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Bioactive Indole Alkaloids and Phenyl Ether Derivatives from a Marine-Derived *Aspergillus* sp. Fungus

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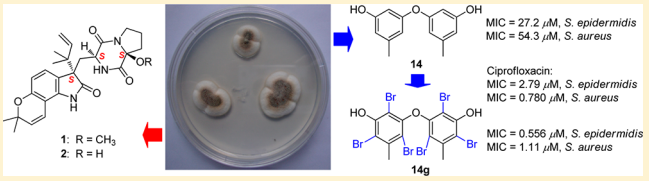
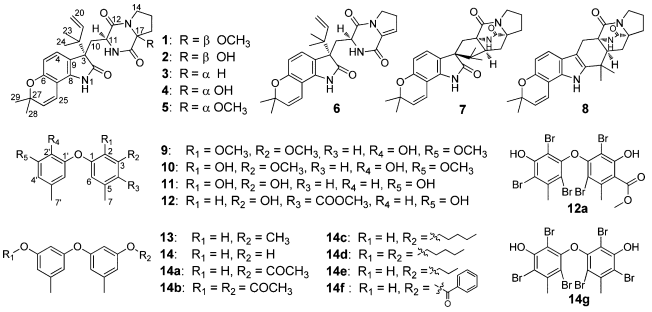
Supporting Information

ABSTRACT: Two new prenylated indole alkaloids, 17-*epi*-notoamides Q and M (**1** and **2**), and two new phenyl ether derivatives, cordyols D and E (**9** and **13**), together with 10 known compounds (**3**−**8**, **10**−**12**, **14**) were isolated from a marine-derived *Aspergillus* sp. fungus. Among them, **1**/**5** and **2**/**4** were pairs of epimers. The planar structures and absolute configurations of the new compounds were determined by extensive NMR spectroscopic data as well as CD spectra. The absolute configuration of **3** was confirmed by single-crystal X-ray diffraction analysis for the first time. All isolated metabolites (**1**−**14**) and eight synthetic phenyl ether derivatives (**12a**, **14a**−**14g**) were evaluated for their antibacterial activities *in vitro*. The polybromide phenyl ether **14g** showed pronounced antibacterial activity against *Staphylococcus epidermidis* with an MIC value of 0.556 μM, stronger than that of the positive control ciprofloxacin (MIC = 3.13 μM).



Marine microorganisms, especially marine fungi, have attracted increasing attention from those seeking new pharmaceutically useful natural products in recent years.[1] During the past decade, a growing number of structurally unique and biologically active compounds have been isolated from marine fungi.[1,2] For example, the notoamides from *Aspergillus* spp. are prenylated indole alkaloids that incorporate complex bicyclo[2.2.2]diazaoctane or diketopiperazine ring systems and show a wide range of biological activities.[3−6]

In our ongoing research on marine fungi from the South China Sea, we have isolated several new compounds with antibacterial, cytotoxic, antiviral, and antifouling activities.[7−11] Recently, a chemical investigation of the marine fungal strain *Aspergillus* sp. XS-20090066, isolated from the gorgonian *Dichotella gemmacea*, was carried out. The EtOAc extract of the fungal culture showed antibacterial activity against a panel of pathogenic bacteria. In this study, we report the isolation, structure elucidation, and antibacterial activities of two new prenylated indole alkaloids, 17-*epi*-notoamides Q and M (**1** and **2**), and two new phenyl ether derivatives, cordyols D and E (**9** and **13**), from the bioactive extract. Ten known compounds, notoamide C (**3**),[6,12] notoamide M (**4**),[13−15] notoamide Q (**5**),[14,15] dehydronotoamide C (**6**),[5] (+)-notoamide B (**7**),[12,16] (−)-stephacidin A (**8**),[16,17] 3,3′-*O*-dimethylviolaceol-I (**10**), cordyol C (**11**),[18] 4-methoxycarbonyldiorcinol (**12**),[19] and diorcinol (**14**),[18] are also described. All isolated metabolites (**1**−**14**) and eight synthetic phenyl ether derivatives (**12a** and **14a**−**14g**) were evaluated for their antibacterial activity *in vitro*. Preliminary structure−activity relationships (SAR) of the phenyl ether derivatives to the antibacterial activity are also discussed.



RESULTS AND DISCUSSION

Compound **1** was isolated as a white powder and has the molecular formula $C_{27}H_{33}N_3O_5$ (13 degrees of unsaturation) as determined by HRESIMS. The ^{1}H NMR spectrum of **1** (Table 1) showed five singlet methyl signals at δ_H 3.31, 1.43, 1.40, 1.08, and 1.01, four doublet olefinic or aromatic signals at δ_H 6.96 (d, J = 7.8 Hz), 6.67 (d, J = 9.6 Hz), 6.37 (d, J = 7.8 Hz), and 5.74 (d, J = 9.6 Hz), three doublet−doublet olefinic signals at δ_H 6.11 (dd, J = 17.4, 10.8 Hz), 5.06 (dd, J = 10.8, 1.2 Hz), and 4.99 (dd, J = 17.4, 1.2 Hz), and two exchangeable proton signals at δ_H 9.65 and 6.22. The ^{13}C NMR and DEPT data revealed that **1** contained three carbonyl groups (δ_C 180.3, 167.8, and 165.7), 10 olefinic or aromatic carbon atoms, and five methyl groups (including a methoxy carbon signal at δ_C 52.1). These spectroscopic features

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Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Spectroscopic Data for Compounds 1 and 2 (acetone-d_6)

