New Bromotyrosine Derivatives from an Association of Two Sponges, *Jaspis wondoensis* and *Poecillastra wondoensis*

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Three new bromotyrosine derivatives (**4**−**6**) were isolated from an association of two sponges, *Jaspis wondoensis* and *Poecillastra wondoensis*, along with the previously described (*E*,*E*)-psammaplin A (**1**), (*E*,*Z*)-psammaplin A (**2**), psammaplin D (**3**), bisaprasin (**7**), and (3-bromo-4-hydroxyphenyl)acetonitrile (**8**). The structures of the new compounds were established on the basis of NMR and MS spectroscopic analysis. The compounds **1**, **3**, and **5**−**7** displayed significant cytotoxicity against human lung (A549), ovarian (SK-OV-3), skin (SK-MEL-2), CNS (XF498), and colon (HCT15) cancer cell lines. Compounds **3**−**7** were further evaluated for antibacterial activity against methicillin- or ofloxacin-resistant *Staphylococcus* strains. Compound **4** exhibited more potent antibacterial activity than meropenem against several strains.

Bromotyrosine derivatives are considered as chemotaxonomic markers of sponges of the order Verongida. Although bromotyrosines were mostly encountered in verongid sponges, they have recently been isolated from nonverongid sponges.[2,12,13] Bromotyrosine derivatives range from simple bromotyrosine monomers represented by aeroplysinin-1[1] to more complex bastadins,[10,11] some of which are macrocycles comprised of several bromotyrosine units. Among these metabolites, psammaplins and related compounds[2−9] comprise a small group of metabolites that appear to be biosynthesized by linear connection of bromotyrosines and modified cysteines. Various biological activities of these compounds were reported, and especially for psammaplin A, bioactivities such as antimicrobial activity,[3,7] cytoxicity against the leukemia cell-line P388,[2,8] and inhibition of DNA topoisomerase II[9] were reported.

We previously isolated pectenotoxin II and (*E*,*E*)-psammaplin A (**1**) from an association of two nonverongid sponges, *Jaspis wondoensis* and *Poecillastra wondoensis*.[2] Pectenotoxin II and (*E*,*E*)-psammaplin A exhibited unique bioactivities such as cytotoxicity against human tumor cell lines[2] and antibacterial activity against methicillin-resistant *Staphylococcus aureus*,[3] respectively. In our continuing search for novel bioactive bromotyrosine derivatives from the two-sponge association, new psammaplin derivatives (**4**−**6**) were isolated. Compound **4** and (*E*,*E*)-bromopsammaplin A (**5**) are the structural analogues of psammaplin A, containing an additional sulfur or bromine in their structures. Bispsammaplin A (**6**) is an ether-linked dimeric form of (*E*,*E*)-psammaplin A (**1**), while bisaprasin (**7**) is a carbon−carbon coupled dimeric form of (*E*,*E*)-psammaplin A. The gross structures of the compounds were elucidated on the basis of NMR and MS analyses. Herein we describe the isolation, structure elucidation, and biological evaluation of the new compounds.

The MeOH extracts of the frozen sponges were evaporated in vacuo and partitioned between H_2O and CH_2Cl_2. Bromotyrosine derivatives were found to be distributed in both the H_2O and CH_2Cl_2 partitions, as evidenced by the ^{1}H NMR spectrum of each partition. Selected fractions from both partitions were successively purified by reversed-phase flash column chromatography, MPLC, and HPLC to afford eight bromotyrosine derivatives (**1**−**8**). Five known compounds, namely, two isomeric psammaplin A (**1**, **2**),[2−9] psammaplin D (**3**),[4] bisaprasin (**7**),[7] and (3-bromo-4-hydroxyphenyl)acetonitrile (**8**),[4−6] were identified by comparison of their spectroscopic data with those reported in the literature.

