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Note

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Antimicrobial Metabolites from the Paracel Islands Sponge *Agelas mauritiana*

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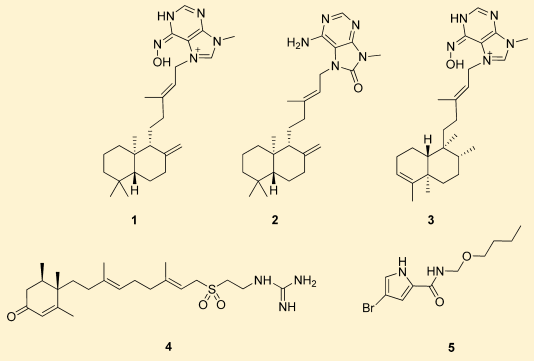
Supporting Information

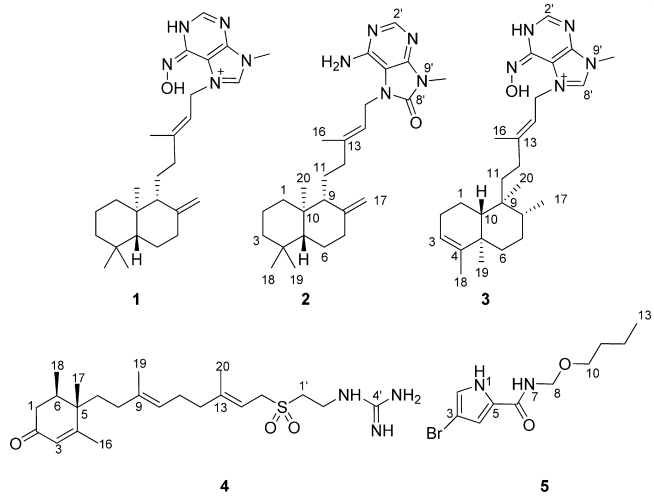
ABSTRACT: Four new alkaloids, (−)-8′-oxo-agelasine D (**2**), ageloxime B (**3**), (+)-2-oxo-agelasidine C (**4**), and 4-bromo-*N*-(butoxymethyl)-1*H*-pyrrole-2-carboxamide (**5**), and the known compound (−)-ageloxime D (**1**) were isolated from the marine sponge *Agelas mauritiana*. Their chemical structures were established on the basis of spectroscopic analysis. Compounds **1** and **3** both showed antifungal activity against *Cryptococcus neoformans* and antileishmanial activity against *Leishmania donovani in vitro*. Compound **3** also exhibited antibacterial activity against *Staphylococcus aureus* and methicillin-resistant *S. aureus in vitro*.



Marine sponges of the genus *Agelas* (order Agelasida, family Agelasidae) have proven to be an excellent source of structurally novel natural products, ranging from diterpene alkaloids[1] to bromopyrrole alkaloids[2] and glycosphingolipids.[3] The diterpene alkaloids derived from this genus include agelines,[1a] agelasines,[1b−e] agelasimines,[4] and agelasidines.[5] They and their analogues have attracted a great deal of attention for their wide range of biological activities such as antimicrobial,[1a,5b,6] antimalarial,[1d] antileukemic,[1c] cytotoxic,[1e,6] and antifouling activities,[1e,7] as well as inhibitory effects on Na^+/K^+-ATPase.[5a]

As part of an ongoing investigation of the chemical constituents from marine sponges collected off the Paracel Islands in the South China Sea, studies on the marine sponge *Agelas mauritiana* led to the isolation and determination of the known compound (−)-ageloxime D (**1**) and four new alkaloids (**2**−**5**). Herein, we report the details of the isolation and structure elucidation of the new compounds and the evaluation of their antimicrobial and antileishmanial activities.