	1		2	
position	δ_C, type	δ_H, mult. (J in Hz)	δ_C, type	δ_H, mult. (J in Hz)
1		9.65, brs		9.78, brs
2	180.3, C		180.1, C	
3	56.9, C		55.8, C	
4	127.1, CH	6.96, d (7.8)	126.3, CH	6.95, d (7.8)
5	109.2, CH	6.37, d (7.8)	108.4, CH	6.35, d (7.8)
6	153.7, C		152.8, C	
7	106.1, C		105.3, C	
8	139.9, C		139.0, C	
9	122.5, C		121.6, C	
10	32.6, CH_2	2.66, dd (14.4, 4.8); 2.46, dd (14.4, 8.4)	31.8, CH_2	2.68, dd (13.8, 5.4); 2.57, dd (13.8, 8.4)
11	56.7, CH	3.60, m	56.3, CH	3.56, ddd (8.4, 5.4, 4.8)
12	167.8, C		167.3, C	
14	46.2, CH_2	3.43−3.49, m	45.1, CH_2	3.52, m; 3.38, m
15	14.3, CH_2	1.83−1.89, m	19.3, CH_2	2.01, m; 1.87, m
16	38.1, CH_2	2.33, m; 1.58, m	37.1, CH_2	2.15, m; 2.09, m
17	91.8, C		86.6, C	
18	165.7, C		167.0, C	
19		6.22, brs		6.25, d (4.8)
20	113.8, CH_2	5.06, dd (10.8, 1.2); 4.99, dd (17.4, 1.2)	113.0,CH_2	5.02, dd (10.8, 1.2); 4.95, dd (17.4, 1.2)
21	144.5, CH	6.11, dd (17.4, 10.8)	143.5, CH	6.08, dd (17.4, 10.8)
22	43.2, C		42.3, C	
23	23.3, CH_3	1.01, s	22.5, CH_3	0.99, s
24	20.4, CH_3	1.08, s	21.2, CH_3	1.05, s
25	118.1, CH	6.67, d (9.6)	117.2, CH	6.66, d (9.6)
26	131.0, CH	5.74, d (9.6)	130.1, CH	5.73, d (9.6)
27	76.5, C		75.7, C	
28	27.7, CH_3	1.40, s	27.2, CH_3	1.39, s
29	28.0, CH_3	1.43, s	26.9, CH_3	1.42, s
17-OCH_3	52.1, CH_3	3.31, s		
17-OH				5.99, brs

suggested that **1** belongs to the family of prenylated indole alkaloids and is very similar to notoamide Q (**5**), which was obtained from the culture of a marine-derived *Aspergillus* sp. and was reported to have the 3*S*,11*S*,17*R* configurations.[14,15] The significant differences between these two compounds were the chemical shifts of H-11 (δ_H 3.60 in **1** vs δ_H 4.25 in **5**) and 17-OCH_3 (δ_H 3.31 in **1** vs δ_H 3.01 in **5**) in their ^{1}H NMR spectra (Table 2). It could be deduced that the relative configuration of C-11 and C-17 in **1** was different from that of **5**. In the NOESY experiments for **1**, no correlation was observed between H-11 and 17-OMe, suggesting that these protons are located on opposite faces of the diketopiperazine ring. This was supported by the selective NOE experiments for **1**, revealing that the irradiation of 17-OMe resulted in no obvious enhancement of H-11. These results suggested that one of the two stereogenic centers in **1** (either C-11 or C-17) was inverted relative to the corresponding center in **5**, indicating two possibilities for the absolute configurations at C-11 and C-17 of **1**, either 11*R*,17*R* or 11*S*,17*S*. The biogenesis of the notoamides and related compounds has been proposed on the basis of the genome sequencing, bioinformatic mining, and biochemical studies.[15,20] Therefore, on the basis of biogenetic considerations, the configuration of C-11 in **1** is proposed as *S* arising from the L-Trp moiety. Accordingly, the configuration at C-17 of **1** is assigned as *S*.

The absolute configuration of C-3 in **1** was established according to its CD spectrum (Figure 1) combined with the X-ray structure (Figure 2) of notoamide C (**3**). As the CD spectrum of **1** matched closely with that of **3** and the X-ray structure of **3** unambiguously determined the *S* configuration at C-3 (see below), the absolute configuration of **1** could also be assigned as 3*S*. Thus, the structure of **1** was determined with the absolute configuration of 3*S*,11*S*,17*S* and named 17-*epi*-notoamide Q.

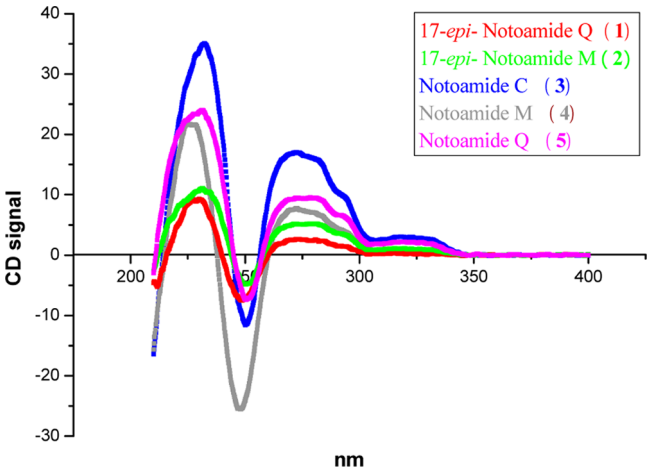

Figure 1. CD spectra of compounds **1**−**5**.

Compound **2** was also isolated as a white powder, possessing the same molecular formula, $C_{26}H_{31}N_3O_5$, as notoamide M (**4**).[13−15] Careful comparison of the ^{1}H NMR spectroscopic

Table 2. Selected ^{1}H NMR (600 MHz) Spectroscopic Data for Compounds 1−5 (acetone-d_6)

	1	2	3	4	5
position	δ_H, mult. (J in Hz)	δ_H, mult. (J in Hz)	δ_H, mult. (J in Hz)	δ_H, mult. (J in Hz)	δ_H, mult. (J in Hz)
10	2.46, dd (14.4, 8.4); 2.66, dd (14.4, 4.8)	2.57, dd (13.8, 8.4); 2.68, dd (13.8, 5.4)	2.73, dd (15.0, 4.8); 2.73, dd (15.0, 4.8)	2.75, dd (14.4,5.4); 2.65, dd (14.4, 3.0)	2.66, dd (14.4, 6.0); 2.90, overlapped
11	3.60, m	3.56, ddd (8.4, 5.4, 4.8)	4.03, brt (4.8)	4.15, m	4.25, dd (6.0, 1.8)
14	3.43−3.49, m	3.52, m; 3.38, m	3.42, m; 3.20, m	3.36−3.41, m	3.48, m; 3.23, m
15	1.83−1.89, m	2.01, m; 1.87, m	1.69−1.74, m	1.71, m; 1.53, m	1.76, m; 1.62, m
16	2.33, m; 1.58, m	2.15, m; 2.09, m	1.99, m; 1.26, m	1.95, m; 1.52, m	1.98, m; 1.20, m
17			3.91, ddd (10.2, 6.6, 1.8)		
17-OCH_3	3.31, s				3.01, s

