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Protuboxepins A and B and Protubonines A and B from the Marine-Derived Fungus *Aspergillus* sp. SF-5044

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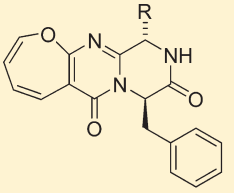
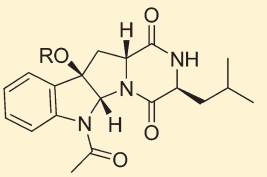
Supporting Information

ABSTRACT: Two new oxepin-containing (**1** and **2**) and two diketopiperazine-type alkaloids (**3** and **4**) have been isolated from an EtOAc extract of the marine-derived fungus *Aspergillus* sp. SF-5044. The structures of these metabolites were determined through analysis of NMR and MS data, along with Marfey's method. Compound **1** showed weak growth inhibitory activity against a small panel of cell lines.



1 R = $CH(CH_3)CH_2CH_3$
2 R = $CH(CH_3)_2$



3 R = H
4 R = Ac

Fungi have proven to be valuable resources for the discovery of novel secondary metabolites. Because the marine environment provides unique ecosystems and living conditions, marine fungi have been recognized as a potential source of diverse novel secondary metabolites.[1−3] As a part of our ongoing studies of bioactive secondary metabolites from marine microorganisms from Korea, we have investigated the chemical constituents of the extracts obtained from cultures of the marine-derived fungus *Aspergillus* sp. SF-5044. This study led to the isolation of two new oxepin-containing diketopiperazine-type metabolites, named protuboxepins A (**1**) and B (**2**), and two new diketopiperazine alkaloids, protubonines A (**3**) and B (**4**).

Protuboxepin A (**1**) was assigned the molecular formula $C_{22}H_{23}N_3O_3$ on the basis of HRESIMS data (*m*/*z* 378.1824 $[M + H]^+$), which was fully supported by the ^{1}H and ^{13}C NMR data (Table 1). In the ^{1}H NMR spectrum, signals appear for $-CH-CH_3$ and $-CH_2CH_3$ units, one additional methine unit, one methylene group, and 10 deshielded protons between δ 5.34 and 7.32. The presence of a benzyl group was suggested by resonances representing a phenyl group along with a benzylic methylene signal at $\delta_{C/H}$ 37.8/3.35. The presence of characteristic signals for an α-amino carbon and proton ($\delta_{C/H}$ 58.4/5.34) and carbonyl/amide carbonyl carbons (δ_C 162.7−169.3) in the NMR spectra of **1** was indicative of the incorporation of amino acid residues in the molecule. Analysis of COSY and HMQC data led to the identification of an isolated proton spin-system corresponding to C9−C12 as well as the $-CHCH(CH_3)-CH_2CH_3$ moiety in structure **1** derived from isoleucine. In addition, the presence of a unit derived from phenylalanine was deduced from the COSY data and an HMBC correlation of H-1 with C-17. The ^{13}C NMR chemical shifts of C-1 and C-4 indicated that these two carbons were attached to nitrogen atoms, and HMBC correlations of H_2-16, H-1, and H-4 with one of the downfield sp^2 carbons (δ 169.3) positioned this carbon at C-2. HMBC correlations of H-1 with C-5 and of H-23 with C-5, in turn, led to the completion of the diketopiperazine ring in the molecule. HMBC correlations of H-9 with C-7, along with its chemical shift (δ 164.4), suggested the connection of C-7 and C-9 via an oxygen atom. The presence of the oxepin ring was revealed by the observation of HMBC correlations of H-11 with C-13 and of H-12 with C-7. The position of the amide carbonyl carbon (C-14) was indicated by HMBC correlations of H-1 with C-14 and of H-12 with C-14. The only remaining heteroatom (N-6) was then connected to C-5 and C-7 to account for the required unsaturation equivalents. A weak four-bond HMBC correlation of H-4 with C-7 supported this assignment. Therefore, the remaining exchangeable proton was attached to the only remaining connection site at the nitrogen atom (N-3), and the planar structure of the compound was assigned as shown in **1**. A literature search revealed several fungal metabolites[4−6] having a carbon skeleton similar to that of **1**, and relevant ^{1}H and ^{13}C NMR chemical shifts of these previously reported metabolites were consistent with those of the proposed structure of protuboxepin A.