The EtOH extract of the marine sponge *A. mauritiana* was subjected to solvent partitioning, column chromatography (on silica gel, ODS, and Sephadex LH-20), and HPLC, to afford compounds **1**−**5**. Their structures were elucidated by MS and 1D and 2D NMR techniques including $^1H-^1H$ COSY, HSQC, HMBC, and NOESY. The known compound (−)-ageloxime D (**1**) was elucidated by comparison of its NMR, MS, and specific rotation data with those in the literature.[1e]



Compound **2** was obtained as a white, amorphous solid. The similarity of the UV absorption pattern (λ_{max} 220, 269 nm, MeOH) to those of agelasines[1d,e] suggested that compound **2** was a related metabolite. The molecular formula $C_{26}H_{39}N_5O$ was deduced from the HRESIMS, ^{13}C NMR, and HSQC data. The ^1H NMR and ^{13}C NMR spectra of compound **2** (Table 1) were similar to those of agelasine D. Comparison of the NMR

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Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Data for 2−4 in $CDCl_3$

	2			3			4		
position	δ_C, mult.	δ_H, mult. (J in Hz)	HMBC (H → C)	δ_C	δ_H	HMBC (H → C)	δ_C	δ_H	HMBC (H→ C)
1	39.1, CH_2	1.71, br d (11.9)	2,9,10,20	18.3, CH_2	1.48, m	2,3,9	42.0, CH_2	2.34, dd (13.8, 10.1)	2,5,6
		0.96, br d (11.4)			1.42, m			2.25, ov[a]	
2	19.3, CH_2	1.54, m	1,10	26.9, CH_2	2.00, ov[a]		199.6, C		
		1.44, m			2.02, ov[a]				
3	42.1, CH_2	1.38, m	4,5,18,19	120.4, CH	5.18, br s	4,18	128.1, CH	5.86, s	5,16
		1.15, br t (13.3)							
4	33.5, C			144.5, C			169.4, C		
5	55.5, CH	1.04, br d (12.3)	4,6,7,9,10,19,20	38.2, C			42.3, C		
6	24.4, CH_2	1.71, br d (12.6)	5,7,8,10	36.8, CH_2	1.70, br d (12.6)	10,19	33.7, CH	2.24, ov[a]	7
		1.30, dd (13.1, 4.1)			1.14, m				
7	38.3, CH_2	2.36, br d (12.1)	5,6,8,9,17	27.4, CH_2	1.40, m	8	34.3, CH_2	1.95, m	
		1.90, m			1.40, m			1.67, ov[a]	
8	148.4, C			36.3, CH	1.41, m	7,10	35.1, CH_2	1.65, ov[a]	7,9,10
9	56.2, CH	1.54, m	5,7,8,10,12,17,20	38.6, C			135.6, C		
10	39.6, C			46.4, CH	1.29, br d (11.8)	2,9,19,20	123.6, CH	5.09, br s	11
11	21.6, CH_2	1.60, m	9,12	36.7, CH_2	1.37, m	8,10	26.1, CH_2	2.12, br s	9,12,13
		1.43, m			1.23, m				
12	38.3, CH_2	2.20, br t (12.3)	11,16	32.9, CH_2	1.80, m	11,13,14	39.7, CH_2	2.12, br s	10,11,14
		1.88, m			1.80,m				
13	141.9, C			144.3, C			147.4, C		
14	120.8, CH	5.32, br t (5.2)	12,15,16	117.1, CH	5.33, t (7.7)	12,15,16	109.1, CH	5.25, t (7.2)	15,20
15	40.2, CH_2	4.65, dd (16.6, 5.9)	13,14,5′,8′	41.5, CH_2	4.14, br d (3.8)	13,14,5′	53.9, CH_2	3.92, br s	
		4.61, dd (16.6, 5.9)			4.12, br d (4.2)				
16	16.8, CH_3	1.81, s	12,13,14	16.5, CH_3	1.61, s	12,13,14	20.4, CH_3	1.92, s	3,4,5
17	106.2, CH_2	4.80, s	7,8,9	16.0, CH_3	0.77, br s	7,8,9	19.7, CH_3	1.02, s	4,5,6,7
		4.45, s							
18	33.5, CH_3	0.87, s	3,4,5,19	18.0, CH_3	1.58, s	3,4,5	15.5, CH_3	0.95, d (5.9)	1,5,6
19	21.7, CH_3	0.79, s	3,4,5,18	19.9, CH_3	0.98, s	4,5,10	16.2, CH_3	1.61, s	8,9,10
20	14.5, CH_3	0.66, s	5,9,10	18.4, CH_3	0.70, s	8,9,10,11	17.1, CH_3	1.75, s	12,13,14
1′							50.7, CH_2	3.41, br s	
2′	151.3, CH	8.18, s	4′,6′	157.6, CH	8.15, s	4′	35.1, CH_2	3.82, br s	
3′									
4′	148.5, C			160.6, C			157.3, C		
5′	106.1, C			99.6, C					
6′	146.0, C			159.8, C					
8′	153.0, C			164.5, CH	7.97, s	15			
9′-*N*Me	26.4, CH_3	3.45, s	4′,8′	28.0, CH_3	2.97, d (4.9)	4′			
6′-*N*H2		5.07, br s	5′						
1′-*N*H					4.78, ov[a]	5′			
6′-*N*OH					4.78, ov[a]				