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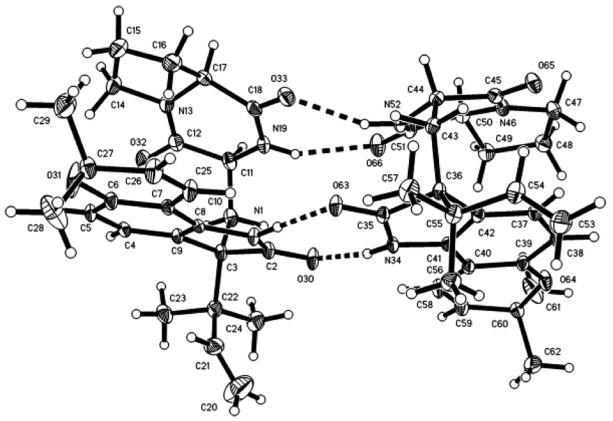

3

Figure 2. Perspective ORTEP drawing for **3**.

features of **2** with those of **4** showed significant differences of the coupling constants of H-11 and H-10 as well as the chemical shifts of H-16 and H-15 (Table 2), indicating a structural pattern for **2** vs **4** similar to **1** vs **5**. The determination of the configurations for **2** was just like that for **1**. The configuration of the C-17 stereocenter in **2** was inverted compared to that of **4**. Combined with its CD spectrum (Figure 1), **2** was finally determined as 17-*epi*-notoamide M, with the absolute configuration of 3*S*,11*S*,17*S*.

Compound **9** was isolated as a colorless oil. Its molecular formula of $C_{17}H_{20}O_5$ (eight degrees of unsaturation) was determined by HRESIMS. The IR spectrum of **9** showed a broad absorption band at 3460 cm^{-1} (OH). The ^1H and ^{13}C NMR data were suggestive of a diphenyl ether derivative for **9** very similar to that of cordyol C (**11**).[18] The significant difference was the presence of three methoxy groups in the ^1H NMR spectrum of **9**. Detailed assignments for proton and carbon signals (Table 3) were unambiguously accomplished by analysis of 1D and 2D NMR data. In the HMBC spectrum, correlations from 2-OCH_3 to C-2, 3-OCH_3 to C-3, and 3′-OCH_3 to C-3′ confirmed the three methoxy groups were attached to C-2, C-3, and C-3′, respectively. However, because the correlations from 2′-OH to C-1′ and C-2′ were observed simultaneously in the HMBC spectrum, it was not possible to unambiguously determine whether these two tetrasubstituted benzene rings were joined via a $C_1-O-C_{1'}$ linkage or a $C_1-O-C_{2'}$ linkage. Thereby, a methylation experiment was carried out for **9**. The ^1H and ^{13}C NMR spectrum of the methylated derivative of **9** indicated a symmetrical diphenyl ether structure, suggesting a $C_1-O-C_{1'}$ linkage for the two benzene rings. The structure of **9** was given the trivial name cordyol D.

Compound **13** was also obtained as a colorless oil with the molecular formula $C_{15}H_{16}O_3$. The 1D and 2D NMR data indicated that **13** consisted of a 3-methoxy-5-hydroxytoluene unit connected to a 3,5-dihydroxytoluene residue via a $C_1-O-C_{1'}$ linkage. Aromatic carbon signals at δ_C 158.7, 158.3, and 140.5 were assigned to C-1′, C-3′, and C-5′ in the dihydroxytoluene residue, respectively. With another methyl group, the 3-methoxy-5-hydroxytoluene unit was observed at δ_C 161.0, 158.2, and 140.5 for the aromatic carbons C-3, C-1, and C-5, respectively. Compound **13** was finally determined by detailed 2D NMR analysis and was named cordyol E.

The structures of the known compounds (**3**–**8**, **10**–**12**, and **14**) were identified on the basis of their ^1H NMR, ^{13}C NMR, ESIMS, and specific rotation data and by comparison with data previously reported in the literature.[5,12−19] A structural revision for notoamide C (**3**) was proposed recently by Williams and coworkers, in which the 3*R* configuration was revised as 3*S* based on biosynthetic discussions.[6] In the present study, the absolute configuration of notoamide C (**3**) was confirmed by single-crystal X-ray diffraction analysis using Cu Kα radiation and was unambiguously determined as the 3*S* configuration (Figure 2). Consequently, as the CD spectra of the known analogues (**4** and

Table 3. ^1H and ^{13}C NMR Spectroscopic Data for 9, 10, and 13 at 600 (^1H) and 150 (^{13}C) MHz

	9[a]		**10**[a]		**13**[b]	
position	δ_C, type	δ_H, mult.	δ_C, type	δ_H, mult.	δ_C, type	δ_H, mult. (*J* in Hz)
1	150.1, C		143.9, C		158.2, C	
2	153.4, C		134.6, C		102.1, CH	6.36, t (1.8)
3	137.9, C		147.7, C		161.0, C	
4	107.9, CH	6.50, brs	108.0, CH	6.49, brs	109.6, CH	6.53, brs
5	133.7, C		129.1, C		140.5, C	
6	112.5, CH	6.36, brs	112.4, CH	6.40, brs	111.6, CH	6.39, brs
7	21.6, CH_3	2.24, s	21.3, CH_3	2.23, s	20.7, CH_3	2.22, s
1′	144.1, C		143.9, C		158.7, C	
2′	134.8, C		134.6, C		103.2, CH	6.26, t (1.8)
3′	147.7, C		147.7, C		158.3, C	
4′	108.6, CH	6.49, brs	108.0, CH	6.49, brs	110.5, CH	6.30, brs
5′	128.9, C		129.1, C		140.5, C	
6′	112.6, CH	6.38, brs	112.4, CH	6.40, brs	111.2, CH	6.44, brs
7′	21.3, CH_3	2.23, s	21.3, CH_3	2.23, s	20.6, CH_3	2.27, s
2-OCH_3	61.2, CH_3	3.86, s				
3-OCH_3	56.1, CH_3	3.85, s	56.3, CH_3	3.88, s	54.8, CH_3	3.75, s
3′-OCH_3	56.2, CH_3	3.89, s	56.3, CH_3	3.88, s		
2-OH				5.62, brs		
2′-OH		5.72, brs		5.62, brs		
3′-OH						8.43, brs

[a]Recorded in $CDCl_3$. [b]Recorded in acetone-d_6.

