Diketopiperazine Alkaloids from a Deep Ocean Sediment Derived Fungus *Penicillium* sp.

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Received March 11, 2009; accepted April 14, 2009; published online May 11, 2009

Five new diketopiperazine alkaloids, brevicompanines D—H (3—7), together with two known analogs, *allo*-brevicompanine B (1) and fructigenine B (2), were isolated from a deep ocean sediment derived fungus *Penicillium* sp. Their structures were established by spectroscopic methods including 2D NMR and chiral HPLC analysis. Compounds 4 and 7 inhibited lipopolysaccharide (LPS)-induced nitric oxide production in BV2 microglial cells.

Key words *Penicillium* sp.; diketopiperazine; brevicompanine

Fungi have proven to be a valuable source of biologically active natural products. The brevicompanine alkaloids,[1,2] isolated from *Penicillium brevicompactum* and *Aspergillus janus*, were all reported as plant growth regulators. Brevicompanine B was also found to be active against the malaria parasite *Plasmodium falciparum* 3D7.[3] We have focused our attention on new bioactive alkaloids from fungi and isolation work on a deep ocean sediment derived fungus *Penicillium* sp. led to the finding of five new diketopiperazine alkaloids brevicompanines D—H (**3**—**7**) and two known analogs *allo*-brevicompanine B (**1**)[4] and fructigenine B (**2**).[5] In this paper, we describe the isolation, structure elucidation, and inhibitory effects of these molecules on lipopolysaccharide (LPS)-induced inflammation in BV2 microglial cells.

The producing strain *Penicillium* sp. F1 cultivated at 28 °C for 17 d in a seawater fermentation broth (40 l) was extracted with aqueous EtOAc. The extract was concentrated and successively chromatographed over a silica gel column and eluted with a gradient chloroform–methanol (100 : 1→1 : 2) to give six fractions. Fraction 2, which eluted with the solvent of chloroform–methanol (20 : 1), was subjected to repeated Sephadex LH-20 column chromatography (chloroform–methanol, 1 : 1) and the subfractions were further purified by HPLC using a reversed-phase C18 column to give compounds **1**—**7**.

Compounds **1** and **2** were identified by comparison with previously reported physical and spectral data for *allo*-brevicompanine B[4] and fructigenine B.[5] *Allo*-brevicompanine B (**1**) was reported as a synthetic isomer of the natural product brevicompanine B[1] and its ^{13}C-NMR data (Table 2) is reported here for the first time.