Compound **4** was obtained as a white amorphous solid. The presence of 3-bromo-4-hydroxyphenyl (δ 7.35, 6.74, 7.05; H-2, 5, 6) and isolated benzylic methylene (δ 3.79; H-7) moieties was deduced from comparison of the ^{13}C and ^{1}H NMR data with those of **1** (Table 1). The NMR data of **4** were almost identical to those of (*E*,*E*)-psammaplin A (**1**) with the only exception that the signals corresponding to the protons and carbons of heteroatom-substituted methylenes were shifted (δ 3.60/2.99, 39.0/37.8; H-11/12, C-11/12) from those observed for **1** (δ 3.51/2.80, 39.5/38.5; H-11/12, C-11/12). The FABMS spectrum of **4** showed a cluster of isotopic $[M + H]^+$ ion peaks at *m*/*z* 695/697/699 in approximate ratio of 1:2:1, respectively, indicating the presence of two bromine atoms in the molecule. The observation of only 11 carbon signals in the ^{13}C NMR spectrum revealed that it has a symmetric structure. The $[M + H]^+$ ion cluster has shifted 32 amu from those of **1** and **2**, indicating the presence of an additional sulfur or two oxygens, which could be placed only at the central unit of the structure to explain the symmetry of **4**. Also ESIMS of **4** showed fragment ions at *m*/*z* 363/365 ($C_{11}H_{12}N_2O_3S_2Br$) and 466/468 ($C_{14}H_{17}N_3O_4S_3Br$), a fragmentation pattern that paralleled that of (*E*,*E*)-psammaplin A (**1**) (Figure 1). The fragment ions at *m*/*z* 363/365 were observed as base peaks in the ESIMS/MS experiment. The exact mass of the pseudomolecular ions could not be measured by FABMS due to weak ion currents. By a more sensitive but less accurate MALDI-TOFMS, the $[M + Na]^+$ ion was detected at *m*/*z* 718.9037, which is within tolerable range (calcd for $C_{22}H_{24}N_4O_6S_3{}^{79}Br^{81}BrNa$, 718.9102). Thus, the most fea-

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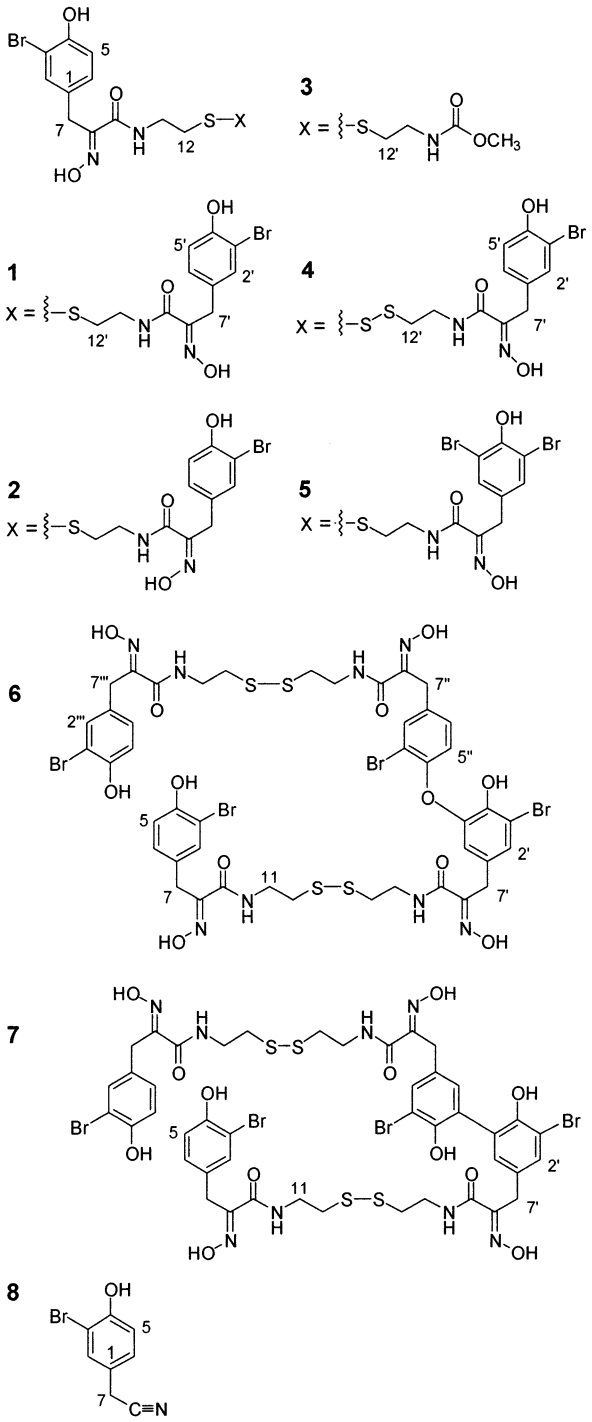

sible structure of compound **4** was proposed as a trisulfide derivative of psammaplin A.

