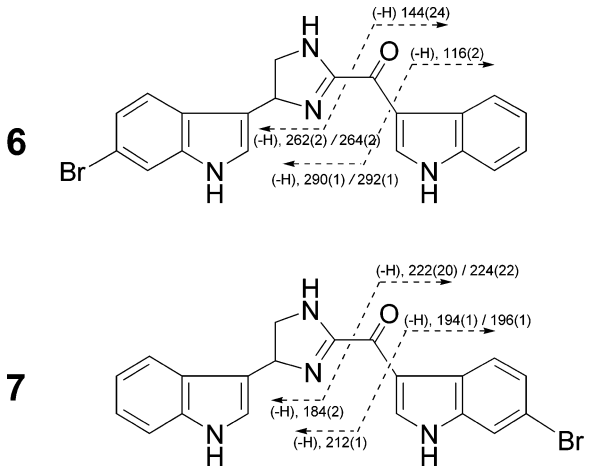

Figure 2. Key fragmentations of the $[M + H]^+$ ions of **6** and **7** in FAB-CID MS/MS (relative intensity in parentheses).

not mentioned for the reported 4,5-dihydro-6″-deoxybromotopsentin,[2] while such phenomena were described for two other topsentin derivatives in the same literature.[2] A prominent α-cleavage (*m*/*z* 144) in the MS/MS of **6** also corroborated the proposed structure

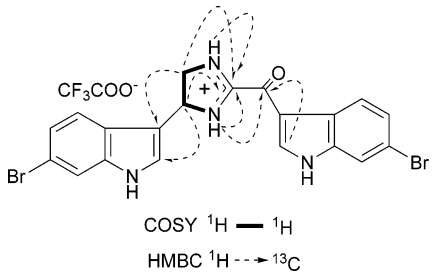

Figure 3. Selected COSY and HMBC correlation of **8**.

(Figure 2). In our study, only the topsentins (**6**, **7**, and **21**) showed the characteristic α-cleavage of an acyclic ketone, while the hamacanthins (**11** and **15**) showed more complex and less significant fragmentations. We believe that the spectral data of compound **6** clearly match with the proposed structure, although distinct spectral data were previously assigned to the same structure. The characteristic tautomerism in the NMR data and the fragmentation in the MS/MS data may serve as strong evidence to distinguish the hamacanthins from the topsentins. The stereochemistry of compound **6** remains to be determined.

Spongotine B (**7**) was isolated as a yellow, amorphous powder, and the structure was erroneously assigned as (*S*)-6″-debromohamacanthin B in our previous report.[8] Its molecular formula was established as $C_{20}H_{15}ON_4Br$ on the basis of the FABMS data. The exact mass of the $[M + H]^+$ ions at *m*/*z* 407.0494 and 409.0530 matched well with the expected molecular formula of $C_{20}H_{16}ON_4{}^{79}Br$ (Δ −1.3 mmu) and $C_{20}H_{16}ON_4{}^{81}Br$ (Δ +4.3 mmu), respectively. The spectroscopic properties of this compound, including the same molecular formula ($C_{20}H_{15}ON_4Br$), were very similar to those of **6** (Tables 3 and 4). The ^{1}H NMR signals of compound **7** also showed broadening or doubling in neutral solution (acetone-d_6 and DMSO-d_6) due to slow interconversion of imidazolylmethanone tautomers and/or rotamers. When we re-examined the NMR data of **7**, an additional NH proton signal at δ_H 11.35 was found overlapped with another NH proton signal with the same chemical shift, and a carbonyl carbon signal at δ_C 173.8 was found in addition to the carbon signal at δ_C 161.1. A typical α-cleavage of an acyclic ketone was observed as a major fragment at *m*/*z* 222/224 (Figure 2). Therefore, our previous structure[8] should be revised to the one shown in Figure 2.