Compound **3** was obtained as a colorless powder. Its molecular formula was established as $C_{24}H_{33}N_3O_3$ by HR-ESI-MS, requiring ten degrees of unsaturation. The UV spectrum showed absorption maxima at λ_{max} 209, 248 and 301 nm, which was similar to those found for **1**.[4] The IR spectrum showed a NH absorption at 3214 cm^{-1} and amide carbonyl absorptions at 1668 and 1604 cm^{-1}. The presence of a 1,1-dimethyl-2-propenyl group (δ_H: geminal methyl, 1.00 (3H, s) and 1.11 (3H, s); terminal olefin, 5.08 (1H, dd, J=17.2, 1.1 Hz), 5.11 (1H, dd, J=10.6, 1.1 Hz), and 5.93 (1H, dd J=17.2, 11.0 Hz)/δ_C: 22.2, 23.0, 41.4, 114.7, and 143.3) and a 1,2-substitued phenyl group (δ_H: 6.62 (1H, d, J=7.8 Hz), 6.77 (1H, ddd, J=7.8, 7.3, 1.1 Hz), 7.15 (1H, ddd, J=7.8, 7.8, 0.9 Hz), and 7.17 (1H, d, J=7.3 Hz)/δ_C: 107.1, 118.9, 125.2, 129.1, 129.6, and 149.5) were established by the ^{1}H- (Table 1) and ^{13}C- (Table 2) NMR spectra. The strong IR absorption band at 1668 cm^{-1} and two signals at δ 164.9 and 168.8 in the ^{13}C-NMR spectrum indicated the presence of two amide functions. In the ^{1}H-NMR spectrum, two sets of ABX patterns due to two –CH_2–CH–N– moieties appeared (δ 2.52 (1H, dd, J=12.4, 5.8 Hz), 2.36 (1H, dd, J=12.4, 11.3 Hz), 4.03 (1H, dd, J=11.3, 5.8 Hz) and 1.64 (1H, ddd, J=13.9, 8.4, 5.1 Hz), 1.98 (1H, ddd, J=13.9, 9.1, 3.7 Hz), 4.06 (1H, dd, J=8.4, 3.7 Hz)). These spectral data indicated the presence of a diketopiperazine system in **3**. One of the amino acid residues was deduced as leucine by analysis of the ^{1}H–^{1}H correlation spectroscopy (COSY) correlations among H-3, 2H-12, H-13, CH_3-14, and CH_3-15 (Fig. 3A). Compound **3** was unstable and could transform into **1** in weakly acidic conditions (Fig. 2) which indicated that their structures were interrelated. Careful comparison of their 1D NMR spectra (Tables 2, 3)[4] indicated they had the same structure, which was confirmed by analysis of the 2D NMR (heteronuclear multiple quantum coherence (HMQC), ^{1}H–^{1}H COSY and heteronuclear multiple bond coherence (HMBC)) spectra of **3** (Fig. 3A), except for the substituents on N-6. The HMBC correlations from a methylene (δ_H 4.87 (1H, d, J=10.0 Hz), 4.96 (1H, d, J=10.0 Hz); δ_C 78.7) to C-5a and C-6a, and those between the methylene and an –OCH_3 (δ_H 3.32 (3H, s); δ_C 55.6) indicated a methoxymethyl (MOM) group was attached to N-6. The structure of **3** was established (Fig. 1), and named brevicompanine D. The MOM group is a common protective group for amines,[6] alcohols,[7] and phenols[8] in organic synthesis but the MOM ether or MOM amine groups are quite rare in natural products.[9,10]

Compound **4**, named brevicompanine E, was obtained as a colorless powder. The molecular formula of **4** was established as $C_{25}H_{33}N_3O_3$ by HR-ESI-MS (m/z 424.2583 $[M+H]^+$, Calcd 424.2600), requiring eleven degrees of un-

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Table 1. ^{1}H-NMR Data for Compounds **3**—**7** in $CDCl_3$