(*E*,*E*)-Bromopsammaplin A (**5**) was obtained as a white amorphous solid. The ESIMS of **5** showed an isotopic cluster of $[M + H]^+$ ion peaks at *m*/*z* 741/743/745/747 in approximate ratio of 1:3:3:1, respectively, indicating the presence of three bromine atoms in the molecule. The NMR data of **5** were simple and highly comparable to those of (*E*,*E*)-psammplin A (**1**). The ^{1}H NMR spectrum (Table 1) revealed that **5** possesses the 3-bromo-4-hydroxybenzyl moiety as found in **1** and a symmetrically substituted benzene ring with a two-proton singlet (δ 7.35; H-2′, H-6′); the deshielding of the resonance suggested that the protons were *ortho* to the bromine substitution. This two-proton singlet showed correlations to C-7′ (δ 28.7) and C-3′/5′ (δ 112.0) in an HMBC experiment. The observed exact mass

Table 1. ^{1}H and ^{13}C NMR Data of Compounds **4** and **5** (CD_3OD)[a]

	4		**5**	
position	δ_H	δ_C	δ_H	δ_C
1		130.3		130.8
2	7.35 (d, 2.0)	134.6	7.36 (d, 2.0)	134.5
3		110.0		110.2
4		153.2		153.7
5	6.74 (d, 8.5)	116.8	6.74 (d, 8.0)	117.0
6	7.05 (dd, 8.5, 2.0)	130.0	7.06 (dd, 8.0, 2.0)	130.6
7	3.79 (s)	28.0	3.76 (s)	28.7
8		152.8		152.8
9		165.8		166.0
11	3.60 (t, 7.0)	39.0	3.51 (t, 7.0)[b]	39.6
12	2.99 (t, 7.0)	37.8	2.81 (t, 7.0)[c]	38.5
12′	2.99 (t, 7.0)	37.8	2.80 (t, 7.0)[c]	38.5
11′	3.60 (t, 7.0)	39.0	3.50 (t, 7.0)[b]	39.6
9′		165.8		166.0
8′		152.8		153.3
7′	3.79 (s)	28.0	3.78 (s)	28.7
6′	7.05 (dd, 8.5, 2.0)	130.0	7.35 (s)	133.0
5′	6.74 (d, 8.5)	116.8		112.0
4′		153.2		151.0
3′		110.0		112.0
2′	7.35 (d, 2.0)	134.6	7.35 (s)	133.0
1′		130.3		128.3

[a] Spectra were recorded in CD_3OD at 500 MHz for ^{1}H and at 50 MHz for ^{13}C, respectively. [b,c] Assignments with the same superscript in the same column may be interchanged.

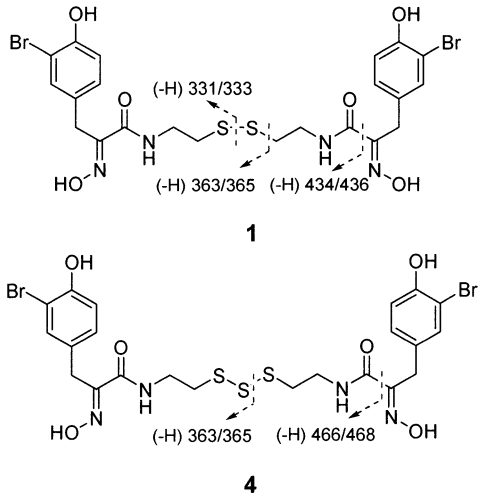

Figure 1. Key MS fragmentations of the $[M + H]^+$ ions of **1** and **4**.

of the $[M + Na]^+$ ion at *m*/*z* 766.8495 closely matched the proposed formula (calcd for $C_{22}H_{23}N_4O_6S_2{}^{79}Br^{81}Br_2Na$, 766.8468, Δ + 2.7 mmu). The geometry of the oxime moieties was defined as *E* on the basis of the ^{13}C chemical shifts of C-7 and -7′ (δ 28.7).[4] Thus, compound **5** was defined as a bromo derivative of (*E*,*E*)-psammplin A (**1**).