[a]ov = overlapped by other signals.

data for compound **2** with those of agelasine D[1b,e] revealed that significant differences were apparent in the adeninium moiety. There was only one aromatic resonance at δ_H 8.18 in the ^{1}H NMR spectrum of compound **2**. Instead of the one sp^2 methine group (δ_C 142.0) in agelasine D,[1e] one quaternary carbon was detected at δ_C 153.0 in the ^{13}C NMR spectrum of compound **2**. These results were confirmed by the HMBC correlations from 9′-*N*CH_3 (δ_H 3.45) to C-4′ (δ_C 148.5) and C-8′ (δ_C 153.0), from H-2′ (δ_H 8.18) to C-4′ (δ_C 148.5) and C-6′ (δ_C 146.0), and from 6′-NH_2 (δ_H 5.07) to C-5′ (δ_C 106.1) (Figure 1). An extensive inspection of the ^{1}H NMR, ^{13}C NMR, and HMBC spectra allowed the establishment of the same labdane skeleton for the diterpene moiety as is seen for agelasine D.[1b] The diterpene moiety was connected to the N-7′ atom of the adeninium unit as commonly occurs in agelasine derivatives, which was verified by the observation of the HMBC cross-peaks of H_2-15 (δ_H 4.59−4.68) with C-8′ (δ_C 153.0), C-13 (δ_C 141.9), C-14 (δ_C 120.8), and C-5′ (δ_C 106.1). Interestingly, H_2-15 (δ_H 4.59−4.68, 2H, dd each, J = 16.6, 5.9 Hz) appears like a quartet of doublets in the ^{1}H NMR spectrum due to the roof effect.[8]

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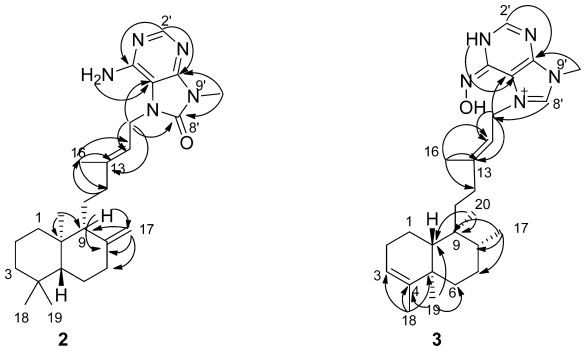

Figure 1. Key HMBC correlations for compounds **2** and **3**.

The relative configuration of compound **2** was deduced from a NOESY experiment. NOE correlations were detected between CH_3-19 (δ_H 0.79)/CH_3-20 (δ_H 0.66), CH_3-20/H-11b (δ_H 1.43), CH_3-18 (δ_H 0.87)/H-5 (δ_H 1.04), and H-5/H-9 (δ_H 1.54), which were identical to those observed for the co-isolated (−)-ageloxime D (**1**).[1e] On the basis of the foregoing analysis, the structure of compound **2** was named (−)-8′-oxo-agelasine D.

Compound **3** was isolated as a white, amorphous solid. The molecular formula was established as $C_{26}H_{41}N_5O$ from HRESIMS and ^{13}C NMR data. Comparison of the NMR data for compound **3** with those of (−)-ageloxime D (**1**)[1e] suggested that changes were in the diterpene moiety. The ^1H NMR and ^{13}C NMR spectra demonstrated that compound **3** possessed a clerodane skeleton, which was confirmed by the HMBC correlations from the five methyl groups (CH_3-16, 17, 18, 19, and 20) to the associated carbons (Figure 1). Similarly to (−)-ageloxime D, protonation occurred at N-9′ when using $CDCl_3$ as a solvent, which can stabilize the tautomer (imino form) of the adeninium moiety of compound **3**.[1e] This fact was supported by the observation of a methyl proton doublet at δ_H 2.97 (J = 4.9 Hz).