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Table 4. Antibacterial Activities of Compounds 8−14, 12a, and 14a−14g[a]

	MIC (μM)				
compound	*S. epidermidis*	*S. aureus*	*V. anguillarum*	*V. parahemolyticus*	*P. putida*
8	14.5	>100	>100	>100	>100
9	>100	>100	>100	>100	>100
10	>100	100	100	>100	>100
11	100	100	100	100	100
12	2.71	10.9	21.7	21.7	21.7
12a	100	100	9.23	>100	>100
13	25.6	51.2	25.6	51.2	51.2
14	27.2	54.3	100	100	100
14a	100	>100	46.0	100	100
14b	>100	>100	>100	>100	>100
14c	20.8	20.8	10.4	10.4	10.4
14d	>100	>100	>100	100	>100
14e	46.0	46.0	23.0	2.87	100
14f	18.7	>100	>100	18.7	>100
14g	0.556	1.11	1.11	1.11	1.11
ciprofloxacin[b]	3.13	0.780	0.0975	0.195	0.195

[a]Data are expressed in MIC values (μM). [b]Ciprofloxacin was used as a positive control.

5) matched closely with that of **3**, the absolute configurations of these two compounds could be assigned as 3*S*. The co-isolated dehydronotoamide C (**6**) was also deduced as 3*S* on the basis of biogenetic considerations. In addition, it seems that **6** may arise from **2** and **4** via dehydration during the extraction or purification processes. However, when **4** was dissolved in a mixed solvent with freshly prepared silica gel and stirred at 40 °C for 1 week, **6** was not detected in the solution. Therefore, **6** is proposed as a new natural product possessing a double bond between C-16 and C-17, although a synthetic sample has been reported.[5] It should be noted that compound **10**, whose structure was listed in SciFinder Scholar, had no associated reference. Thus we report its spectroscopic and MS data in the Experimental Section.

The antibacterial activities of the isolated compounds **1**−**14** were evaluated with four Gram-positive bacteria (*Bacillus cereus*, *Micrococcus tetragenus*, *Staphylococcus epidermidis*, *S. aureus*) and four Gram-negative bacteria (*Escherichia coli*, *Vibrio anguillarum*, *V. parahemolyticus*, and *Pseudomonas putida*), using a standard screening protocol (Table 4).[21] The results demonstrated that most of prenylated indole alkaloids (**1**−**8**) showed weak or no activity against these pathogenic bacteria except for stephacidin A (**8**), which revealed selectivity toward *S. epidermidis* (MIC = 14.5 μM).

Most of the phenyl ether derivatives (**11**−**14**) exhibited moderate to strong inhibitory activity against five of the tested pathogenic bacteria (*S. epidermidis*, *S. aureus*, *V. anguillarum*, *V. parahemolyticus*, and *P. putida*). Especially, **12** showed pronounced antibacterial activity against *S. epidermidis* with an MIC value of 2.71 μM, which was close to the positive control ciprofloxacin (MIC = 3.13 μM). It is noteworthy that the activity of **13** was stronger than that of **14**, and the only difference between their structures is the methylation of the 3-OH group in **13**. To elucidate the contribution of the two hydroxy groups (3-OH and 3′-OH) to antibacterial activity and also to further study the SAR of these compounds, a series of derivatives (**12a**, **14a**−**14g**) was designed and synthesized. As expected, different chemically modified analogues exhibited different inhibitory activities. It was found that the alkylation of a single hydroxy group (**14c** and **14e**) was helpful for the activity. The corresponding benzoylation (**14f**) resulted in stronger activity against *S. epidermidis* and *V. parahemolyticus* than observed for the parent compound **14**. The dialkylation or diacylation of two hydroxy groups (**14b** and **14d**) reduced activity. These results suggested that one free hydroxy plays a critical role in antibacterial activity.

Interestingly, compound **12**, with a methoxycarbonyl group at C-4, was more active than **14**, while the antibacterial intensities of the polybromide derivatives of **12** and **14** (**12a** and **14g**) were reversed. 2,4,6,2′,4′,6′-Hexabromodiorcinol (**14g**) exhibited promising antibacterial activity against five tested pathogenic bacteria (*S. epidermidis*, *S. aureus*, *V. anguillarum*, *V. parahemolyticus*, and *P. putida*) with MIC values ranging from 0.556 to 1.11 μM (Table 4), 1 order of magnitude more active than that of the parent compound **14**. In particular, **14g** showed the highest activity toward *S. epidermidis*, with an MIC value of 0.556 μM, approximately 5-fold more potent than that of ciprofloxacin (MIC = 3.13 μM). However, comparison of the antibacterial activity of 2,6,2′,4′,6′-pentabromo-4-methoxycarbonyldiorcinol (**12a**) with that of its parent compound (**12**) indicated that bromination of the benzene ring decreased the activity. The above results revealed that an ester functionality or bromination could increase activity, but the simultaneous presence of both an ester and a brominated benzene ring caused loss of activity.

Halogenated biphenols are well known as antibacterial agents. Triclosan, with chlorine substitutions, is a topical antibacterial ingredient added to many consumer products such as clothing, kitchenware, furniture, soaps, and body washes to reduce or prevent bacterial contamination.[22] The polybrominated phenyl ether derivative **14g** displayed potent antibacterial activity against a panel of pathogenic bacteria and has potential for antibacterial agent development.

Compounds **1**−**14** were also assessed for their cytotoxic activity against human erythroleukemia K562 and human promyelocytic leukemia HL-60 cell lines. Compound **12** showed cytotoxic activity against the K562 cell line with an IC_{50} value of 5.94 μM. Other compounds were found to be inactive (IC_{50} > 10 μM) against these tumor cell lines.