Bispsammaplin A (**6**) was isolated as a yellow oil. Analysis of the ^{1}H NMR and COSY spectra of **6** revealed that it possessed four separate sets of aromatic proton resonances (Table 2). Two identical proton spin systems, which could be designated as two 3-bromo-4-hydroxybenzyl moieties as in **1**, were seen at δ 7.35 (2H, d, J = 2.0 Hz, H-2, 2‴), 6.74 (2H, d, J = 8.5 Hz, H-5, 5‴), and 7.05 (2H, dd, J = 8.5 Hz, 2.0, H-6, 6‴). A third benzene ring with a 1,3,4-trisubstitution was indicated by the resonances at δ 7.54 (1H, d, J = 2.0 Hz, H-2″), 6.75 (1H, d, J = 8.5 Hz, H-5″), and 7.20 (1H, dd, J = 8.5 Hz, 2.0, H-6″), while the final benzene ring was 1,3,4,5-tetrasubstituted as gauged by resonances at δ 7.14 (1H, d, J = 2.0 Hz, H-2′) and 6.57 (1H, d, J = 2.0 Hz, H-6′). Also, the chemical shifts of the partial structure with a catechol-phenol hemisphere were

Table 2. ^{1}H and ^{13}C NMR Data of Compound **6** (CD_3OD, 500 MHz)[a]

position	δ_H	δ_C	position	δ_H	δ_C
1, 1‴		130.8			
2, 2‴	7.35 (d, 2.0)	133.3			
3, 3‴		109.5			
4, 4‴		152.8			
5, 5‴	6.74 (d, 8.5)	116.0			
6, 6‴	7.05 (dd, 8.5, 2.0)	129.2			
7, 7‴	3.78 (s)	27.5			
8, 8‴		152.0			
9, 9‴		164.7			
11, 11‴	3.51 (t, 7.0)	38.5			
12, 12‴	2.80 (t, 7.0)	37.4			
12′	2.81 (t, 7.0)[b]	37.4	12″	2.76 (t, 7.0)[b]	37.4
11′	3.54 (t, 7.0)[c]	38.5	11″	3.47 (t, 7.0)[c]	38.5
9′		164.6	9″		164.6
8′		151.7	8″		151.5
7′	3.70 (s)	27.6	7″	3.88 (s)	27.9
6′	6.57 (d, 2.0)	118.3	6″	7.20 (dd, 8.5, 2.0)	129.6
5′		145.3	5″	6.75 (d, 8.5)	119.6
4′		145.3	4″		152.3
3′		110.9	3″		113.6
2′	7.14 (d, 2.0)	128.2	2″	7.54 (d, 2.0)	134.0
1′		128.7	1″		129.3

[a] Spectra were recorded in CD_3OD at 500 MHz for ^{1}H and at 125 MHz for ^{13}C, respectively. [b,c] Assignments with the same superscript may be interchanged.

Table 3. Cytotoxicities (ED_{50}, μg/mL) of the Compounds against Human Solid Tumor Cells[a]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	0.57	0.14	0.13	0.57	0.68
3	0.80	0.17	0.20	0.60	1.23
5	1.34	1.38	0.90	0.92	3.31
6	1.53	1.52	1.02	1.10	3.35
7	3.40	2.78	2.94	2.44	6.00
doxorubicin	0.04	0.15	0.03	0.10	0.09

[a] A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT 15, human colon cancer.