No.	3	4	5	6	7
2	6.14 (1H, s)	6.39 (1H, s)	6.05 (1H, s)	6.56 (1H, s)	6.07 (1H, s)
3	4.06 (1H, dd, 8.4, 3.7)	3.95 (1H, dd, 9.7, 2.8)	3.97 (1H, br s)	3.92 (1H, br s)	3.93 (1H, br s)
5a	5.89 (1H, s)	6.10 (1H, s)	6.11 (1H, s)	6.14 (1H, s)	6.23 (1H, s)
7	6.62 (1H, d, 7.8)	8.02 (1H, br s)	8.03 (1H, br s)	8.03 (1H, br s)	8.05 (1H, br s)
8	7.15 (1H, ddd, 7.8, 7.8, 0.9)	7.31 (1H, ddd, 7.3, 7.3, 1.1)	7.31 (1H, dd, 7.2, 7.2)	7.30 (1H, ddd, 7.3, 1.1)*a)*	7.30 (1H, ddd, 7.3, 7.3, 1.1)
9	6.77 (1H, ddd, 7.8, 7.3, 1.1)	7.13 (1H, dd, 7.6, 7.3, 0.8)	7.13 (1H, dd, 7.2, 7.2)	7.13 (1H, ddd, 7.6, 7.3, 0.8)	7.12 (1H, ddd, 7.6, 7.3, 1.1)
10	7.17 (1H, d, 7.3)	7.28 (1H, d, 7.6)	7.28 (1H, d)*a)*	7.29 (1H, d)*a)*	7.28 (1H, d, 7.6)
11	2.52 (1H, dd, 12.4, 5.8, H_a)	2.59 (1H, dd, 12.6, 5.7, H_a)	2.59 (1H, br d, H_a)	2.56 (1H, dd, 12.4, 5.5, H_a)	2.58 (1H, dd, 12.5, 5.5, H_a)
	2.36 (1H, dd, 12.4, 11.3, H_b)	2.38 (1H, dd, 12.6, 11.2, H_b)	2.39 (1H, br t, H_b)	2.29 (1H, dd, 12.4, 11.4, H_b)	2.29 (1H, dd, 12.5, 11.7, H_b)
11a	4.03 (1H, dd, 11.3, 5.8)	3.84 (1H, dd, 11.2, 5.7)	3.84 (1H, br s)	3.80(1H, dd, 11.4, 5.1)	3.81 (1H, dd, 11.7, 5.5)
12	1.64 (1H, ddd, 13.9, 8.4, 5.1)	1.56 (1H, ddd, 14.2, 9.6, 5.0)	1.57 (1H, br s)	2.61 (2H, m)*a)*	2.63 (2H, m)*a)*
	1.98 (1H, ddd, 13.9, 9.1, 3.7)	1.99 (1H,ddd, 14.2, 8.9, 3.8)	2.00 (1H, br s)		
13	1.72 (2H, m)	1.72 (2H, m)	1.70 (1H, br s)	0.91 (3H, d, 6.6)	0.91 (3H, d, 6.6)
14	0.93 (3H, d, 6.6)	0.90 (3H, d, 6.6)	0.90 (3H, br d)	1.04 (3H, d, 7.3)	1.04 (3H, d, 6.9)
15	0.97 (3H, d, 6.6)	0.98 (3H, d, 6.6)	0.99 (3H, br d)		
16				5.80 (1H, dd, 16.8, 10.6)	5.80 (1H, dd, 17.1, 11.0)
17	5.93 (1H, dd, 17.2, 11.0)	5.80 (1H, dd, 17.4, 10.7)	5.81 (1H, dd, 17.0, 10.9)	5.12 (1H, d, 17.2, *trans*)	5.12 (1H, d, 17.1, *trans*)
				5.12 (1H, d, 10.6, *cis*)	5.12 (1H, d, 11.0, *cis*)
18	5.08 (1H, dd, 17.2, 1.1, *trans*)	5.09 (1H, d, 17.4, *trans*)	5.09 (1H, d, 17.0, *trans*)	1.00 (3H, s)	0.99 (3H, s)
	5.11 (1H, dd, 10.6, 1.1, *cis*)	5.11 (1H, d, 10.7, *cis*)	5.12 (1H, d, 10.9, *cis*)		
19	1.00 (3H, s)	0.97 (3H, s)	0.98 (3H, s)	1.16 (3H, s)	1.15 (3H, s)
20	1.11 (3H, s)	1.14 (3H, s)	1.15 (3H, s)		
21	4.87 (1H, d, 10.0)			2.62 (3H, s)*a)*	2.62 (1H, m)*a)*
	4.96 (1H, d, 10.0)				3.41 (1H, br s)
22	3.32 (3H, s)	2.55 (1H, m)	2.55 (1H, m)		1.22 (3H, t, 7.3)
		3.54 (1H, br s)	3.44 (1H, br s)		
23		1.23 (3H, t, 7.4)	1.77 (2H, m)		
24			1.03 (3H, t, 6.6)		

a) Signals overlapped.