In conclusion, two new prenylated indole alkaloids (**1** and **2**) and two new phenyl ether derivatives (**9** and **13**), together with 10 known compounds (**3**−**8**, **10**−**12**, and **14**), were isolated from the marine-derived fungus *Aspergillus* sp. XS-20090066. Eight chemically modified phenyl ether derivatives (**12a**, **14a**−**14g**)

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were also prepared. The absolute configuration of **3** was confirmed by single-crystal X-ray diffraction for the first time, and the absolute configurations of **1**−**5** were unambiguously determined as 3*S* based on their CD spectra. The antibacterial activities of the isolated metabolites and eight synthesized derivatives were evaluated *in vitro*, and the preliminary SAR of phenyl ether derivatives was also examined. Particularly, the polybrominated phenyl ether derivative **14g** showed pronounced antibacterial activity against *S. epidermidis*, approximately 5-fold more potent than that of ciprofloxacin.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on an X-6 micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were obtained on a Beckman DU 640 spectrophotometer. CD spectra were recorded on a JASCO J-810 circular dichroism spectrometer. IR spectra were recorded on a Nicolet-Nexus-470 spectrometer using KBr pellets. NMR spectra were acquired using a JEOL JEM-ECP NMR spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C), using TMS as internal standard. ESIMS spectra were obtained from a Micromass Q-TOF spectrometer. Single-crystal data were measured on an Agilent Gemini Ultra diffractometer (Cu Kα radiation). Semipreparative HPLC was performed on a Waters 1525 system using a semipreparative C_{18} (Kromasil, 5 μm, 10 × 250 mm) column coupled with a Waters 2996 photodiode array detector. Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200−300 mesh), Sephadex LH-20 (Amersham Biosciences), and octadecylsilyl silica gel (Unicorn; 45−60 μm) were used for column chromatography (CC). Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co.; G60, F-254) were used for thin layer chromatography (TLC).

Extraction and Isolation. The fungus *Aspergillus* sp. XS-20090066 was isolated from the inner part of the fresh gorgonian *Dichotella gemmacea*, which was collected from the Xisha Islands coral reef in the South China Sea in December 2009. The strain was deposited at the Key Laboratory of Marine Drugs, the Ministry of Education of China, School of Medicine and Pharmacy, Ocean University of China, Qingdao, PR China, with the GenBank (NCBI) accession number HM535361. The fermentation was carried out statically in a rice medium (100 mL seawater, 100 g rice, 0.6 g peptone) in 1 L Erlenmeyer flasks for 28 days at room temperature (rt). The fermented rice substrate (30 flasks) was extracted repeatedly with EtOAc (3 × 300 mL for each flask), and the solvent was combined and concentrated *in vacuo* to afford a residue (21.5 g), which was subjected to silica gel column chromatography using a step gradient elution with EtOAc−petroleum ether (0−100%) and then with MeOH−$CHCl_3$ (0−100%) to provide seven fractions (Fr.1−Fr.7). Fr.2 was subjected to Sephadex LH-20 CC eluting with a mixture of $CHCl_3$−MeOH (v/v, 1:1) and then was further purified by using semipreparative HPLC (Kromasil, 5 μm, 10 × 250 mm, 2 mL/min) eluting with 90% MeOH−H_2O to give compounds **13** (4.5 mg) and **12** (11.9 mg). Fr.3 was isolated on silica gel CC by using repeated gradient elution of petroleum ether−EtOAc to yield **14** (190 mg) and subfraction Fr.3-1, which was further purified by HPLC (80% MeOH−H_2O) to give **9** (6.2 mg). Fr.4 was subjected to silica gel CC using gradient elution with petroleum ether−EtOAc and then was subjected to an ODS column eluting with 95% MeOH−H_2O to provide **10** (4.9 mg) and **11** (5.1 mg). Fr.5 was separated on silica gel CC by using gradient elution of petroleum ether−EtOAc and then further purified on an ODS column eluting with 85% MeOH−H_2O to produce **7** (24 mg) and **8** (65 mg). Fr.6 was isolated by silica gel CC eluting with $CHCl_3$−MeOH (v/v, 30:1) to obtain **3** (16 mg) and two subfractions, Fr.6-1 and Fr.6-2. Fr.6-1 was further purified by HPLC (62% MeOH−H_2O) to afford **1** (3.1 mg) and **5** (6 mg). Fr.6-2 was further purified on HPLC (55% MeOH−H_2O) to afford **2** (2.7 mg), **4** (4.9 mg), and **6** (3.7 mg).

*17-epi-Notoamide Q (**1**):* white powder; mp 179−180 °C; $[\alpha]^{25}_D$ −9.3 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 245 (2.30), 278 (1.10), 296 (0.67) nm; CD (0.64 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 230 (9.3), 248 (−7.5), 272 (2.7) nm; IR (KBr) ν_{max} 3445, 2921, 1647, 1447, 1154 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz) and ^{13}C NMR (acetone-d_6, 150 MHz), see Table 1; ESIMS *m*/*z* 502.4 [M + Na]$^+$; HRESIMS *m*/*z* 502.2319 (calcd for $C_{27}H_{33}N_3O_5Na$, 502.2312).

*17-epi-Notoamide M (**2**):* white powder; mp 194−196 °C; $[\alpha]^{25}_D$ −8.6 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 252 (2.52), 282 (1.65), 294 (1.14) nm; CD (0.83 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 231 (11.1), 250 (−4.9), 278 (5.2) nm; IR (KBr) ν_{max} 3465, 2940, 1653, 1457, 1118 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz) and ^{13}C NMR (acetone-d_6, 150 MHz), see Table 1; ESIMS *m*/*z* 488.2 [M + Na]$^+$; HRESIMS *m*/*z* 466.2343 (calcd for $C_{26}H_{32}N_3O_5$, 466.2336).

*Cordyol D (**9**):* colorless oil; UV (MeOH) λ_{max} (log ε) 234 (1.87), 276 (0.46) nm; IR (KBr) ν_{max} 3460, 2926, 1637, 1452, 1103, 821 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 3; ESIMS *m*/*z* 327.1 [M + Na]$^+$, 631.3 [2 M + Na]$^+$; HRESIMS *m*/*z* 327.1197 (calcd for $C_{17}H_{20}O_5Na$, 327.1203).

*3,3′-O-Dimethylviolaceol-I (**10**):* colorless oil; UV (MeOH) λ_{max} (log ε) 238 (2.15), 280 (0.93) nm; IR (KBr) ν_{max} 3440, 2962, 1658, 1457, 1098, 795 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 3; ESIMS *m*/*z* 313.1 [M + Na]$^+$, 603.3 [2 M + Na]$^+$; HRESIMS *m*/*z* 313.1041 (calcd for $C_{16}H_{18}O_5Na$, 313.1046).

*Cordyol E (**13**):* colorless oil; UV (MeOH) λ_{max} (log ε) 232 (1.84), 274 (0.72) nm; IR (KBr) ν_{max} 3416, 2921, 1606, 1452, 1149, 810 cm^{-1}; ^{1}H NMR (acetone-d_6, 600 MHz) and ^{13}C NMR (acetone-d_6, 150 MHz), see Table 3; ESIMS *m*/*z* 245.1 [M + H]$^+$; HRESIMS *m*/*z* 245.1172 (calcd for $C_{15}H_{17}O_3$, 245.1172).