similar to those of related portions of the bastadins.[9,10] HSQC and HMBC data were used to confirm structures of the three different dipeptide units. The HMBC spectrum of **6** showed self-consistent correlations from benzylic methylene proton signals (δ 3.78, 3.70, 3.88, 3.78; H-7, 7′, 7″, 7‴) to the respective *ortho* aryl carbon signals (δ 133.3/129.2, 128.2/118.3, 134.0/129.6, 133.3/129.2; C-2/6, C-2′/6′, C-2″/6″, C-2‴/6‴). Three bond ^{13}C−^{1}H correlations of the proton signals at δ 7.14 (H-2′) and 6.57 (H-6′) to oxygenated aromatic carbon signals (δ 145.3, C-4′, 5′) were observed. Supporting evidence for the connectivity between two phenyl groups was provided by the correlation between H-6′ (δ 6.57) and H-5″ (δ 6.75) in the NOESY spectrum. The stereochemistry of the oxime groups in **6** was assigned as *E* on the basis of the characteristic ^{13}C chemical shifts of the benzylic carbons (δ 27.9, 27.6, 27.5).[3] The ESIMS spectrum of **6** showed a complex isotopic cluster of $[M + Na]^+$ ion peaks centered at *m*/*z* 1349 in an approximate ratio of 1:4:6:4:1. In MALDI-TOFMS, the $[M + Na]^+$ ion detected at *m*/*z* 1348.9407 was within tolerable range (calcd for $C_{44}H_{46}N_8O_{12}S_4{}^{79}Br_2{}^{81}Br_2Na$, 1348.8714, Δ + 69.3 mmu, Δ + 51.4 ppm). Thus, the structure of **6** was defined as a dimer of (*E*,*E*)-psammplin A (**1**) of which two (*E*,*E*)-psammaplin A units were linked by an ether bond.

Compounds **1**, **3**, **5**, **6**, and **7** were evaluated for cytotoxicity against five human solid tumor cell lines. Significant cytotoxicity against human lung (A549), ovarian (SK-OV-3), skin (SK-MEL-2), CNS (XF498), and colon (HCT15) cancer cell lines (Table 3) was observed. (*E*,*E*)-Psammaplin A (**1**) exhibited the highest potency among the compounds tested. Antibacterial activity against antibiotic-resistant pathologic strains was determined for compounds **3**−**7**, since significant antibacterial activity in (*E*,*E*)-psammaplin was previously observed.[3] Compound **4** showed the highest potency among the compounds tested, and the potency was higher than that of meropenem against several strains (Table 4).

Experimental Section

General Experimental Procedures. Optical rotations were recorded using a JASCO DIP-370 digital polarimeter. IR spectra were measured by a JASCO FT/IR-410 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200 and Varian Inova 500 instruments. Chemical shifts were reported with reference to the respective solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). FABMS data were obtained on a JEOL JMS-SX-102A double focusing MS. ESIMS data were obtained using a Finnigan DecaXP. MALDI-TOFMS data were obtained on an Applied Biosystems Voyager-DESTR. HPLC was performed with a YMC-Pack ODS column (preparative, 250 × 20 mm, 4 μm, 80 Å) and a C18-5E Shodex packed column (semipreparative, 250 ×10 mm, 5 μm, 100 Å) using a Shodex RI-71 detector.

Animal Material. The sponges were collected in July 1999 (15−25 m depth), off the coast of Gomun Island, Korea. Prof. C. J. Sim, Hannam University, identified the specimen as an association of two sponges, *Jaspis wondoensis* Sim & Kim (family Jaspidae) and *Poecillastra wondoensis* Sim & Kim (family Pachastrellidae).[14] The voucher specimen (J99K-1) of the sponge was deposited in the Natural History Museum, Hannam University, Daejon, Korea.