Spongotine C (**8**) was isolated as a colorless solid. Its molecular formula was established as $C_{20}H_{14}ON_4Br_2$ on the basis of the ^{1}H NMR, ^{13}C NMR, and MS data. In the EIMS of **8**, the $[M]^+$ ion cluster was observed at *m*/*z* 484/486/488 in the ratio of 1:2:1, which is characteristic of a dibrominated compound. The exact mass of the $[M]^+$ ions at *m*/*z* 483.9532, 485.9542, and 487.9542 matched

Table 5. Cytotoxicity of Compounds **1**−**14**[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	8.30	11.50	5.00	17.10	4.10
2	3.71	8.50	7.60	8.30	4.20
3[b]	11.70	12.60	13.70	24.10	4.79
9	4.49	5.24	5.44	5.60	4.66
11[b]	4.20	6.00	7.10	6.80	6.30
13[b]	7.50	12.10	13.10	19.10	6.30
doxorubicin	0.02	0.14	0.07	0.07	0.02
5	>10.00	9.64	>10.00	>10.00	>10.00
6	6.82	3.71	5.04	7.22	9.80
12	9.67	5.67	>10.00	9.74	>10.00
14	>10.00	4.92	>10.00	>10.00	>10.00
doxorubicin	0.04	0.05	0.05	0.06	0.32
7	>30.00	>30.00	>30.00	>30.00	>30.00
doxorubicin	0.01	0.03	0.01	0.01	0.05
4[b]	7.86	7.85	7.71	9.21	6.31
8[b]	5.22	4.81	4.82	5.16	4.88
10[b]	2.14	2.61	1.59	2.93	1.52
doxorubicin	0.02	0.14	0.03	0.04	0.10

[a] Data expressed in ED_{50} values (μg/mL). A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT15, human colon cancer. [b]The solubility of the sample was not good.

well with the expected molecular formula of $C_{20}H_{14}ON_4{}^{79}Br_2$ (Δ −0.2 mmu), $C_{20}H_{14}ON_4{}^{79}Br^{81}Br$ (Δ +2.8 mmu), and $C_{20}H_{14}ON_4{}^{81}Br_2$ (Δ +4.9 mmu), respectively. The spectroscopic properties of this compound were very similar to those of **6** and **7** (Tables 3 and 4). The clear difference was the presence of an additional bromine substitution and, accordingly, the presence of two independent 6-bromoindol-3-yl moieties. The ^{1}H NMR signals of compound **8** also showed broadening or doubling in neutral solution (acetone-d_6 and DMSO-d_6). Therefore, compound **8** was defined as a 4,5-dihydro-6′,6″-dibromotopsentin, and the structure was confirmed by COSY and HMBC data (Figure 3).