*Specific rotations for known compounds **3**−**8**:* notoamide C (**3**): $[\alpha]^{25}_D$ +97 (*c* 0.40, MeOH) [lit. $[\alpha]^{27}_D$ +23 (*c* 0.255, MeOH)];[12] notoamide M (**4**): $[\alpha]^{25}_D$ +34 (*c* 0.10, MeOH) [lit. $[\alpha]^{23}_D$ +51 (*c* 1.7, MeOH)];[13] notoamide Q (**5**): $[\alpha]^{25}_D$ +78 (*c* 0.10, MeOH) [lit. $[\alpha]^{20}_D$ +82 (*c* 2.2, MeOH)];[13] dehydronotoamide C (**6**): $[\alpha]^{25}_D$ +30 (*c* 0.10, CH_2Cl_2) [lit. $[\alpha]^{25}_D$ +81.3 (*c* 0.07, $CHCl_3$)];[5] notoamide B (**7**): $[\alpha]^{25}_D$ +118 (*c* 0.20, MeOH) [lit. $[\alpha]_D$ +102 (*c* 0.05, MeOH); $[\alpha]^{27}_D$ −118 (*c* 0.064, MeOH)];[16,12] stephacidin A (**8**): $[\alpha]^{25}_D$ −67 (*c* 0.06, MeOH−CH_2Cl_2, 1:1) [lit. $[\alpha]_D$ −32 (*c* 0.05, MeOH−CH_2Cl_2, 1:1); $[\alpha]_D$ +61.5 (*c* 0.26, MeOH−CH_2Cl_2, 1:1)].[16]

X-ray Crystallographic Analysis of 3. Colorless crystals of **3** were obtained from MeOH. Single-crystal X-ray diffraction data were collected at 150 K on an Agilent Gemini Ultra diffractometer with Cu Kα radiation (λ = 1.54178 Å). The structure was solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined relatively isotropically with a riding model. Crystallographic data for **3** have been deposited in the Cambridge Crystallographic Data Centre with the deposition number 886124. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK [fax: t44-(0)1223-336033, or e-mail: deposit@ccdc.cam.ac.uk].

*Crystal data for **3**:* $C_{26}H_3N_{31}O_4$, M_r = 449.54, monoclinic, *a* = 14.9294(2) Å, *b* = 10.36530(10) Å, *c* = 15.2344(2) Å, α = 90°, β = 92.6220(10)°, γ = 90°, *V* = 2355.02(5) Å^3, space group $P2_1$, *Z* = 4, D_x = 1.268 mg/m^3, μ(Cu Kα) = 0.695 mm^{-1}, and *F*(000) = 960. Crystal dimensions: 0.43 × 0.39 × 0.34 mm^3. Independent reflections: 8378 (R_{int} = 0.0293). The final R_1 values were 0.0370, wR_2 = 0.0970 ($I > 2\sigma(I)$). Flack parameter = 0.01(12).

Methylation Experiment for 9. To a solution of **9** (3 mg) in dry acetone (1.0 mL) were added $(CH_3)_2SO_4$ (5 μL) and K_2CO_3 (10 mg) at rt, and the reaction mixture was stirred for 5 h. The solvent was evaporated *in vacuo*, and the residue was purified by HPLC (95% MeOH−H_2O) to give 1.9 mg of the methylated derivative of **9** as a colorless oil: ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm) 6.49 (2H, brs), 6.30 (2H, brs), 3.87 (6H, s), 3.85 (6H, s), 2.23 (6H, s); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm) 153.4 (C × 2), 150.1 (C × 2), 137.8 (C × 2), 133.6 (C × 2), 112.4 (CH × 2), 108.2 (CH × 2), 61.1 (CH_3 × 2), 56.1 (CH_3 × 2), 21.6 (CH_3 × 2); ESIMS *m*/*z* 319.1 [M + H]$^+$.

Preparation of Derivatives of 12 and 14. Brominated Derivatives 12a and 14g. To a stirred solution of **12** or **14** (10 mg, respectively) in acetone (1.0 mL) was added slowly bromine (2.0 mL) at rt, and the reaction mixture was stirred for 30 min. After the starting material was consumed, the mixture was concentrated *in vacuo* to give a

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residue, which was purified by HPLC (70% MeOH−H_2O) to give compound **12a** or **14g** (9 or 11 mg, 30.9% or 36.4%) as a white solid.

Alkylated Derivatives 14c, 14d, and 14e. A mixture of **14** (10 mg), 1-bromopentane (10 μL) or 1-bromopropane (4 μL), and K_2CO_3 (10 mg) in dry acetone (2.0 mL) was stirred at 30 °C overnight. The solvent was evaporated *in vacuo* to give a residue, which was purified by silica gel column chromatography (petroleum ether−EtOAc, v/v, 9:1 to 5:1) to give **14c** (3.7 mg, 28.7%), **14d** (8.6 mg, 54.1%), or **14e** (4.9 mg, 42.2%).

Acylated Derivatives 14a, 14b, and 14f. To a solution of **14** (10 mg) in dry acetone (2.0 mL) were added Ac_2O (9 μL) or BzCl (5 μL) and K_2CO_3 (10 mg) at rt, and the reaction mixture was stirred for 2 h. The solvent was evaporated *in vacuo*, and the residue was purified by silica gel column chromatography (petroleum ether−EtOAc, v/v, 9:1 to 5:1) to give **14a** (4.1 mg, 35.1%), **14b** (6.9 mg, 51.1%), or **14f** (4.9 mg, 34.2%).

2,6,2′,4′,6′-Pentabromo-4-methoxycarbonyldiorcinol (12a): white powder; mp 180−181 °C; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm) 11.77 (1H, s), 6.10 (1H, brs), 4.01 (3H, s), 2.68 (3H, s), 2.61 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm) 170.4 (C), 158.3 (C), 152.8 (C), 148.8 (C), 148.6 (C), 140.1 (C), 137.5 (C), 110.3 (C), 110.3 (C), 108.3 (C), 107.1 (C), 100.6 (C), 100.0 (C), 52.4 (CH_3), 24.1 (CH_3), 22.8 (CH_3); ESIMS *m/z* 682.6 $[M - H]^-$; HRESIMS *m/z* 682.6375 (calcd for $C_{16}H_{10}{}^{79}Br_2{}^{81}Br_3O_5$, 682.6378).