Extraction and Isolation. The frozen sponges (10 kg) were extracted with MeOH at room temperature. The crude extract was partitioned between H_2O and CH_2Cl_2, followed by partitioning of the CH_2Cl_2 layer between aqueous MeOH and

Table 4. Antibacterial Activity (MIC, μg/mL) of the Compounds against Methicillin-Resistant or Ofloxacin-Resistant *Staphylococcus* Strains[a]

strain	**3**	**4**	**5**	**6**	**7**	meropenem
S. aureus KIST 1[b]	>25.0	3.1	12.5	>50.0	12.5	25.0
S. aureus KIST 2[b]	>25.0	3.1	12.5	>50.0	6.3	3.1
S. aureus KIST 3[b]	>25.0	6.3	12.5	>50.0	6.3	50.0
S. aureus KIST 4[b]	>25.0	3.1	12.5	>50.0	6.3	50.0
S. aureus 003[c]	>25.0	3.1	12.5	>50.0	12.5	3.1
S. aureus 004[c]	>25.0	3.1	12.5	>50.0	6.3	3.1
S. aureus Y-80-12-1109[d]	>25.0	6.3	12.5	>50.0	50.0	50.0
S. aureus Y-80-12-1999[d]	>25.0	6.3	12.5	>50.0	50.0	50.0
S. aureus Y-80-12-844[d]	>25.0	25.0	25.0	>50.0	25.0	50.0
S. epidermidis 178[e]	>25.0	0.8	3.2	>50.0	6.3	0.8
S. epidermidis 291[e]	>25.0	0.8	3.2	>50.0	12.5	1.6

[a] A total of 40 strains were employed for testing, and only the strains to which compound **4** exhibited equipotency to or higher potency than meropenem were registered. [b] Strains were obtained from KIST (Korea Institute of Science and Technology). [c] Strains were obtained from LG Chemical, Korea. [d] Strains were obtained from Yon-sei Medical Center, Korea. [e] Ofloxacin-resistant strains.

n-hexane, to yield aqueous methanol (6.3 g) and *n*-hexane-soluble (11.9 g) fractions. The aqueous MeOH layer was then subjected to successive reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 500/400 mesh), eluting with a step gradient solvent system of 67−100% MeOH/H_2O to afford 16 fractions. The fraction eluted with 75% MeOH was dried, and the residue (1.6 g) was separated by reversed-phase MPLC. Subfractions 5−7 were further purified by repeated HPLC (C18-5E Shodex packed column, 250 × 10 mm, 5 μm, 100 Å) using 65% MeOH/H_2O as a mobile phase to give compounds **1** (100 mg), **2** (7 mg), and **3** (4 mg). Compound **8** was obtained by HPLC (C18-5E Shodex packed column, 250 × 10 mm, 5 μm, 100 Å) purification of fraction 4 using 45% MeOH/H_2O as a mobile phase.

The H_2O layer of the extract was also abundant in psammaplin derivatives. The H_2O layer was extracted with *n*-BuOH. A portion (2 g) of the *n*-BuOH extract (8 g) was subjected to MPLC (YMC ODS 60 Å, 400/230 mesh) eluting with a solvent system of 35−100% MeOH/H_2O, to afford 30 fractions. Fraction 12 was repeatedly chromatographed on HPLC (YMC ODS-H80, 250 × 20 mm, 4 μm, 80 Å) eluting with 72% MeOH/H_2O, to yield compounds **4** (1 mg), **5** (4 mg), and **7** (10 mg). Fraction 14 was separated by HPLC (YMC ODS-H80, 250 × 20 mm, 4 μm, 80 Å) eluting with 80% MeOH/H_2O, to afford compound **6** (5 mg).

***(E,E)*-Psammaplin A (1):** white amorphous solid; ^{1}H NMR (500 MHz, CD_3OD) δ 7.35 (2H, d, J = 2.0 Hz, H-2, 2′), 7.05 (2H, dd, J = 8.5, 2.0 Hz, H-6, 6′), 6.75 (2H, d, J = 8.5 Hz, H-5, 5′), 3.78 (4H, s, H-7, 7′), 3.51 (4H, t, J = 6.8 Hz, H-11, 11′), 2.80 (4H, t, J = 6.8 Hz, H-12, 12′); ^{13}C NMR (50 MHz, CD_3OD) δ 165.8 (C-9, 9′), 153.7 (C-8, 8′), 153.1 (C-4, 4′), 134.4 (C-2, 2′), 130.6 (C-6, 6′), 130.4 (C-1, 1′), 117.0 (C-5, 5′), 110.5 (C-3, 3′), 39.5 (C-11, 11′), 38.5 (C-12, 12′), 28.7 (C-7, 7′); FABMS *m*/*z* 663 (48), 665 (100), 667 (58) $[M + H]^+$, 685 (35), 687 (78), 689 (43) $[M + Na]^+$.