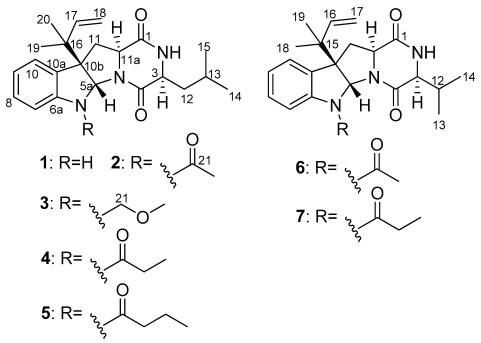

Fig. 1. Structures of Compounds **1**—**7**

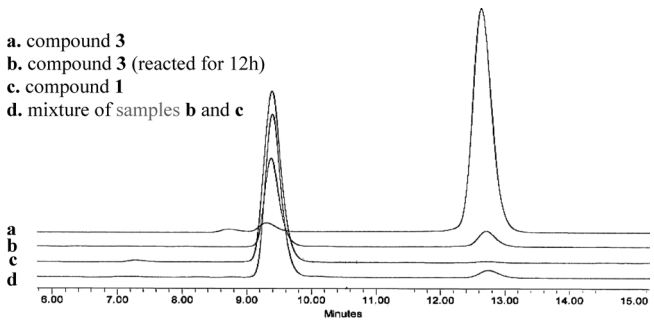

Fig. 2. HPLC Profiles of Compound **3** and Its Acid-Catalyzed Product

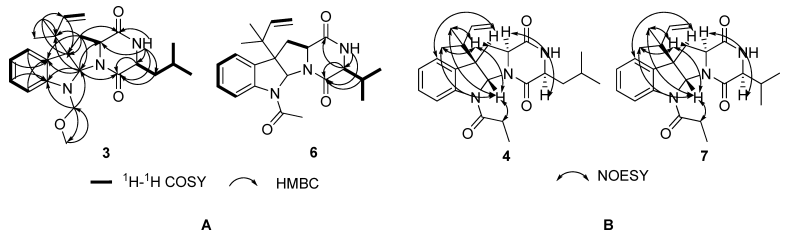

Fig. 3. (A) Key ^{1}H–^{1}H COSY and HMBC Correlations of **3**, **6** and (B) Key NOESY Correlations of **4**, **7**

saturation. The ^{1}H- and ^{13}C-NMR data were similar to those of fructigenine B (**2**)[5] except that the signals of the singlet CH_3-22 (δ_H 2.63 (3H, s); δ_C 22.9) in **2** were absent and those of a methylene (δ_H 2.55 (1H, m) and 3.54 (1H, br s); δ_C 27.9) and a coupled methyl (δ_H 1.23 (3H, t, J=7.4 Hz); δ_C 8.9) were observed in **4** instead. These data indicated that they had the same structure except that the acetyl group on N-6 in **2** was replaced by a propionyl group (Fig. 1).

Compound **5**, named brevicompanine F, had the molecular formula $C_{26}H_{35}N_3O_3$ established by HR-ESI-MS (m/z 438.2761 $[M+H]^+$, Calcd 438.2757). Its 1D NMR data were similar to those of **4** except that resonances characteristic for a butyroyl group appeared in place of the propionyl group (Fig. 1).

Table 2. ^{13}C-NMR Data for Compounds **1** and **3**—**7** in $CDCl_3$

No.	1	3	4	5	6	7
1	169.7, qC	168.8, qC	169.0, qC	169.0, qC	168.5, qC	168.1, qC
3	53.5, CH	53.6, CH	53.2, CH	53.3, CH	60.2, CH	60.3, CH
4	166.6, qC	164.9, qC	165.9, qC	165.8, qC	164.6, qC	164.4, qC
5a	77.7, CH	80.3, CH	78.4, CH	78.5, CH	79.2, CH	78.3, CH
6a	150.0, qC	149.5, qC	143.5, qC	143.5, qC	143.6, qC	143.9, qC
7	109.2, CH	107.1, CH	119.1, CH	119.3, CH	118.8, CH	118.6, CH
8	128.8, CH	129.1, CH	129.0, CH	129.1, CH	129.1, CH	129.1, CH
9	118.8, CH	118.9, CH	124.3, CH	124.4, CH	124.5, CH	124.4, CH
10	125.0, CH	125.2, CH	124.4, CH	124.4, CH	124.7, CH	124.7, CH
10a	129.0, qC	129.6, qC	132.1, qC	132.1, qC	131.9, qC	131.8, qC
10b	61.4, qC	60.5, qC	60.8, qC	60.8, qC	61.2, qC	61.2, qC
11	35.9, CH_2	38.7, CH_2	35.9, CH_2	36.0, CH_2	37.6, CH_2	37.9, CH_2
11a	58.7, CH	58.1, CH	58.9, CH	59.2, CH	58.7, CH	58.7, CH
12	38.7, CH_2	38.5, CH_2	38.9, CH_2	39.1, CH_2	29.3, CH	29.3, CH
13	24.5, CH	24.4, CH	24.3, CH	24.5, CH	15.9, CH_3	15.8, CH_3
14	21.1, CH_3	21.4, CH_3	21.0, CH_3	21.0, CH_3	19.0, CH_3	19.0, CH_3
15	23.2, CH_3	23.2, CH_3	23.2, CH_3	23.4, CH_3	40.6, qC	40.7, qC
16	40.8, qC	41.4, qC	40.4, qC	40.5, qC	143.1, CH	143.1, CH
17	143.5, CH	143.3, CH	143.0, CH	143.1, CH	114.7, CH_2	114.8, CH_2
18	114.5, CH_2	114.7, CH_2	114.6, CH_2	114.7, CH_2	23.3, CH_3	23.4, CH_3
19	22.8, CH_3	23.0, CH_3	23.3, CH_3	23.3, CH_3	22.3, CH_3	22.3, CH_3
20	22.4, CH_3	22.2, CH_3	22.3, CH_3	22.4, CH_3	170.3, qC	173.7, qC
21		78.7, CH_2	173.4, qC	172.7, qC	23.8, CH_3	28.3, CH_2
22		55.6, CH_3	27.9, CH_2	36.6, CH_2		9.0, CH_3
23			8.9, CH_3	18.1, CH_2		
24				14.0, CH_3		

