Spirobisnaphthalene Analogues from the Endophytic Fungus *Preussia* sp.

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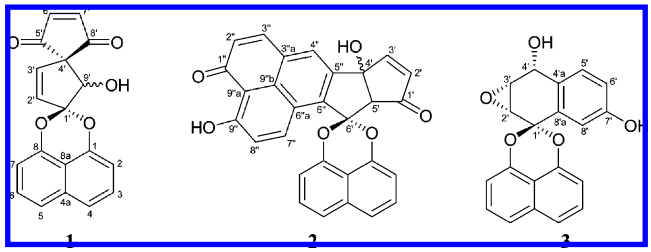
A study on the chemical constituents of the endophytic fungus *Preussia* sp. led to the isolation of three new spirobisnaphthalene analogues, spiropreussione A (**1**), spiropreussione B (**2**), and spiropreussomerin A (**3**). Compound **2** is a spirobisnaphthalene analogue with a cyclopenteno-naphthoindene fragment bridged to a 1,8-dioxygenated naphthalene fragment, and compound **1** is the second compound in this series with a spiro-nonadiene skeleton. The structures of **1**−**3** were elucidated using spectroscopic data interpretation. Compound **1** showed cytotoxicity toward A2780 and BEL-7404 cells with IC_{50} values of 2.4 and 3.0 μM, respectively, and weak activity against *Staphylococcus aureus* (CMCC B26003) with a MIC value of 25 μM.

The spirobisnaphthalenes are a series of fungal secondary metabolites consisting of a 1,8-dihydroxynaphthalene-derived spiroketal unit linked to a second, oxidized naphthalene moiety that show different biological activities. These compounds may be divided into three structural subclasses. The first contains two oxygen bridges connecting two C_{10} units through a spiroketal carbon, and many of these show antibacterial and/or antifungal activities.[1−4] It has been observed that the introduction of an oxygen function at the 8-position can increase antifungal activity significantly.[1,2] The second group, the bis-spirobisnaphthalenes, contains substances with three oxygen bridges joining two units through two spiroketal carbons, and some compounds exhibit antifungal and antiparasitic activities, as well as ras farnesyl-protein transferase (FPTase) activity.[5−10] The presence of the bis-spirobisnaphthalene core structure is considered essential to antiparasitic activity.[10] The last structural group contains compounds with two oxygen bridges and one carbon−carbon bridge, forming a bisnaphthospiroketal octacyclic ring system, and some display antibacterial activity and cytotoxicity.[11] Two other compounds, palmarumycin C6[2] and spiromamakone A,[12] containing the C_9 unit of an indenone and a spirononadiene unit, respectively, instead of a C_{10} naphthalene, have been reported in the literature. During a search for new antimicrobials, the EtOAc extracts from the fermentation broth and mycelium of the endophytic fungus *Preussia* sp. (Sporormiaceae, Ascomycota) (CGMCC No. 2022, CPCC 810274) showed inhibitory activities against *Candida albicans* (ATCC 10231) and *Staphylococcus aureus* (CMCC B26003). In the further investigation, three new spirobionaphthalene analogues, designated as spiropreussione A (**1**), spiropreussione B (**2**), and spiropreussomerin A (**3**), were isolated from a liquid culture of *Preussia* sp.



1 **2** **3**

The endophytic fungus *Preussia* sp. was isolated from a mature stem of *Aquilaria sinensis* (Lour.) Gilg (Thymelaeaceae), collected from Guangxi Medicinal Arboretum, and fermented in wheat bran broth (500 × 100 mL, 50 L). The EtOH extract of the dried mycelium was extracted sequentially with petroleum ether and EtOAc. The petroleum ether and EtOAc extracts were subjected to series of column chromatographic steps to give compound **1** and compounds **2** and **3**, respectively.