***(E,Z)*-Psammaplin A (2):** white amorphous solid; ^{1}H NMR (500 MHz, CD_3OD) δ 7.36 (1H, d, J = 2.0 Hz, H-2), 7.31 (1H, d, J = 2.0 Hz, H-2′), 7.07 (1H, dd, J = 8.5, 2.0 Hz, H-6′), 7.05 (1H, dd, J = 8.5, 2.0 Hz, H-6), 6.78 (1H, d, J = 8.5 Hz, H-5), 6.76 (1H, d, J = 8.5 Hz, H-5′), 3.79 (2H, s, H-7), 3.57 (2H, s, H_2-7′), 3.54 (2H, t, J = 7.0 Hz, H-11), 3.36 (2H, t, J = 7.0 Hz, H-11′), 2.81 (2H, t, J = 7.0 Hz, H-12), 2.78 (2H, t, J = 7.0 Hz, H-12′); ^{13}C NMR (50 MHz, CD_3OD) δ 165.9 (C-9′) 165.8 (C-9), 154.2 (C-8′), 153.7 (C-4, 4′), 152.7 (C-8), 134.5 (C-2, 2′), 130.6 (C-6′), 130.4 (C-6), 130.4 (C-1, 1′), 117.4 (C-5) 117.0 (C-5′), 110.7 (C-3), 110.5 (C-3′), 39.6 (C-11′), 39.3 (C-11), 38.3 (C-12, 12′), 37.5 (C-7′) 28.7 (C-7); FABMS *m*/*z* 663 (48), 665 (100), 667 (58) $[M + H]^+$, 685 (35), 687 (78), 689 (43) $[M + Na]^+$.

Psammaplin D (3): white amorphous solid; ^{1}H NMR (500 MHz, CD_3OD) δ 7.36 (1H, d, J = 2.0, H-2), 7.07 (1H, dd, J = 8.5, 2.0, H-6), 6.76 (1H, d, J = 8.5, H-5), 3.78 (2H, s, H-7), 3.61 (3H, s, OCH_3), 3.54 (2H, t, J = 7.0, H-11), 3.36 (2H, t, J = 7.0, H-11′), 2.81 (2H, t, J = 7.0, H-12), 2.78 (2H, t, J = 7.0, H-12′); ^{13}C NMR (50 MHz, CD_3OD) δ 165.8 (C-9′) 164.1 (C-9), 153.7 (C-8), 153.2 (C-4), 134.5 (C-2), 130.4 (C-6), 130.6 (C-1), 117.0 (C-5), 110.5 (C-3), 52.2 (C-OCH_3), 39.6 (C-11′), 39.3 (C-11), 39.7(C-12), 38.5 (C-12′), 28.7 (C-7); FABMS *m*/*z* 466 (91), 468 (100) $[M + H]^+$.

Compound 4: white amorphous solid; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 695 (46), 697 (100), 699 (60) $[M + H]^+$, 717 (35), 719 (74), 721 (45) $[M + Na]^+$, 466 (19), 468 (20), 363 (19), 365 (22); MALDI-TOFMS *m*/*z* 718.9037 $[M + Na]^+$ (calcd for $C_{22}H_{24}N_4O_6S_3{}^{79}Br^{81}BrNa$, 718.9102).

***(E,E)*-Bromopsammaplin A (5):** white amorphous solid; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 741 (25), 743 (81), 745 (100), 747 (35) $[M + H]^+$; MALDI-TOFMS *m*/*z* 766.8495 $[M + Na]^+$ (calcd for $C_{22}H_{23}N_4O_6S_2{}^{79}Br^{81}Br_2Na$, 766.8468), 764.8561 $[M + Na]^+$ (calcd for $C_{22}H_{23}N_4O_6S_2{}^{79}Br_2{}^{81}BrNa$, 764.8487).