In the NOESY spectrum, correlations between CH_3-20 (δ_H 0.70)/CH_3-19 (δ_H 0.98), CH_3-20/CH_3-17 (δ_H 0.77), CH_3-19/CH_3-17, H-8 (δ_H 1.41)/H-11b (δ_H 1.23), and H-10 (δ_H 1.29)/H_2-11a (δ_H 1.37) suggested the three methyl groups were on the same face of the ring system. Further comparison of the ^{13}C NMR data for **3** and the reported agelasine B revealed the diagnostic high-field signal of CH_3-19 (δ_C = 19.9), confirming the *trans* ring juncture. For the *cis* isomer, the carbon chemical shift of CH_3-19 resonates at ca. 32−33 ppm.[1b,9] Strong NOE correlations between 6′-*N*OH (δ_H 4.78) and H_2-15 (δ_H 4.12−4.14) were also detected, which indicated the oxime group was *E* configured. Therefore, compound **3** was elucidated as the oxime derivative of agelasine B, which we named ageloxime B.

Compound **4** was obtained as a light yellow oil. The molecular formula was established as $C_{23}H_{39}N_3O_3S$ from the HRESIMS peak at m/z 438.2787 $[M + H]^+$ and the ^{13}C NMR data. It was determined to be a guanidine derivative by the characteristic ^{13}C NMR signal (C-4′, δ_C 157.3) and positive coloration with Sakaguchi reagents.[5] Its ^1H NMR and ^{13}C NMR spectra were similar to those of agelasidine C, except for a ketone group at δ_C 199.6 in **4** replacing the methylene group at C-2 (δ_C 28.0) in agelasidine C.[5] The HMBC correlations from H_2-1 (δ_H 2.34, 2.25) to C-2, H-3 (δ_H 5.86) to C-1 and C-16, and CH_3-16 (δ_H 1.92) to C-3, C-4, and C-5 suggested the presence of an α,β-unsaturated carbonyl group, and the ketone group was located at C-2. High-field olefinic methyl resonances at δ_C 16.2 (C-19) and 17.1 (C-20) indicated the 9,10- and 13,14-double bonds were both *E* configured.[1b]

The relative configuration of compound **4** was found to be the same as in agelasidine C on the basis of comparison of their NMR data. In the NOESY spectrum, correlations between CH_3-17 (δ_H 1.02)/CH_3-18 (δ_H 0.95) and CH_2-7 (δ_H 1.95, 1.67)/H-6 (δ_H 2.24) were observed, which revealed that the two methyl groups were cofacial. The absolute configuration of compound **4** can be assigned by comparing its circular dichroism (CD) curve with those of the known compounds. The signs of the short-wavelength region (200−220 nm) of the CD spectra for this type of α,β-unsaturated cyclohexenones depend mainly on the configuration at the C-6 position of compound **4**.[10] The CD spectrum of **4** showed a positive Cotton effect ($\Delta\varepsilon$ +49) around 212 nm due to the overlap of Cotton effects attributed to π−π^* and n−σ^* transitions, which was opposite the negative Cotton effect of (−)-(5*R*)-methyl-2-cyclohexenone[10] in a similar spectral region. This would suggest the *S* configuration at C-6 of **4**, but due to the more highly substituted ring, a 6*R* configuration is assigned for **4**. In this case, the positive specific rotation for the 5*S*,6*R*-compound **4** ($[\alpha]_D^{26}$ +26.0, MeOH) correlates with the sign of rotation for (+)-5*S*,6*R*-agelasidine C ($[\alpha]_D^{25}$ +8.5, MeOH) and is opposite that for (−)-5*R*,6*S*-agelasidine C ($[\alpha]_D^{29}$ −5.6, MeOH).[5] Accordingly, the new compound was named (+)-2-oxo-agelasidine C.