The molecular formula of spiropreussione A (**1**) was determined to be $C_{19}H_{12}O_5$ by HREIMS analysis (*m*/*z* 320.0690, $[M]^+$; Δ −0.5 mmu), and this conclusion was consistent with the number of proton and carbon signals observed in the ^1H and ^{13}C NMR spectrum (Table 1). Detailed examination of the ^1H, ^{13}C, and HSQC NMR data revealed the presence of one oxymethine carbon, two carbonyl carbons, two quaternary carbons (one of which was doubly oxygenated at δ_C 110.1), and 14 aromatic/olefinic carbons (10 of which were protonated). Analysis of the COSY NMR data (Table 2) led to the identification of two isolated three-proton spin systems corresponding to the C-2−C-4 and C-5−C-7 subunits of structure **1**, which showed the coupling constants for *ortho* coupling (J = 7.8, 8.4 Hz). HMBC correlations of H-4 and H-5 with two nonprotonated carbons, C-4a and C-8a, as well as correlations of H-2 and H-3 with C-1, and H-6 and H-7 with C-8, indicated that the subunits C-1−C-4 and C-5−C-8 are attached at C-4a and C-8a, leading to the determination of a naphthalene moiety. The chemical shifts of C-1 (δ_C 147.2) and C-8 (δ_C 146.9) suggested 1,8-dioxygenation of naphthalene. The HMBC correlations of olefinic protons H-2′ (δ_H 6.00, dd, J = 6.0, 1.8 Hz) and H-3′ (δ_H 6.41, dd, J = 6.0, 1.8 Hz) with C-1′, C-4′, and C-9′, and the oxymethine proton H-9′ (δ_H 5.38, d, J = 10.8 Hz) with C-2′ and C-3′, suggested the C-1′−C-4′ and C-9′ subunit as a cyclopentenol. The weak COSY correlations of H-2′ and H-3′ with H-9′ confirmed the assignment and also explained the doublet of doublets signals of H-2′ and H-3′ in the ^1H NMR spectrum. The HMBC correlations of the last two olefinic protons H-6′ (δ_H 7.19, d, J = 6.0 Hz) and H-7′ (δ_H 7.13, d, J = 6.0 Hz) with the carbonyl carbons C-5′ and C-8′, respectively, and with the quaternary carbon C-4′ (δ_C 66.8) indicated the C-4′−C-8′ subunit to be a cyclopentenedione and suggested C-4′ as a spiro center between the two subunits, forming a spiro-nonadiene skeleton. Both the chemical shift of C-1′ (δ_C 110.1) and the naphthalene moiety with two oxygenated carbons suggested the presence of a ketal moiety at C-1′ between the spiro-nonadienedione and the naphthalene systems. On the basis of the above analysis, the structure of spiropreussione A (**1**) was proposed as depicted. This new compound has a similar molecular arrangement to spiro-mamakone A,[12] with differences being in the position of hydroxy substitution at 4′ and a spiro center at 5′ in the spirononadienedione moiety.

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Table 1. NMR Spectroscopic Data for **1**−**3** in $CDCl_3$