Application of Marfey's method[7] enabled assignment of the D-absolute configuration of the phenylalanine residue in **1**. In addition, NOESY correlations of H-4 with H_2-16 and H-18/H-22 indicated that these protons are on the same face of the C-ring. Thus, the absolute configurations of C-1 and C-4 were assigned as *R* and *S*, respectively. However, at present, the absolute configuration at C-23 has not been determined.

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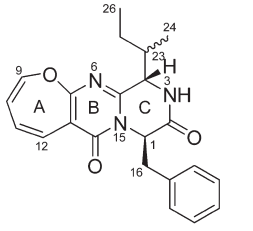
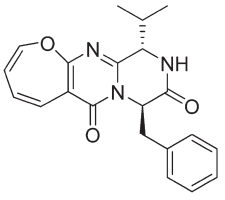
The molecular formula of protuboxepin B (**2**) was established as $C_{21}H_{21}N_3O_3$ by analysis of its HRESIMS (*m/z* 364.1672 $[M + H]^+$) data. The ^1H NMR spectrum of **2** was almost identical to that of **1** except for the presence of signals corresponding to a $-CH-CH(CH_3)_2$ unit instead of the $-CH-CH(CH_3)CH_2CH_3$ unit observed in **2**. This was further confirmed by analysis of its COSY data. Therefore, protuboxepin B (**2**) was proposed to differ from **1** by incorporation of a valine residue rather than an isoleucine unit. Detailed 2D NMR analysis of compound **2** was not pursued due to the clear analogy with **1** and the limited quantity of material, but the relative configuration of **2** was proposed by the observation of NOESY correlations of H-4 with H-18/H-22.



1



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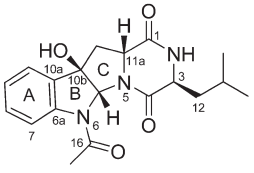

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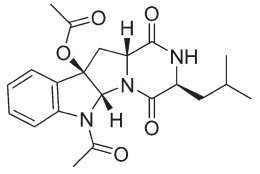

4

The molecular formula of protubonine A (**3**) was established as $C_{19}H_{23}N_3O_4$ on the basis of HRESIMS (*m/z* 358.1768 $[M + H]^+$) analysis and NMR data, which required 10 degrees of unsaturation. Analysis of ^1H, ^{13}C NMR and DEPT NMR data of **3** indicated the presence of three methyl groups, two methylene units, eight methines, four of which are aromatic protons, one quaternary sp^3 carbon, two nonprotonated aromatic carbons, and three carbonyl carbons. These data accounted for all of the ^1H and ^{13}C NMR resonances except for one exchangeable signal at δ_H 5.86. In addition, interpretation of coupling patterns in the aromatic region of the ^1H NMR spectrum suggested the presence of a 1,2-disubstituted aromatic ring. Considering the presence of three carbonyl groups, the compound must have three additional rings to account for the required unsaturation equivalents. The two methine peaks at $\delta_{C/H}$ 53.1/3.91 and $\delta_{C/H}$ 58.8/3.81 were suggestive of two amino acid α-CH units. Analysis of the COSY data revealed the presence of a leucine (Leu) residue and a $-CH-CH_2-$ unit and confirmed the 1,2-disubstituted aromatic ring. HMBC correlations of the amide proton at δ 5.86 with two amide carbonyls and two methines (C-1 and C-4), together with HMBC correlations of H_2-12 with C-4 and of H-11a with C-1, established the presence of a diketopiperazine ring. An indoline ring moiety in the compound was established by key HMBC correlations of H-10 with C-10b, of H-11 with C-10a, C-10b, and C-5a, and of H-5a with C-10a, C-10b, and C-6a, together with the downfield chemical shift of C-5a (δ_C 82.1). HMBC correlations of H-5a with C-4 and C-11a enabled the connection to N-5 of the diketopiperazine ring, thus allowing establishment of the C-ring. The presence of an acetyl group was identified by a HMBC correlation of H_3-17 with C-16, and this group was attached to the nitrogen atom of the indoline moiety (N-6) on the basis of a HMBC correlation of H-5a with C-16. At this point, all structural features were accounted for except for a hydroxy group and one connection site at C-10b. Therefore, C-10b was assigned to bear a hydroxy group to complete the gross structure of **3** as shown.