Six known (**2** and **9**−**14**) bisindole alkaloids of the hamacanthin class were isolated as yellow, amorphous powders. Compound **9** was identified as hamacanthin A, and the optical rotation of **9** (+58, *c* 0.05, MeOH) was similar to that of the natural hamacanthin A (+84, *c* 0.1, MeOH)[6] and opposite that of the synthetic counterpart (*R*)-3,6-bis(bromoindol-3-yl)-5,6-dihydro-1*H*-pyrazin-2-one (−79, *c* 0.1, MeOH),[14] indicating that compound **9** has an *S* configuration. Compound **10** was identified as hamacanthin B, and the optical rotation of **10** (+56, *c* 0.2, MeOH) was similar to that of the natural hamacanthin B (+176, *c* 0.1, MeOH) and the synthetic (*S*)-hamacanthin B (+183, *c* 0.1, MeOH),[15] indicating that compound **10** has a 5*S* configuration. Compounds **11** and **12** were identified as 6′-debromo-*cis*-3,4-dihydrohamacanthin B and 6″-debromo-*cis*-3,4-dihydrohamacanthin B,[7] respectively. The relative stereochemistry at positions C-3 and C-5 of the central piperazinone ring of compounds **11** and **12** was established as *cis* on the basis of the diaxial coupling (J = 11.0 Hz) between the H-6_{ax} (δ_H 3.56) and H-5_{ax} (δ_H 4.51) protons and the dipolar correlation between H-3 (δ_H 4.87) and H-5_{ax} in the ROESY spectrum (similar to compound **5** in Figure 1). The absolute configuration of compounds **11** and **12** was determined as (3*S*,5*R*) on the basis of optical rotation. The specific rotation of **11** (+80, *c* 0.25, MeOH) and **12** (+105, *c* 0.20, MeOH) was similar to that of the natural *cis*-3,4-dihydrohamacanthin B (+98.7, *c* 0.2, MeOH),[7] but opposite that of the synthetic enantiomer, (3*R*,5*S*)-3,5-bis(6-bromoindol-3-yl) piperazinone-2-one (−92.3, *c* 0.66, acetone).[16] Compounds **13** and **14** were identified as 6′-debromo-*trans*-3,4-dihydrohamacanthin A and 6″-debromo-*trans*-3,4-dihydrohamacanthin A,[7] respectively. The relative stereochemistry at positions C-3 and C-6 of the central piperazinone ring of compounds **13** and **14** was established as *trans* on the basis of the diaxial coupling (J = 9.5 Hz) between the H-6_{ax} (δ_H 5.01) and H-5_{ax} (δ_H 2.98, **13**; δ_H 3.02, **14**) protons and the dipolar correlation between H-3 (δ_H 4.71) and H-5_{ax} in the ROESY spectrum (Figure 1). The absolute configuration of compounds **13** and **14** was determined as (3*S*,6*R*) by comparison of optical rotations of compounds **13** (+31, *c* 0.38, MeOH) and **14** (+34, *c* 0.15, MeOH) with the synthetic enantiomer,[18] (3*R*,6*S*)-3,6-bis(6-bromo-1*H*-indol-3-yl)piperazin-2-one (−6, *c* 0.275, MeOH/acetone = 1:1).

It is noteworthy that both *R* and *S* isomers were isolated for the hamacanthins (**1**−**4**, **9**, **10**, **15**, and **16**), while a single stereoisomer was isolated for the dihydrohamacanthins (**5**, **11**−**14**, **17**, and **18**).

Compounds **1**−**14** were evaluated for cytotoxicity against a panel of five human solid tumor cell lines. Compounds **1**−**4**, **6**, and **8**−**14** showed marginal cytotoxicity to the cancer cell lines tested (Table 5). Compounds **1**−**3**, **7**, and **11**−**14** were also evaluated for antibacterial activity against 20 clinically isolated methicillin-resistant strains, and compound **2** showed weak antibacterial activity against 10 of the 20 strains to various degrees (Table 6).

Experimental Section

General Experimental Procedures. Optical rotations were measured using a JASCO DIP-370 digital polarimeter. CD spectra were recorded using a JASCO J-715 spectropolarimeter (sensitivity 50 mdeg, resolution 0.2 nm). IR spectra were recorded using a JASCO FT/IR-410 spectrometer. UV spectra were recorded using a Shimadzu Uvmini 1240 UV/visible spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC300 and Varian INOVA 500 instruments. Chemical shifts were reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 2.5 and δ_C 39.5 for DMSO-d_6). FABMS data were obtained on a JEOL JMS SX-102A; EIMS data were obtained on a Shimadzu QP5050. HPLC was performed with an YMC ODS-H80 column (250 × 10 mm i.d., 4 μm, 80 Å) and a C18-5E Shodex packed column (250 × 10 mm i.d., 5 μm, 100 Å) using a Shodex RI-71 detector.

Animal Material. The sponges were collected by hand using scuba (20 m depth) in October 2002, off the coast of Jeju Island, Korea. The

Table 6. Antibacterial Activity of Compounds **1**−**3**, **7**, and **11**−**13**[a]

compound	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
1	25.0	25.0	>25.0	>25.0	>25.0	>25.0	25.0	25.0	>25.0	>25.0
2	6.3	12.5	12.5	12.5	12.5	25.0	>25.0	25.0	25.0	25.0
3	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	25.0	25.0	>25.0	>25.0
11	25.0	>25.0	>25.0	>25.0	>25.0	>25.0	25.0	25.0	>25.0	>25.0
13	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	25.0	25.0	>25.0	>25.0
meropenem	0.004	0.004	0.049	0.098	0.049	0.013	0.098	0.391	0.049	0.049
7	25.0	25.0	25.0	25.0	25.0	25.0	>25.0	>25.0	25.0	25.0
meropenem	0.007	0.007	0.098	0.195	0.049	0.025	0.098	0.391	0.098	0.049
12	25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0	>25.0
meropenem	0.013	0.007	0.098	0.195	0.049	0.025	0.098	0.391	0.049	0.049