Compound **5** was obtained as a white, amorphous solid. The ESI mass spectrum showed two pseudomolecular ion peaks at m/z 273 and 275 $[M - H]^-$ in a 1:1 ratio, suggesting the presence of one bromine atom in the molecule. The molecular formula of compound **5** was determined to be $C_{10}H_{15}{}^{79}BrN_2O_2$ by HRESIMS and ^{13}C NMR data. The ^{13}C NMR and DEPT spectra displayed 10 signals including three quaternary carbons, two methines, four methylenes (of which two were oxygenated), and one methyl. The presence of a 4-bromopyrrole-2-carboxamide moiety was indicated by the aromatic resonances at δ_H 6.95 (1H, br s) and 6.60 (1H, s) in the ^1H NMR spectrum and the characteristic pattern of resonances (δ_C 122.0, 97.1, 111.6, 125.5, and 160.2), which was similar to the values of reported bromopyrrole alkaloids.[11] The partial structure of C6−C13 was assigned on the basis of ^1H−^1H COSY and HMBC spectrometric data. The connection of C10−C13 was deduced from the COSY correlations between H_2-10 (δ_H 3.53) and H_2-11 (δ_H 1.56), H_2-11 and H_2-12 (δ_H 1.35), and H_2-12 and CH_3-13 (δ_H 0.91). The HMBC correlations from H_2-8 (δ_H 4.88) to C-6 (δ_C 160.2) and C-10 (δ_C 68.5) and from H-4 (δ_H 6.60) to C-5 (δ_C 125.5) completed the assignment of the structure of compound **5** as depicted. Therefore, compound **5** was elucidated as 4-bromo-*N*-(butoxymethyl)-1*H*-pyrrole-2-carboxamide.

Compounds **1**−**4** were assessed for antimicrobial activity against 10 organisms. The agelasine oxime derivatives (**1** and **3**) both showed activity against *Cryptococcus neoformans* with IC_{50}/MIC values of 5.94/10.00 and 4.96/10.00 μg/mL, respectively. Compound **3** also exhibited antibacterial activity against *Staphylococcus aureus* (IC_{50}/MIC = 7.21/10.00 μg/mL) and methicillin-resistant *S. aureus* (IC_{50}/MIC = 9.20/20.00 μg/mL). The antileishmanial activity of compounds **1**−**4** was also tested *in vitro*. Only compounds **1** and **3** exhibited antileishmanial activity against *Leishmania donovani*, with IC_{50}/IC_{90} values of 29.28/33.96 and 28.55/33.19 μg/mL, respectively.

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EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotation data were obtained on a JASCO P-1030 polarimeter. The CD spectrum was obtained on a JASCO J-715 spectropolarimeter. UV spectra were acquired using a Shimadzu UV-240 spectrophotometer. NMR experiments were performed on Bruker AVANCE-500 spectrometers. HRESIMS and ESIMS spectra were acquired using a Q-Tof micro YA019 mass spectrometer. HPLC purifications were carried out on a Waters 1525/2996 liquid chromatograph. Column chromatography was performed on Sephadex LH-20 (Pharmacia) and YMC ODS-A (50 μm). Fractions were monitored by TLC (HSGF 254, Yantai, China), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Animal Material. The specimen of *Agelas mauritiana* was collected around Yongxing Island and Seven Connected Islets in the South China Sea in June 2007. The sponge was identified by Prof. Jin-He Li (Institute of Oceanology, Chinese Academy of Sciences, P. R. China). A voucher sample (No. JNF07) was deposited in the Laboratory of Marine Drugs, Department of Pharmacy, Changzheng Hospital, Second Military Medical University, Shanghai, P. R. China.