The relative configuration assigned for protubonine A (**3**) was deduced by analysis of NOESY data. NOESY correlations of H-11a with H-5a and H_2-12 indicated that these protons were close in space. Marfey's analysis of the acid hydrolysates of **3** indicated that the Leu residue in the molecule has the L-configuration. Therefore, the configuration at C-3 was determined as *S*. Considering the relative configuration established for **3** by NOESY analysis, the absolute configurations of the remaining stereogenic centers were assigned as 5a*R*, 11a*R*. In addition, in a view of a presumed cis-ring junction of the B and C rings, the absolute configuration at C-10b was proposed to be *R*.

Compound **4** was readily identified as the O-acetylated derivative of protubonine A by analysis of its NMR and MS data. Interpretation of COSY, HMQC, and HMBC NMR data of **4** allowed assignment of all ^1H and ^{13}C NMR resonances as well as the location of the acetoxy group at C-10b. The relative configuration of **4** was also deduced from the observation of key NOESY correlations of H-11a with H-5a and thus proposed to be identical with that of compound **3**. A literature search indicated that the planar structure of compound **4** was reported in a recent Chinese patent.[8] However, the absolute configuration of the compound is proposed for the first time in this report.

Protuboxepins A (**1**) and B (**2**) are new members of a rarely observed class of natural products containing both oxepin and diketopiperazine ring systems. Previously described fungal metabolites containing these structural features include cinereain,[5] oxepinamides A–C,[6] and janoxepin.[7] Various biological activities such as anti-inflammatory,[6] antiplasmodial,[7] and liver X-activated receptor (LXR) agonist[8] effects have been reported for these compounds. Protuboxepins A and B possess the same ring system, but differ from those reported previously by incorporating different amino acid residues. Moreover, protuboxepins contain the relatively rare D-Phe residue.

Compounds **1**, **3**, and **4** were evaluated for growth inhibitory activity against five cell lines. Protuboxepin A (**1**) showed weak inhibitory activity against human acute promyelocytic leukemia cells (HL-60), human breast cancer adenocarcinoma cells (MDA-MB-231), hepatocellular carcinoma cells (Hep3B), rat fibroblast cells (3Y1), and chronic myelogenous leukemia cells (K562) with IC_{50} values of 75, 130, 150, 180, and 250 μM, respectively. On the other hand, compounds **3** and **4** showed no inhibitory activity against these cells at the 250 μM level.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 341 digital polarimeter. UV spectra were recorded on a Biochrom 1300 UV/visible spectrophotometer. NMR spectra (1D and 2D) were recorded in CD_3OD or $CDCl_3$ using a JEOL JNM ECP-400 spectrometer (400 MHz for ^1H and 100 MHz for ^{13}C), and chemical shifts were referenced relative to the corresponding residual solvent signals (CD_3OD: δ 3.30/49.0; $CDCl_3$: δ 7.26/77.0). HMQC and HMBC experiments were optimized for $^1J_{CH}$ = 140 Hz and $^nJ_{CH}$ = 8 Hz, respectively. ESIMS data were obtained using a Q-TOF micro LC-MS/MS instrument (Waters) at Korea University, Seoul, Korea. Solvents for extractions and flash column chromatography were