3-*O*-Acetyldiorcinol (14a): colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz) 6.70 (1H, brs), 6.64 (1H, brs), 6.54 (1H, t, *J* = 2.4 Hz), 6.42 (2H, brs), 6.30 (1H, t, *J* = 2.4 Hz), 5.00 (1H, brs), 2.32, (3H, s), 2.27, (3H, s), 2.26, (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm) 168.7 (C), 156.8 (C), 155.8 (C), 150.3 (C), 140.2 (C), 139.8 (C), 116.2 (CH), 116.2 (CH), 111.3 (CH), 110.6 (CH), 108.6 (CH), 102.6 (CH), 20.5 (CH_3), 20.5 (CH_3), 20.2 (CH_3); ESIMS *m/z* 295.0 $[M + Na]^+$, 567.1 $[2\ M + Na]^+$; HRESIMS *m/z* 295.0941 (calcd for $C_{16}H_{16}O_4Na$, 295.0941).

3,3′-*O*-Diacetyldiorcinol (14b): colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz) 6.71 (2H, brs), 6.67 (2H, brs), 6.55 (2H, t, *J* = 2.4 Hz), 2.32 (6H, s), 2.26 (6H, s); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm) 168.7 (C × 2), 156.8 (C × 2), 150.8 (C × 2), 140.2 (C × 2), 116.8 (CH × 2), 116.5 (CH × 2), 109.1 (CH × 2), 20.8 (CH_3 × 2), 20.5 (CH_3 × 2); ESIMS *m/z* 337.1 $[M + Na]^+$, 651.0 $[2\ M + Na]^+$; HRESIMS *m/z* 337.1051 (calcd for $C_{18}H_{18}O_5Na$, 337.1046).

3-*O*-Pentyldiorcinol (14c): colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz) 6.48 (1H, brs), 6.40 (2H, brs), 6.38 (2H, brs), 6.29 (1H, d, *J* = 2.4 Hz), 4.87 (1H, brs), 3.89 (2H, t, *J* = 6.6 Hz), 2.28 (3H, s), 2.26 (3H, s), 1.76 (2H, m), 1.34−1.42 (4H, m), 0.92 (3H, t, *J* = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm) 159.7 (C), 157.9 (C), 157.2 (C), 155.9 (C), 140.3 (C), 139.9 (C), 111.5 (CH), 111.4 (CH), 110.3 (CH), 110.1 (CH), 102.6 (CH), 102.3 (CH), 67.5 (CH_2), 28.4 (CH_2), 27.6 (CH_2), 21.9 (CH_3), 21.1 (CH_3), 20.8 (CH_3), 13.4 (CH_2); ESIMS *m/z* 299.5 $[M - H]^-$, 599.2 $[2\ M - H]^-$; HRESIMS *m/z* 299.1649 (calcd for $C_{19}H_{23}O_3$, 299.1642).

3,3′-*O*-Dipentyldiorcinol (14d): colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz) 6.46 (2H, brs), 6.39 (2H, brs), 6.36 (2H, brs), 3.88 (4H, t, *J* = 6.6 Hz), 2.27 (6H, s), 1.74 (4H, dt, *J* = 13.8, 6.6 Hz), 1.34−1.43 (8H, m), 0.91 (6H, t, *J* = 6.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm) 159.6 (C × 2), 157.5 (C × 2), 139.8 (C × 2), 111.2 (CH × 2), 109.8 (CH × 2), 101.9 (CH × 2), 67.4 (CH_2 × 2), 28.3 (CH_2 × 2), 27.6 (CH_2 × 2), 21.8 (CH_3 × 2), 21.0 (CH_3 × 2), 13.4 (CH_2 × 2); ESIMS *m/z* 371.2 $[M + H]^+$; HRESIMS *m/z* 371.2583 (calcd for $C_{24}H_{35}O_3$, 371.2581).

3-*O*-Propyldiorcinol (14e): colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz) 6.48 (1H, brs), 6.40 (2H, brs), 6.38 (2H, brs), 6.29 (1H, brs), 4.85 (1H, brs), 3.86 (2H, t, *J* = 6.6 Hz), 2.28 (3H, s), 2.26 (3H, s), 1.78 (2H, m), 1.01 (3H, t, *J* = 7.2 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm) 159.5 (C), 157.7 (C), 157.1 (C), 155.8 (C), 140.2 (C), 139.8 (C), 111.4 (CH), 111.2 (CH), 110.2 (CH), 109.9 (CH), 102.5 (CH), 102.1 (CH), 68.9 (CH_2), 29.0 (CH_2), 21.9 (CH_3), 20.9 (CH_3), 9.8(CH_2); ESIMS *m/z* 273.1 $[M + H]^+$; HRESIMS *m/z* 273.1486 (calcd for $C_{17}H_{21}O_3$, 273.1485).

3-*O*-Benzoyldiorcinol (14f): colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, *J*/Hz) 8.17 (2H, dd, *J* = 7.2, 1.8 Hz), 7.63 (1H, dt, *J* = 7.2, 1.8 Hz), 7.49 (2H, t, *J* = 7.2 Hz), 6.78 (1H, brs), 6.75 (1H, brs), 6.67 (1H, t, *J* = 1.2 Hz), 6.44 (1H, brs), 6.41 (1H, brs), 6.32, (1H, t, *J* = 1.2 Hz), 5.36 (1H, brs), 2.35 (3H, s), 2.26 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm) 165.3 (C), 157.8 (C), 156.7 (C), 151.6 (C), 141.1 (C), 140.8 (C), 133.8 (C), 133.7 (C), 130.3 (CH), 130.2 (CH), 129.4 (CH), 128.7 (CH), 128.6 (C), 117.3 (CH), 117.2 (CH), 112.2 (CH), 111.6 (CH), 109.8 (CH), 103.6 (CH), 21.5 (CH_3), 21.5 (CH_3); ESIMS *m/z* 335.1 $[M + H]^+$, 357.0 $[M + Na]^+$; HRESIMS *m/z* 357.1103 (calcd for $C_{21}H_{18}O_4Na$, 357.1097).

2,4,6,2′,4′,6′-Hexabromodiorcinol (14g): white powder; mp 204−205 °C; ^{1}H NMR (600 MHz, CD_3OD, δ, ppm) 2.58 (s, 6H); ^{13}C NMR (150 MHz, CD_3OD, δ, ppm) 154.4 (C × 2), 152.6 (C × 2), 139.4 (C × 2), 107.5 (C × 2), 105.5 (C × 2), 104.0 (C × 2), 23.0 (CH_3 × 2); ESIMS *m/z* 702.3 $[M - H]^-$; HRESIMS *m/z* 702.5440 (calcd for $C_{14}H_7{}^{79}Br_3{}^{81}Br_3O_3$, 702.5429).