[a] Data expressed in MIC values (μg/mL). MIC values > 25 μg/mL were determined for *Streptococcus faecium* MD 8b; *Escherichia coli* 078; *E. coli* DC0; *E. coli* TEM; *E. coli* 1507 E; *Pseudomonas aeruginosa* 9027; *Salmonella typhimurium*; *Klebsiella aerogenes* 1522 E; *Enterobcter cloacae* P 99; *E. cloacae* 1321 E. **A**, *Streptococcus pyogenes* 308A; **B**, *S. pyogenes* 77A; **C**, *S. aureus* SG 511; **D**, *S. aureus* 285; **E**, *S. aureus* 503; **F**, *Escherichia coli* DC 2; **G**, *Pseudomonas aeruginosa* 1592E; **H**, *P. aeruginosa* 1771; **I**, *P. aeruginosa* 1771M; **J**, *Klebsiella oxytoca* 1082 E.

collected sample was a loose association of two sponges, *Spongosorites* sp. and *Halichondria* sp. The two sponges were separated, and only *Spongosorites* sp. was subjected to chemical analysis. The morphology of the sponge was described elsewhere.[8] A voucher specimen (registry No. Spo. 44) is deposited at the Natural History Museum, Hannam University.

Extraction and Isolation. The frozen sponge (0.8 kg) was chopped into small pieces and extracted with MeOH at room temperature. The MeOH extract showed significant toxicity to brine shrimp larvae (LD_{50} 23.7 μg/mL). The MeOH extract was partitioned between CH_2Cl_2 and water. The CH_2Cl_2 layer was further partitioned between aqueous MeOH and *n*-hexane. The aqueous MeOH fraction was subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 230 mesh) with a stepped gradient solvent system of 60 to 100% MeOH/H_2O to afford 16 fractions. Fraction 2 (0.80 g), one of the bioactive fractions (LD_{50} 33.9 μg/mL, brine shrimp assay), was subjected to a reversed-phase HPLC (YMC ODS-H80 column) eluting with 75% MeOH to afford 13 subfractions. Compounds **1** (7.2 mg) and **11** (2.5 mg) were obtained by separation of subfraction 2-6 on a reversed-phase HPLC eluting with 45% CH_3CN. Subfraction 2-8 was subjected to successive reversed-phase HPLC eluting with 63% CH_3CN and further purification with 60% and then 55% to afford compounds **2** (2.4 mg) and **6** (1.4 mg). Compounds **3** (4.7 mg) and **14** (1.5 mg) were obtained by separation of subfraction 2-4 on a reversed-phase HPLC (Shodex C18 M10E column) eluting with 38% CH_3CN. Compound **5** (0.8 mg) was obtained by separation of subfraction 2-2 on a reversed-phase HPLC (Shodex C18 M10E column) eluting with 35% CH_3CN. Compound **12** (1.9 mg) was obtained by separation of subfraction 2-5 on a reversed-phase HPLC (Shodex C18 M10E column) eluting with 42% CH_3CN and further purification with 39% CH_3CN. Compound **13** (3.7 mg) was obtained by separation of subfraction 2-3 on a reversed-phase HPLC (Shodex C18 M10E column) eluting with 38% CH_3CN. Subfraction 2-12 was subjected to successive reversed-phase HPLC eluting with 71% MeOH to afford **7** (3.0 mg). Fraction 5 (1.20 g), one of the bioactive fractions (LD_{50} 39.2 μg/mL), was subjected to reversed-phase HPLC (YMC ODS-H80 column) eluting with 62% CH_3CN to afford compounds **2** (9.3 mg), **4** (11.5 mg), **8** (103.0 mg), **9** (28.9 mg), and **10** (49.5 mg). Fraction 11 (3.45 g), one of the bioactive fractions (LD_{50} 14.7 μg/mL), was subjected to reversed-phase HPLC (YMC ODS-H80 column) eluting with 78% MeOH to afford compound **9** (0.5 mg).