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Table 1. NMR Spectroscopic Data (^{1}H 400 MHz, CD_3OD) for Protuboxepins A (1) and B (2)

	protuboxepin A (**1**)		protuboxepin B (**2**)
position	δ_C	δ_H, mult. (J in Hz)	δ_H, mult. (J in Hz)
1	58.4	5.34, br d (4.0)	5.34, br d (4.0)
2	169.3		
4	59.4	2.75, br d (1.4)	2.74, br d (1.8)
5	157.7		
7	164.4		
9	144.6	6.08, d (5.5)	6.08, d (5.5)
10	118.4	5.72, dd (5.9, 5.5)	5.72, dd (5.5, 5.5)
11	129.3	6.21, dd (11.4, 5.9)	6.22, dd (11.0, 5.5)
12	126.1	6.73, d (11.4)	6.73, d (11.0)
13	111.4		
14	162.7		
16	37.8	3.35, m	3.36, m
17	135.8		
18/22	130.9	6.93, br d, (7.0)	6.93, br d, (7.0)
19/21	129.9	7.25, br t (7.7)	7.24, br t (7.7)
20	129.1	7.32, br t, (7.3)	7.31, br t, (7.3)
23	38.8	2.13, m	2.46, m
24	15.4	0.84, d (7.0)	0.87, d (7.4)
25	24.8	1.14, m	0.69, d (7.0)
26	12.3	0.75, t (7.3)	

Table 2. NMR Spectroscopic Data (^{1}H 400 MHz, $CDCl_3$) for Protubonines A (3) and B (4)

	protubonine A (**3**)		protubonine B (**4**)	
position	δ_C	δ_H, mult. (J in Hz)	δ_C	δ_H, mult. (J in Hz)
1	168.2		167.4	
2		5.86, br s		5.99, br s
3	53.1	3.91, dd (9.9, 3.3)	53.3	4.02, dd (9.3, 3.0)
4	166.6		166.1	
5a	82.1	5.92, s	80.1	6.39, s
6a	143.2		144.6	
7	119.7	7.98, d (8.1)	119.2	8.05, d (7.6)
8	131.2	7.37, dd (8.1, 7.6)	131.4	7.40, dd (7.6, 7.4)
9	125.5	7.17, dd (7.6, 7.3)	125.1	7.18, dd (7.4, 7.4)
10	123.2	7.42, d (7.3)	124.9	7.54, d (7.4)
10a	131.5		127.9	
10b	83.8		88.0	
11	39.0	2.89, dd (12.8, 5.5)	39.6	3.26, dd (12.4, 5.6)
		2.59, dd (12.8, 11.4)		2.59, dd (12.4, 11.8)
11a	58.8	3.81, dd (11.4, 5.5)	58.1	3.94, dd (11.8, 5.6)
12	38.9	1.99, m	39.5	2.02, m
		1.53, m		1.59, m
13	24.5	1.69, m	24.6	1.71, m
14	23.4	1.00, d (6.2)	23.3	1.00, d (6.5)
15	21.1	0.91, d (6.6)	21.1	0.93, d (6.5)
16	171.5		171.3	
17	23.8	2.64, s	23.6	2.65, s
O-Ac			169.5	
			21.3	2.04, s

reagent grade and used without further purification. Solvents used for HPLC were analytical grade. Flash column chromatography was carried out using YMC octadecyl-functionalized silica gel (C_{18}). HPLC separations were performed on an Agilent semiprep-C_{18} column (21.2 × 150 mm; 5 μm particle size; 5 mL/min). Compounds were detected by UV absorption at 210 nm.

Fungal Strain Isolation and Identification. *Aspergillus* sp. SF-5044 (deposited at the College of Medical and Life Sciences fungal strain repository, Silla University) was isolated from an intertidal sediment sample collected from Dadaepo Beach, Busan, Korea, in April 2006. The sediment sample was stored in a sterile plastic bag and transported to the laboratory, where it was kept frozen until processed. The sample was diluted 10-fold using sterile seawater. A 1 mL amount of the diluted sample was processed utilizing the spread plate method in potato dextrose agar (PDA) medium containing 3% NaCl. The plate was incubated at 25 °C for 14 days. After purifying the isolates several times, the final pure cultures were selected and preserved at −70 °C. A GenBank search with the 28S rRNA gene of SF-5044 (Genbank accession number FJ935999) indicated *Aspergillus protuberus* (FJ176897) as the closest match, showing a sequence identity of 99.64%. Therefore, the marine-derived fungal strain SF-5044 was identified as an *Aspergillus* sp.