Antibacterial Assays. Eight bacterial strains, including Gram-positive *Bacillus cereus* (ACCC 11077), *Micrococcus tetragenus* (ATCC 13623), *Staphylococcus epidermidis* (ATCC 12228), and *S. aureus* (ATCC 27154) and Gram-negative *Escherichia coli* (ATCC 25922), *Vibrio anguillarum* (ATCC 19019), *V. parahemolyticus* (ATCC 17802), and *Pseudomonas putida* (ATCC 17848), were used for antibacterial assay. The specific antibacterial assay was carried out as described previously.[21]

Cytotoxicity Assays. The cytotoxic activities against the K562 and HL-60 cell lines were determined by the MTT method.[23] Adriamycin was used as a positive control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H, ^{13}C, HMQC, HMBC, and MS spectra of compounds **1**, **2**, **9**, **10**, and **13**; NOESY spectra of **1** and **2**; 1D NOE spectrum of **1**; ^{1}H, ^{13}C, and MS spectra of compounds **12a** and **14a**−**14g**; CIF file and X-ray crystallographic data for **3**; specific rotations for known compounds **3**−**8**. These materials 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) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2012**, *75*, 311−335.
(2) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2012**, *29*, 144−222.
(3) Grubbs, A. W.; Artman, G. D., 3rd; Tsukamoto, S.; Williams, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2257−2261.
(4) Greshock, T. J.; Grubbs, A. W.; Tsukamoto, S.; Williams, R. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2262−2265.
(5) Miller, K. A.; Tsukamoto, S.; Williams, R. M. *Nat. Chem.* **2009**, *11*, 63−69.

(6) Li, S. Y.; Finefield, J. M.; Sunderhaus, J. D.; McAfoos, T. J.; Williams, R. M.; Sherman, D. H. *J. Am. Chem. Soc.* **2012**, *134*, 788−791.
(7) Zheng, C.-J.; Shao, C.-L.; Guo, Z.-Y.; Chen, J.-F.; Deng, D.-S.; Yang, K.-L.; Chen, Y.-Y.; Fu, X.-M.; She, Z.-G.; Lin, Y.-C.; Wang, C.-Y. *J. Nat. Prod.* **2012**, *75*, 189−197.
(8) Shao, C.-L.; Wu, H.-X.; Wang, C.-Y.; Liu, Q.-A.; Xu, Y.; Wei, M.-Y.; Qian, P.-Y.; Gu, Y.-C.; Zheng, C.-J.; She, Z.-G.; Lin, Y.-C. *J. Nat. Prod.* **2011**, *74*, 629−633.
(9) Yang, K.-L.; Wei, M.-Y.; Shao, C.-L.; Fu, X.-M.; Guo, Z.-Y.; Xu, R.-F.; Zheng, C. -J.; She, Z.-G.; Lin, Y.-C.; Wang, C.-Y. *J. Nat. Prod.* **2012**, *75*, 935−941.
(10) Shao, C.-L.; Wang, C.-Y.; Wei, M.-Y.; Gu, Y.-C.; She, Z.-G.; Qian, P.-Y.; Lin, Y.-C. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 690−693.
(11) Sun, L.-L.; Shao, C.-L.; Chen, J.-F.; Guo, Z.-Y.; Fu, X.-M.; Chen, M.; Chen, Y.-Y.; Li, R.; Nicole, J.; She, Z.-G.; Lin, Y.-C.; Wang, C.-Y. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1326−1329.
(12) Kato, H.; Yoshida, T.; Tokue, T.; Nojiri, Y.; Hirota, H.; Ohta, T.; Williams, R. M.; Tsukamoto, S. *Angew. Chem., Int. Ed.* **2007**, *46*, 2254−2256.
(13) Tsukamoto, S.; Kawabata, T.; Kato, H.; Greshock, T. J.; Hirota, H.; Ohta, T.; Williams, R. M. *Org. Lett.* **2009**, *11*, 1297−1300.
(14) Tsukamoto, S.; Umaoka, H.; Yoshikawa, K.; Ikeda, T.; Hirota, H. *J. Nat. Prod.* **2010**, *73*, 1438−1440.
(15) Finefield, J. M.; Frisvad, J. C.; Sherman, D. H.; Williams, R. M. *J. Nat. Prod.* **2012**, *75*, 812−833.
(16) Greshock, T. J.; Grubbs, A. W.; Jiao, P.; Wicklow, D. T.; Gloer, J. B.; Williams, R. M. *Angew. Chem., Int. Ed.* **2008**, *47*, 3573−3577.
(17) Qian-Cutrone, J.; Huang, S.; Shu, Y.-Z.; Vydas, D.; Fairchild, C.; Menendez, A.; Krampitz, K.; Dalterio, R.; Klohr, S. E.; Gao, Q. *J. Am. Chem. Soc.* **2002**, *124*, 14556−14557.
(18) Taridaporn, B.; Seangaroon, Y.; Kamolphan, I.; Kanokarn, K. *Chem. Pharm. Bull.* **2007**, *55*, 304−307.
(19) Gong, D.-L.; Wang, X.-J.; Xiang, Z.-D.; Wang, J.-D.; Zhang, H.; Liu, C.-X.; Zhang, J.; Xiang, W.-S. *J. Antibiot.* **2011**, *64*, 465−467.
(20) Li, S.; Anand, K.; Tran, H.; Yu, F.; Finefield, J. M.; Sunderhaus, J. D.; McAfoos, T. J.; Tsukamoto, S.; Williams, R. M.; Sherman, D. H. *Med. Chem. Commun.* **2012**, *3*, 987−996.
(21) Pierce, C. G.; Uppuluri, P.; Teistan, A. R.; Wormley, J. F. L.; Mowat, E.; Ramage, G.; Lopez-ribot, J. L. *Nat. Protoc.* **2008**, *3*, 1494−1500.
(22) Jones, R. D.; Jampani, H. B.; Newman, J. L.; Lee, A. S. *Am. J. Infect. Control* **2000**, *28*, 184−196.
(23) Mosmann, T. *J. Immunol. Meth.* **1983**, *65*, 55−63.