(*S*)-6′,6″-Didebromohamacanthin A (1): yellow, amorphous powder; $[\alpha]^{25}_D$ +59 (*c* 0.72, MeOH); IR (film) ν_{max} 3266 (br), 1667 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 330 (3.73), 268 (3.92), 221 (4.17) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRFABMS *m*/*z* 329 $[M + H]^+$; HRFABMS *m*/*z* 329.1393 (calcd for $C_{20}H_{17}ON_4$, 329.1403).

(*R*)-6′-Debromohamacanthin B (2): yellow, amorphous powder; $[\alpha]^{25}_D$ −194 (*c* 0.25, MeOH); ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRFABMS *m*/*z* 407/409 $[M + H]^+$; HRFABMS *m*/*z* 407.0385/409.0462 (calcd for $C_{20}H_{16}ON_4{}^{79}Br$, 407.0507; $C_{20}H_{16}ON_4{}^{81}Br$, 409.0487).

(*R*)-6′,6″-Didebromohamacanthin B (3): yellow, amorphous powder; $[\alpha]^{25}_D$ −288 (*c* 0.4, MeOH); IR (film) ν_{max} 3263 (br), 1675 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 329 (3.84), 267 (3.95), 227 (4.03) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRFABMS *m*/*z* 329 $[M + H]^+$; HRFABMS *m*/*z* 329.1398 (calcd for $C_{20}H_{17}ON_4$, 329.1403).

(*R*)-6″-Debromohamacanthin B (4): yellow, amorphous powder; $[\alpha]^{25}_D$ −83 (*c* 0.5, MeOH); IR (film) ν_{max} 3239 (br), 1671 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 322 (3.78), 277 (3.98), 230 (4.06) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LREIMS *m*/*z* 406/408 $[M]^+$; HREIMS *m*/*z* 406.0429/408.0444 (calcd for $C_{20}H_{15}ON_4{}^{79}Br$, 406.0429; $C_{20}H_{15}ON_4{}^{81}Br$, 408.0411).

(3*S*,5*R*)-6′,6″-Didebromo-3,4-dihydrohamacanthin B (5): yellow, amorphous powder; $[\alpha]^{25}$ +127 (*c* 0.08, MeOH); IR (film) ν_{max} 3267 (br), 1654 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 278 (3.68), 219 (4.17) nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; LRFABMS *m*/*z* 331 $[M]^+$; HRFABMS *m*/*z* 331.1577 (calcd for $C_{20}H_{19}ON_4$, 331.1559).

Spongotine A (6): yellow, amorphous powder; $[\alpha]^{25}_D$ −14 (*c* 0.2, MeOH); IR (film) ν_{max} 3223 (br), 1681 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 320 (3.75), 268 (3.92), 224 (4.27) nm; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 4; LRFABMS *m*/*z* 407/409 $[M + H]^+$; HRFABMS *m*/*z* 407.0464/409.0508 (calcd for $C_{20}H_{16}ON_4{}^{79}Br$, 407.0507; $C_{20}H_{16}ON_4{}^{81}Br$, 409.0487).

Spongotine B (7): yellow, amorphous powder; $[\alpha]^{23}_D$ +43 (*c* 0.3, MeOH); CD (*c* 1 × 10^{-4} M, MeOH) $\Delta\epsilon$ (nm) +1.6 (290), 0.0 (315), −5.40 (338), −1.5 (378), 0.0 (413), 0.0 (500); IR (film) ν_{max} 1693 cm^{-1}; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 4; LRFABMS *m*/*z* 407/409 $[M + H]^+$; HRFABMS *m*/*z* 407.0494/409.0530 (calcd for $C_{20}H_{16}ON_4{}^{79}Br$, 407.0507; $C_{20}H_{16}ON_4{}^{81}Br$, 409.0487).