Compound **6**, obtained as a colorless powder, had the molecular formula $C_{23}H_{29}N_3O_3$ established by HR-ESI-MS (*m/z* 396.2284 $[M+H]^+$, Calcd 396.2287). Comparison of the ^{1}H- and ^{13}C-NMR data with those of **2** indicated they had the same carbon skeleton and acetyl substitution on N-6. The ^{1}H–^{1}H COSY correlations among H-3, H-12, CH_3-13, and CH_3-14 afforded an isopropyl group attached to C-3 (Fig. 3A). HMBC correlations confirmed the structure of **6** (Fig. 1), named brevicompanine G.

Compound **7**, with the molecular formula $C_{24}H_{31}N_3O_3$ established by HR-ESI-MS (*m/z* 410.2439 $[M+H]^+$, Calcd 410.2444), was the 6-propionyl analog of **6**. The structure (Fig. 1), named brevicompanine H, was inferred from extensive comparison of the ^{1}H- and ^{13}C-NMR data with those of **4** and **6** (Tables 1, 2).

The relative configuration of **4** was determined by interpreting its nuclear Overhauser effect spectroscopy (NOESY) spectrum. NOEs from CH_3-19 and CH_3-20 to H-5a and from H-5a to H-17 indicated that the B and C rings were connected with a *cis* junction. The coupling constant between H-11a and H_b-11 (J=11.2 Hz) as well as NOEs from H_b-11 to H-5a and from H-3 to H-11a suggested that the vinyl allyl group on C-10b and the methane protons of C-3, C-5a, and C-11a were of β, α, β, and α orientation, respectively. The interpretation of the NOESY spectrum of **7** gave the same result (Fig. 3B).

Acidic hydrolysis and chiral HPLC analysis[11,12] of the resulting hydrolysate using D- and L-leucine reference samples confirmed the absolute configuration of **4**. The absolute configuration of C-3 was determined to be *S*, as one of the products was identified as L-leucine. Hence, the absolute configuration of **4** was established as 3*S*, 5a*R*, 10b*R*, and 11a*S*. A similar procedure was performed on **7** using D- and DL-valine reference samples and it established the same absolute configuration as **4**. The absolute configurations of **3**, **5**, and **6** are assumed to be the same as those of **4** and **7** on biogenetic grounds.

The cellular events in microglia cells (*e.g.*, BV2 cell) induced by lipopolysaccharide (LPS) are regarded as useful *in vitro* models for evaluating the potency of anti-inflammatory compounds.[13] Inhibitory effects of compounds **1**—**7** on LPS-induced inflammation in BV2 microglial cell lines were evaluated by the Griess assay (reference). Compounds **4** and **7** displayed moderate activities with IC_{50} values of 27 and 45 μg/ml, respectively. These results suggested that substituents at the N-6 position are important for inhibition of NO production. These compounds showed no cytotoxic effect at concentrations that inhibited NO production. Although the specific activity of the brevicompanine alkaloids was less than other anti-inflammatory agents, the lack of cytotoxicity at high concentrations warrants further investigation to look for more potent N-6 derivatives which might be useful as anti-inflammatory agents.