Bispsammaplin A (6): yellow oil; $[\alpha]^{23}_D$ −3° (*c* 0.21, MeOH); IR (KBr disk) ν_{max} 3369, 1655, 1534, 1490, 1426, 1228, 1016, 669 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; ESIMS *m*/*z* 1323 (8), 1325 (38), 1327 (52), 1329 (43), 1331 (15) $[M + H]^+$, 1345 (13), 1347 (54), 1349 (100), 1351 (77), 1353 (25) $[M + Na]^+$; MALDI-TOFMS *m*/*z* 1348.9407 $[M + Na]^+$ (calcd for $C_{44}H_{46}N_8O_{12}S_4{}^{79}Br_2{}^{81}Br_2Na$, 1348.8714), 1350.9407 $[M + Na]^+$ (calcd for $C_{44}H_{46}N_8O_{12}S_4{}^{79}Br^{81}Br_3Na$, 1350.8699).

Bisaprasin (7): yellow oil; ^{1}H NMR (500 MHz, CD_3OD) δ 7.40 (1H, d, J = 2.0 Hz, H-2′), 7.35 (1H, d, J = 2.0 Hz, H-2), 7.06 (1H, d, J = 8.5 Hz, H-6′), 7.05 (1H, dd, J = 8.5, 2.0 Hz, H-6), 6.74 (1H, d, J = 8.5 Hz, H-5), 3.84 (2H, s, H-7′), 3.78 (2H, s, H-7), 3.51 (2H, t, J = 7.0 Hz, H-11), 3.49 (2H, t, J = 7.0 Hz, H-11′), 2.79 (2H, J = 7.0 Hz, H-12), 2.77 (2H, t, J = 7.0 Hz, H-12′); ^{13}C NMR (50 MHz, CD_3OD) δ 164.8, 164.6 (C-9, 9′), 152.3 (C-4), 151.9 (C-8, 8′), 150.3 (C-4′), 133.4 (C-2), 132.6 (C-2′), 131.5 (C-6′), 129.3, 129.2 (C-6, 1), 127.5 (C-1′), 116.0 (C-5), 111.9 (C-3′), 109.2 (C-3), 38.3 (C-11, 11′), 37.2 (C-12, 12′), 27.6, 27.5 (C-7, 7′).

(3-Bromo-4-hydroxyphenyl)acetonitrile (8): white amorphous solid; ^{1}H NMR (500 MHz, CD_3OD) δ 7.44 (1H, d, J = 2.0 Hz, H-2), 7.14 (1H, dd, J = 8.5, 2.0 Hz, H-6), 6.86 (1H, d, J = 8.5 Hz, H-5), 3.76 (2H, s, H-7); ^{13}C NMR (50 MHz, CD_3OD) δ 155.7 (C-4), 133.6 (C-2), 129.3 (C-6), 125.0 (C-1), 119.0 (C-8), 117.7 (C-5), 111.3 (C-3), 22.2 (C-7); ESIMS *m*/*z* 210 (91), 212 (100) $[M - H]^-$.

Antibacterial Assay. Forty clinically isolated 40 bacterial strains were inoculated into 3 mL of Fleisch extract broth (beef extract 1%, peptone 1%, NaCl 0.3%, $Na_2HPO_4{\cdot}12H_2O$ 0.2%, pH 7.4−7.5. 10% horse serum was supplemented for *Streptococcus pyogenes* and *S. faecium*) and cultured on a shaking incubator at 37 °C for 18 h. Tested compounds and a standard compound, meropenem (Yuhan, Korea), were 2-fold serially diluted from 100 μg/mL to 0.02 μg/mL (17 tubes). Each 1.5 mL of the diluted compounds was mixed with 13.5 mL of Muller Hinton agar (Difco), and agar plates were prepared. The overnight-cultured broths were 100 times diluted and transferred into a 96-well plate. Using an automatic inoculator (Dynatech), the diluted culture strains were inoculated (10^4 CFU/spot) on the prepared agar plates. The plates were incubated at 37 °C for 18 h. At the end of incubation, the growth state of each inoculum on the Petri dish plate was observed and the MIC was determined.

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