Extraction and Isolation. The sponge (6.7 kg, wet weight) was extracted with 95% EtOH at room temperature. The EtOH extract was suspended in H_2O and extracted with EtOAc. The EtOAc-soluble extract was partitioned between MeOH–H_2O (9:1) and petroleum ether. The MeOH–H_2O (9:1) phase was diluted to 3:2 with H_2O and extracted with CH_2Cl_2 to afford the CH_2Cl_2-soluble extract (100.6 g). This CH_2Cl_2-soluble extract was subjected to VLC on silica gel using CH_2Cl_2–MeOH (50:1, 20:1, 10:1, 0:100) as eluent to give four fractions (A–D). Fraction A (23.3 g) was chromatographed on a Sephadex LH-20 column with CH_2Cl_2–MeOH (1:1) as eluting solvent to afford five fractions (A1–A5). Fraction A4 (6.4 g) was subjected to column chromatography (CC) on YMC ODS-A (50 μm) using MeOH–H_2O (1:1–1:0) to give 13 fractions (A401–A413). Fraction A411 (596.7 mg) was separated by repeated CC on silica gel followed by HPLC (SunFire silica, 5 μm, 10 × 250 mm, 2 mL/min, UV detection at 220 and 269 nm) using *n*-hexane–2-propanol (82:18) as eluent to yield compound **2** (15.6 mg). Fraction A410 (1.84 g) was subjected to chromatography repeatedly on silica gel and purified by HPLC (YMC Pack B C-18, 5 μm, 10 × 250 mm, 1.5 mL/min, UV detection at 225 and 259 nm), eluting with CH_3CN–H_2O (54:46), to afford compounds **1** (16.2 mg) and **3** (12.7 mg). The separation of fraction A405 (109.0 mg) was performed by using HPLC (YMC Pack B C-18, 5 μm, 10 × 250 mm, 1.5 mL/min, UV detection at 220 nm), eluting with CH_3CN–H_2O (30:70), to obtain compound **5** (1.2 mg). Similarly, fraction B (14.4 g) was subjected to CC on silica gel repeatedly and further purified by HPLC (YMC Pack B C-18, 5 μm, 10 × 250 mm, 1.5 mL/min, UV detection at 240 nm) with CH_3CN–H_2O (25:75) as the elute to yield compound **4** (5.8 mg).

*(−)-8′-Oxo-agelasine D (**2**):* white, amorphous solid; $[\alpha]_D^{26}$ −18 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 269 (3.47) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 1; HRESIMS *m*/*z* 438.3235 $[M + H]^+$ (calcd for $C_{26}H_{40}N_5O$, 438.3233).

*(−)-Ageloxime B (**3**):* white, amorphous solid; $[\alpha]_D^{26}$ −110 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 259 (2.31) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 1; HRESIMS *m*/*z* 440. 3392 $[M + H]^+$ (calcd for $C_{26}H_{42}N_5O$, 440.3389).

*(+)-2-Oxo-agelasidine C (**4**):* light yellow oil; $[\alpha]_D^{26}$ +26 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.18) nm; CD (6.86×10^{-4} M, EtOH) λ_{max} ($\Delta\varepsilon$) 212 (+49), 247 (−1.37), 317 (−0.07) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) data, see Table 1; HRESIMS *m*/*z* 438.2787 $[M + H]^+$ (calcd for $C_{23}H_{40}N_3O_3S$, 438.2790).

*4-Bromo-N-(butoxymethyl)-1H-pyrrole-2-carboxamide (**5**):* white, amorphous solid; UV (MeOH) λ_{max} (log ε) 220 (3.05) nm; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.95 (1H, br s, H-2), 6.60 (1H, s, H-4), 4.88 (2H, d, *J* = 7.2 Hz, H_2-8), 3.53 (2H, t, *J* = 6.5 Hz, H_2-10), 1.56 (2H, m, H_2-11), 1.35 (2H, m, H_2-12), 0.91 (3H, t, *J* = 7.0 Hz, H-13); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.2 (C, C-6), 125.5 (C, C-5), 122.0 (CH, C-2), 111.6 (CH, C-4), 97.1 (C, C-3), 70.1 (CH_2, C-8), 68.5 (CH_2, C-10), 31.7 (CH_2, C-11), 19.2 (CH_2, C-12), 13.9 (CH_3, C-13); HRESIMS *m*/*z* 297.0214 $[M + Na]^+$ (calcd for $C_{10}H_{15}{}^{79}BrN_2O_2Na$, 297.0215) and 299.0200 $[M + Na]^+$ (calcd for $C_{10}H_{15}{}^{81}BrN_2O_2Na$, 299.0194).

Antimicrobial Assays. All organisms were obtained from the American Type Culture Collection (Manassas, VA, USA), including the fungi *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Candida krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 204305 and the bacteria *Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* ATCC 33591, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068. All organisms were tested using modified versions of the CLSI (formerly NCCLS) methods as described previously.[12] The control drugs ciprofloxacin for bacteria and amphotericin B for fungi were included in each assay.

Antileishmanial Assay. Antileishmanial activities of the compounds were assessed *in vitro* against a culture of *Leishmania donovani* promastigotes. In a 96-well microplate assay, compounds with appropriate dilution were added to the leishmania promastigotes culture (2×10^6 cell/mL). The plates were incubated at 26 °C for 72 h, and growth of the leishmania promastigotes was determined by the Alamar blue assay.[13] Pentamidine and amphotericin B were used as the standard antileishmanial drugs. IC_{50} and IC_{90} values for each compound were computed from the growth inhibition curve.