Spongotine C (8): colorless crystals; $[\alpha]^{25}_D$ −9 (*c* 0.8, MeOH); IR (film) ν_{max} 3354 (br), 1703 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 315 (3.57), 276 (3.85), 221 (4.21) nm; ^{1}H NMR data, see Table 3; ^{13}C NMR data, see Table 4; LREIMS *m*/*z* 484/486/488 $[M]^+$; HREIMS *m*/*z* 483.9532/485.9542/487.9542 (calcd for $C_{20}H_{14}ON_4{}^{79}Br_2$, 483.9534; $C_{20}H_{14}ON_4{}^{79}Br\ {}^{81}Br$, 485.9514; $C_{20}H_{14}ON_4\ {}^{81}Br_2$, 487.9493).

(*S*)-Hamacanthin A (9): yellow, amorphous powder; $[\alpha]^{25}_D$ +58 (*c* 0.05, MeOH); LRFABMS *m*/*z* 485/487/489 $[M + H]^+$.

(*S*)-Hamacanthin B (10): yellow, amorphous powder; $[\alpha]^{25}_D$ +56 (*c* 0.2, MeOH); LREIMS *m*/*z* 484/486/488 $[M]^+$.

(3*S*,5*R*)-6″-Debromo-3,4-dihydrohamacanthin B (11): yellow, amorphous powder; $[\alpha]^{25}_D$ +80 (*c* 0.25, MeOH); LRFABMS *m*/*z* 409/411 $[M + H]^+$.

(3*S*,5*R*)-6′-Debromo-3,4-dihydrohamacanthin B (12): yellow, amorphous powder; $[\alpha]^{25}_D$ +105 (*c* 0.20, MeOH); LRFABMS *m*/*z* 409/411 $[M + H]^+$.

(3*S*,6*R*)-6′-Debromo-3,4-dihydrohamacanthin A (13): yellow, amorphous powder; $[\alpha]^{25}_D$ +31 (*c* 0.38, MeOH); LRFABMS *m*/*z* 409/411 $[M + H]^+$.

(3*S*,6*R*)-6″-Debromo-3,4-dihydrohamacanthin A (14): yellow, amorphous powder; $[\alpha]^{25}_D$ +34 (*c* 0.15, MeOH); LRFABMS *m*/*z* 409/411 $[M + H]^+$.

Evaluation of Cytotoxicity. The rapidly growing cells were harvested, counted, and inoculated at the appropriate concentrations ((1−2) × 10^4 cells/well) into 96-well microtiter plates. After incubation for 24 h, the compounds dissolved in culture medium (RPMI 1640, Gibco; 10% FBS, Gibco) were applied to the culture wells in triplicate followed by incubation for 48 h at 37 °C under a 5% CO_2 atmosphere. The culture was fixed with cold TCA and was stained by 0.4% SRB (sulforhodamine B, Sigma) dissolved in 1% acetic acid. After solubilizing the bound dye with 10 mM unbuffered Tris base by a gyrotatory shaker, the absorbance at 520 nm was measured with a microplate reader (Dynatech Model MR 700). Fifty percent inhibitory concentration (ED_{50}) was defined as the concentration that reduced absorbance by 50% compared to the control level in the untreated wells.

Evaluation of Antibacterial Activity. Mueller Hinton agar plates were impregnated with 17 serial dilutions of the sample and standards (meropenem and imipenem) to make a final concentration of 25 to 0.002 μg/mL. The strains were inoculated into Fleisch extract broth (containing 10% horse serum depending on strains) and incubated at 37 °C for 18 h. The cultured strains were inoculated onto the Mueller Hinton agar plates with 104 cfu per spot population by automatic inoculater (Dynatech, U.S.A.). The MIC was measured after 18 h of incubation.

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Supporting Information Available: ^{1}H and ^{13}C NMR data of compounds **9**−**14**, ^{1}H NMR data of compounds **15**−**18**, and a color photograph of the sponge *Spongosorites* sp. This material is available free of charge via the Internet at http:// pubs.acs.org.

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