Fermentation, Extraction, and Isolation. The fungal strain was cultured on 110 Petri plates (90 mm), each containing 20 mL of PDA with 3% NaCl. Plates were individually inoculated with 2 mL of seed cultures of the fungal strain and incubated at 25 °C for a period of 10 days. Extraction of the combined agar media with EtOAc (2 L) provided an organic phase, which was then concentrated *in vacuo* to yield 2.0 g of an extract. The EtOAc extract was subjected to C_{18} flash column chromatography (5 × 26 cm), eluting with a stepwise gradient of 20%, 40%, 60%, 80%, and 100% (v/v) MeOH in H_2O (500 mL each). The fraction that eluted at 80% MeOH (261 mg) was subjected to silica flash column chromatography (3.5 × 10 cm), eluting with a stepwise gradient of 0% to 20% (v/v) MeOH in CH_2Cl_2 (200 mL each, 1% increment for each fraction). The fraction that eluted with 1% MeOH (30.0 mg) was further purified by semipreparative reversed-phase HPLC eluting with a gradient from 70% to 80% MeOH in H_2O (0.1% formic acid) over 25 min to yield **1** (8.5 mg, t_R = 22.0 min). Compound **2** was purified by additional semipreparative HPLC of the fraction collected between 1 and 10 min of the above HPLC step using a gradient from 50% to 100% MeOH in H_2O (0.1% formic acid) over 50 min (1.0 mg, t_R = 32.4 min). The fraction that eluted with 2% MeOH (17.5 mg) from the flash silica gel column was further purified by semipreparative reversed-phase HPLC eluting with a gradient from 70% to 90% MeOH in H_2O (0.1% formic acid) over 25 min to yield **4** (6.2 mg, t_R = 11.2 min). The fraction that eluted with 4% MeOH (49.2 mg) from the flash silica gel column was further purified by semipreparative reversed-phase HPLC eluting with a gradient from 60% to 73% MeOH in H_2O (0.1% formic acid) over 15 min to yield **3** (5.6 mg, t_R = 13.2 min).

*Protuboxepin A (**1**):* yellow solid; $[\alpha]^{25}_D$ −327 (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ε) 257 (3.59), 336 (3.39); ^{1}H and ^{13}C NMR data, Table 1; HRESIMS *m/z* 378.1824 $[M + H]^+$ (calcd for $C_{22}H_{24}N_3O_3$, 378.1818).

*Protuboxepin B (**2**):* yellow solid; $[\alpha]^{25}_D$ −143 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 260 (3.69), 329 (3.42); ^{1}H NMR data, Table 1; HRESIMS *m/z* 364.1672 $[M + H]^+$ (calcd for $C_{21}H_{22}N_3O_3$, 364.1661).

*Protubonine A (**3**):* yellow solid; $[\alpha]^{25}_D$ −155 (*c* 0.51, MeOH); UV (MeOH) λ_{max} (log ε) 243 (4.12), 280 (3.31); ^{1}H and ^{13}C NMR data, Table 2; HRESIMS *m/z* 358.1768 $[M + H]^+$ (calcd for $C_{19}H_{24}N_3O_4$, 358.1767).

*Protubonine B (**4**):* yellow solid; $[\alpha]^{25}_D$ −269 (*c* 0.55, MeOH); UV (MeOH) λ_{max} (log ε) 243 (3.97), 283 (3.22); ^{1}H and ^{13}C NMR data, Table 2; HRESIMS *m/z* 400.1854 $[M + H]^+$ (calcd for $C_{21}H_{26}N_3O_5$, 400.1872).