ASSOCIATED CONTENT

Supporting Information

NMR spectra and HRESIMS data for compounds **2**–**5** are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) (a) Capon, R. J.; Faulkner, D. J. *J. Am. Chem. Soc.* **1984**, *106*, 1819–1822. (b) Wu, H.; Nakamura, H.; Kobayashi, J.; Kobayashi, M.; Ohizumi, Y.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2495–2504. (c) Ishida, K.; Ishibashi, M.; Shigemori, H.; Sasaki, T.; Kobayashi. *J. Chem. Pharm. Bull.* **1992**, *40*, 766–767. (d) Appenzeller, J.; Mihci, G.; Martin, M.; Gallard, J.; Menou, J.; Boury-Esnault, N.; Hooper, J.; Petek, S.; Chevalley, S.; Valentin, A.; Zaparucha, A.; Al-Mourabit, A.; Debitus, C. *J. Nat. Prod.* **2008**, *71*, 1451–1454. (e) Hertiani, T.; Edrada-Ebel, R. A.; Ortlepp, S.; van Soest, R. W. M.; de Voogd, N. J.;

Wray, V.; Hentschel, U.; Kozytska, S.; Müller, W. E. G.; Proksch, P. *Bioorg. Med. Chem.* **2010**, *18*, 1297−1311.
(2) Endo, T.; Tsuda, M.; Okada, T.; Mitsuhashi, S.; Shima, H.; Kikuchi, K.; Mikami, Y.; Fromont, J.; Kobayashi, J. *J. Nat. Prod.* **2004**, *67*, 1262−1267.
(3) Natori, T.; Koezuka, Y.; Higa, T. *Tetrahedron Lett.* **1993**, *34*, 5591−5592.
(4) Fathi-Afshar, R.; Allen, T. *Can. J. Chem.* **1988**, *66*, 45−50.
(5) (a) Nakamura, H.; Wu, H.; Kobayashi, J.; Kobayashi, M.; Ohizumi, Y.; Hirata, Y. *J. Org. Chem.* **1985**, *50*, 2494−2497. (b) Morales, J. J.; Rodriguez, A. D. *J. Nat. Prod.* **1992**, *55*, 389−394.
(6) Vik, A.; Hedner, E.; Charnock, C.; Samuelsen; Larsson, R.; Gundersen, L. L.; Bohlin, L. *J. Nat. Prod.* **2006**, *69*, 381−386.
(7) Hattori, T.; Adachi, K.; Shizuri, Y. *J. Nat. Prod.* **1997**, *60*, 411−413.
(8) (a) Jacobsen, N. E. *NMR Spectroscopy Explained*; Wiley-Interscience: Hoboken, NJ, 2007; p 66. (b) Balc, M. *Basic ^{1}H- and ^{13}C-NMR Spectroscopy*; Elsevier Science, 2005; pp 162−163.
(9) De Rosa, S.; Minale, L.; Riccio, R.; Sodano, G. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1408−1414.
(10) Gawroski, J. K. *Tetrahedron* **1982**, *38*, 3−26.
(11) Aiello, A.; D'Esposito, M.; Fattorusso, E.; Menna, M.; Muller, W. E. G.; Perovic-Ottstadt, S.; Schroder, H. C. *Bioorg. Med. Chem.* **2006**, *14*, 17−24.
(12) (a) NCCLS, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard M27-A2. National Committee on Clinical Laboratory Standards, 2002; *22*. (b) NCCLS, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi; Approved standard, M38-A. National Committee on Clinical Laboratory Standards, 2002; *22*. (c) NCCLS, Susceptibility Testing of Mycobacteria, Nocardia, and Other Aerobic Actinomycetes; Tentative Standard−Approved Standard, M24-A. National Committee on Clinical Laboratory Standards, 2003; *23*. (d) NCCLS, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard, Seventh Edition M7-A7. National Committee on Clinical Laboratory Standards, 2006; *26*. (e) Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. *J. Clin. Microbiol.* **1998**, *36*, 362−366.
(13) Mikus, J.; Steverding, D. *Parasitol. Int.* **2000**, *48*, 265−269.