Experimental

General Experimental Procedures Specific rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on Beckman DU® 640 spectrophotometer. IR spectra were taken on a NICOLET NEXUS 470 spectrophotometer in KBr discs. ^{1}H-, ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra and 2D-NMR were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard and chemical shifts were recorded as δ values. ESI-MS were measured on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column [YMC-pak ODS-A, 10×250 mm, 5 μm, 4 ml/min].

Fungal Material The fungus, strain F1, was obtained from a deep ocean sediment sample (depth 5080 m). It was identified as *Penicillium* sp. on the basis of its ribosomal internal transcribed spacers and the 5.8S ribosomal RNA gene (ITS1-5.8S-ITS2) which was deposited in Genbank (EU770317). Working stocks were prepared on Potato Dextrose agar slants stored at 4 °C.

Fermentation, Extraction, and Isolation Spores were directly inoculated into 500 ml Erlenmeyer flasks containing 200 ml fermentation media (mannitol 20 g, maltose 20 g, glucose 10 g, monosodium glutamate 10 g, KH_2PO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.3 g, yeast extract 3 g and corn steep liquor 1 g, dissolved in 1 l seawater, pH 6.5). The flasks were incubated on a rotatory shaker at 165 rev/min at 28 °C. After 17 d of cultivation, 40 l of whole broth was filtered through cheesecloth to separate the broth supernatant and mycelia. The former was extracted with ethyl acetate, while the latter was extracted with acetone. The acetone extract was evaporated under reduced pressure to afford an aqueous solution, and then extracted with ethyl acetate. The two ethyl acetate extracts were combined and concentrated *in vacuo* to give a crude gum (55 g).

The crude gum (55 g) was subjected to silica gel column chromatography (chloroform–methanol, v/v, gradient) and the fraction 2 eluted with the solvent of chloroform–methanol (20 : 1) was subjected to repeating Sephadex LH-20 column chromatography (chloroform–methanol, 1 : 1). The subfractions 2-2-1, 2-2-7, 2-9-1, and 2-6-1 were further purified respectively by HPLC using a reversed-phase C18 column (70% MeOH, 65% MeOH, 70% MeOH, and 65% MeOH, 4.0 ml/min), to give compounds **1** (20 mg), **4** (30 mg), and **5** (10 mg); **2** (430 mg); **3** (12 mg) and **6** (30 mg); **7** (11 mg).

Acidic Transformation of Compound 3 Compound **3** (1 mg) was stirred with MeOH (500 μl), H_2O (500 μl), and AcOH (50 μl) at 25 °C for 12 h. HPLC analysis revealed that compound **3** transformed into compound **1** (HPLC conditions: 75% MeOH/H_2O; 237 nm; 1 ml/min).

Acidic Hydrolysis of 4 and 7 Compound **4** (2.0 mg) was dissolved in 6 N HCl (1 ml) and the mixture was heated at 105 °C for 12 h in a sealed tube. The solution was diluted with 5 ml of H_2O and evaporated to dryness under reduced pressure. The residue was dissolved in 5 ml of H_2O and the solution was then analyzed by chiral HPLC (CROWNPAK CR(+), Daicel Chemical, Japan): flow rate 0.8 ml/min; solvent, aqueous $HClO_4$ (pH=1.5); detection, 200 nm; temperature 30 °C. The retention time of hydrolysate was 16.71 min, while the retention times of D- and L-leucine were 7.29 and 16.71 min, respectively. By the same procedure except that the flow rate was 0.4 ml/min, the hydrolysate of compound **7** (2.0 mg) was compared with D- and DL-valine (hydralyzate, 7.52 min; D-valine, 6.20 min; L-valine, 7.52 min).