Preparation and Analysis of Marfey's Derivatives. Protuboxepin A (**1**, 0.6 mg) and protubonine A (**3**, 0.5 mg) were separately hydrolyzed by heating in 6 N HCl (1 mL) at 110 °C for 24 h. After cooling, the solutions were evaporated to dryness and redissolved in H_2O (50 μL). To each mixture from acid hydrolysis was added a 1% (w/v) solution (100 μL) of FDAA (Marfey's reagent; 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) in acetone. After the addition of $NaHCO_3$ solution (1 M, 20 μL), the mixture was incubated at 40 °C for 1 h. The reactions were stopped by the addition of HCl (2 N, 10 μL), the solvents were evaporated to dryness, and the resulting residues were dissolved in MeOH/H_2O (1:1; 1 mL). Separately, L-Phe, D-Phe, L-Leu, and D-Leu were derivatized with FDAA in the same manner as that of **1**. Aliquots of the resulting solutions (20 μL for **1** and 10 μL for the Phe standards) were analyzed by HPLC [Capcell Pak C_{18} column, linear gradient from 30% to 60% CH_3CN in H_2O (0.1% formic acid) over 200 min; 1 mL/min; 25 °C; 340 nm]. The retention times of the FDAA amino acid derivatives used as standards were 100 (L-Phe) and 117 min (D-Phe). The retention time of the observed peak in the HPLC trace of the FDAA derivatized hydrolysis product mixture from **1** was 117 min.

Similarly, aliquots of solution (20 μL for **1** and 10 μL for the Leu standards) were analyzed by HPLC [Capcell Pak C_{18} column, linear gradient from 70% to 100% MeOH in H_2O (0.1% formic acid) over 60 min; 1 mL/min; 25 °C; 340 nm]. Separately, L-Leu and D-Leu were derivatized with FDAA in the same manner as that of **3**. Retention times of the FDAA amino acid derivatives used as standards were 32 (L-Leu) and 41 min (D-Leu). The retention time of the observed peak in the HPLC trace of the FDAA derivatized hydrolysis product mixture from **3** was 32 min.

Cell Proliferation Assay Procedures. MDA-MB-231 (5×10^3 cell per well), Hep3B (5×10^3 cell per well), 3Y1 (5×10^3 cell per well), K562 (3×10^4 cell per well), and HL-60 (1×10^5 cell per well) cells were seeded on a 96-well microplate. Test compounds were dissolved in DMSO at appropriate concentrations and were treated for 48 h. Cell proliferation assays were carried out using the Enhanced Cell Viability Assay Kit EZ-CyTox (Daeil Lab Service Co., Ltd.) protocol. The absorbance (A450) of each well was measured using a Power WaveX 340 (Bio-Tek Instruments).

■ ASSOCIATED CONTENT

Supporting Information. HMBC data for compounds **1**, **3**, and **4** and 1D- and 2D-NMR spectra for compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) Fenical, W.; Jensen, P. R. *Nat. Chem. Biol.* **2006**, *2*, 666–673.
(2) Bugni, T. S.; Ireland, C. M. *Nat. Prod. Rep.* **2004**, *21*, 143–163.
(3) Saleem, M.; Ali, M. S.; Hussain, S.; Jabbar, A.; Ashraf, M.; Lee, Y. S. *Nat. Prod. Rep.* **2007**, *24*, 1142–1152.
(4) Cutler, H. G.; Springer, J. P.; Arrendale, R. F.; Arison, B. H.; Cole, P. D.; Roberts, R. G. *Agric. Biol. Chem.* **1988**, *52*, 1725–1733.
(5) Belofsky, G. N.; Anguera, M.; Jensen, P. R.; Fenical, W.; Kock, M. *Chem.—Eur. J.* **2000**, *6*, 1355–1360.
(6) Sprogoe, K.; Manniche, S.; Larsen, T. O.; Christophersen, C. *Tetrahedron* **2005**, *61*, 8718–8721.
(7) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.
(8) Lu, X.; Zheng, Z.; Li, Y.; Ke, A.; Ma, Y.; Cui, X.; Shi, Y.; Zhu, J.; Ren, X.; Lin, J.; Ding, Y.; Mu, D.; Xu, Y.; Cao, L.; Zhang, X.; Shan, Y.; Cai, C.; Fan, Y.; Zhang, H.; He, J. Chinese Patent CN 101456863, 2009.