Brevicompanine D (**3**): Colorless powder (methanol), $[\alpha]_D^{17}$ −342.9° (c=0.50, MeOH), IR (KBr) cm^{-1}: 3214, 3081, 2959, 2872, 1668, 1604, 1487, 1434, 1315, 1263, 1221, 1148, 1072, 1008, UV λ_{max} (MeOH) nm (log ε): 301 (3.63), 248 (4.11), 209 (4.43), ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 150 MHz), see Tables 1 and 2, HR-ESI-MS m/z: 420.2253 $[M-MeOH+H_2O+Na]^+$ (Calcd for $C_{23}H_{31}N_3O_3Na$: 420.2263).

Brevicompanine E (**4**): Colorless powder (methanol), $[\alpha]_D^{17}$ −140.0° (c=1.35, MeOH), IR (KBr) cm^{-1}: 3204, 3077, 2960, 2930, 2869, 1686, 1670, 1596, 1476, 1413, 1386, 1300, 1276, 1197, 1152, 1011, UV λ_{max} (MeOH) nm (log ε): 247 (4.02), 205 (4.44), ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 150 MHz), see Tables 1 and 2, HR-ESI-MS m/z: 424.2583 $[M+H]^+$ (Calcd for $C_{25}H_{34}N_3O_3$: 424.2600).

Brevicompanine F (**5**): Colorless powder (methanol), $[\alpha]_D^{17}$ −129.6° (c=0.60, MeOH), IR (KBr) cm^{-1}: 3207, 3080, 2963, 2868, 1687, 1671, 1601, 1476, 1409, 1387, 1326, 1301, 1276, 1239, 1197, 1152, 1012, UV λ_{max} (MeOH) nm (log ε): 247 (3.99), 205 (4.43), ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 150 MHz), see Tables 1 and 2, HR-ESI-MS m/z: 438.2761 $[M+H]^+$ (Calcd for $C_{26}H_{36}N_3O_3$: 438.2757).

Brevicompanine G (**6**): Colorless powder (methanol), $[\alpha]_D^{17}$ −164.0° (c=1.43, MeOH), IR (KBr) cm^{-1}: 3329, 3200, 3069, 2964, 2879, 1668, 1593, 1473, 1458, 1383, 1349, 1282, 1207, 1183, 1157, 1135, 1095, 1006, UV λ_{max} (MeOH) nm (log ε): 246 (4.02), 206 (4.43), ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 150 MHz), see Tables 1 and 2, HR-ESI-MS m/z: 396.2284 $[M+H]^+$ (Calcd for $C_{23}H_{30}N_3O_3$: 396.2287).

Brevicompanine H (**7**): Colorless powder (methanol), $[\alpha]_D^{17}$ −142.0° (c=0.50, MeOH), IR (KBr) cm^{-1}: 3337, 3202, 3080, 2968, 2737, 2878, 1681, 1598, 1476, 1460, 1413, 1289, 1243, 1199, 1150, 1006, UV λ_{max} (MeOH) nm (log ε): 247 (3.98), 206 (4.42), ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR ($CDCl_3$, 150 MHz), see Tables 1 and 2, HR-ESI-MS m/z: 410.2439 $[M+H]^+$ (Calcd for $C_{24}H_{32}N_3O_3$: 410.2444).

Lipopolysaccharide (LPS)-Induced Inflammation Inhibition Assay Nitrite in culture supernatants, a stable by-product of NO, was measured by Griess reagent (iNtRON) according to the instructions provided by the manufacturer. Briefly, cells (1×10^5 cells per well) were seeded in 96-well microtiter plates and incubated for 24 h in growth medium under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Cells were then pretreated with compounds at various concentrations in serum-free medium for 45 min. After pretreatment, LPS (0.1 μg/ml) was added, and the cells were incubated for 16 h. After LPS exposure, duplicates of supernatants (100 μl) were collected and mixed with 50 μl of Griess reagent. The absorbance was measured with a 595-nm filter by a microplate spectrophotometer. The concentration of nitrite released in the supernatants was calculated with the linear equation derived from the standard curve generated by known concentrations.

Acknowledgments This work was funded by the Chinese Ocean Mineral Resource R & D Association (DY105-2-04). Thank professor Linda J. Van Eldik of Northwestern University (Chicago, IL) for providing us BV2 cell lines.

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