	compound **1**		compound **2**		compound **3**	
position	δ_H[a] (J in Hz)	δ_C[b], mult.	δ_H[a] (J in Hz)	δ_C[b], mult.	δ_H[a] (J in Hz)	δ_C[b], mult.
1		147.2, qC		148.2, qC		149.1, qC
2	6.89, d (7.8)	110.0, CH	6.94, d (7.2)	109. 6, CH	7.05, d (7.8)	110.4, CH
3	7.38, t (7.8,8.4)	127.6, CH	7.56, t (7.2,7.2)	127.7, CH	7.50, t (7.8,8.4)	128.7, CH
4	7.46, d (8.4)	121.2, CH	7.66, d (7.2)	120.8, CH	7.57, d (8.4)	121.9, CH
5	7.45, d (8.4)	120.9, CH	7.67, d (7.2)	122.2, CH	7.54, d (8.4)	121.7, CH
6	7.37, t (7.8, 8.4)	127.3, CH	7.47, t (7.2,7.2)	127.0, CH	7.44, t (7.8,8.4)	128.7, CH
7	6.85, d (7.8)	109.6, CH	7.02, d (7.2)	109.8, CH	6.95, d (7.8)	110.1, CH
8		146.9, qC		146.5, qC		149.0, qC
4a		134.1, qC		134.4, qC		135.7, qC
8a		113.2, qC		113.6, qC		114.3, qC
1′		110.1, qC		198.7, qC		99.1, qC
2′	6.00, dd (6.0, 1.8)	129.6, CH	6.04, d (5.4)	133.2, CH	3.64, d (4.2)	51.7, CH
3′	6.41, dd (6.0, 1.8)	140.8, CH	7.82, d (5.4)	160.7, CH	3.53, dd (2.4,4.2)	54.5, CH
4′		66.8, qC		88.2, qC	5.49, d (2.4)	61.6, CH
5′		198.5, qC	3.37, s	65. 7, CH	7.25, d (7.8)	130.5, CH
6′	7.19, d (6.0)	150.2, CH		106.6, qC	6.95, d (7.8)	117.3, CH
7′	7.13, d (6.0)	150.8, CH				157.2, qC
8′		197.6, qC			7.25, s	119.0, CH
9′	5.38, d (10.8)	77.7, CH				
4′a						123.2, qC
8′a						134.0, qC
1″				183.1, qC		
2″			7.10, d (9.0)	126.6, CH		
3″			8.02, d (9.0)	141.2, CH		
4″			8.14, s	127.2, CH		
5″				139.2, qC		
6″				138.2, qC		
7″			8.70, d (9.0)	137.4, CH		
8″			7.22, d (9.0)	123.9, CH		
9″				175.0, qC		
3″a/9″b				129.0, qC		
6″a				122.1, qC		
9″a				111.3, qC		
9″b/3″a				129.0, qC		
OH-4′			3.17, s			
OH-9′	2.66, d (10.8)					
OH-9″			15.93, s			

[a] Recorded at 600 MHz. [b] Recorded at 150 MHz.

Table 2. COSY and HMBC Data for Compounds **1**−**3** in $CDCl_3$

	compound **1**		compound **2**		compound **3**	
position	COSY (H→H#)	HMBC (H→C#)	COSY (H→H#)	HMBC (H→C#)	COSY (H→H#)	HMBC (H→C#)
2	3	1, 4, 4a, 8a	3	1, 4, 8a	3	1, 4, 8a
3	2, 4	1, 2, 4a, 8a	2, 4	1, 4a	2, 4	1, 4a
4	3	1, 2, 5, 4a, 8a	3	1, 2, 5, 4a	3	1, 2, 5, 4a, 8a
5	6	4, 7, 8, 4a, 8a	6	4, 7, 8, 4a	6	4, 7, 8, 4a, 8a
6	5, 7	7, 8, 4a, 8a	5, 7	8, 4a	5, 7	8, 4a
7	6	5, 8, 4a, 8a,	6	5, 8, 8a	6	5, 8, 8a,
2′	3′	1′, 3′, 4′, 9′	3′	1′, 3′, 4′, 5′	3′	1′, 3′, 8′a
3′	2′	1′, 2′, 4′, 9′	2′	1′, 2′, 4′, 5′, 5″	2′, 4′	4′, 4′a
4′					3′	2′, 3′, 4′a, 8′a,
5′				1′, 2′, 3′, 4′, 6′, 5″, 6″	6′	7′, 8′a
6′	7′	4′, 5′, 7′			5′	7′, 8′, 4′a
7′	6′	4′, 6′, 8′				
8′						1′, 6′, 4′a
9′	2′, 3′, OH-9′	2′, 3′				
2″			3″	3″a, 9″a		
3″			2″	1″, 4″, 9″b		
4″				4′, 3″, 6″, 9″b		
7″			8″	6″, 9″, 9″b,		
8″			7″	6″a, 9″a		
9″				1″, 2″, 8″, 9″, 9″a		
OH-9′	9′	9′				

Reactions of spiropreussione A (**1**) with (*R*)- and (*S*)-α-methoxy-α-trifluoromethyl-α-phenylacetic acid chloride [(*R*)- and (*S*)-MTPA chloride], respectively, gave the (*S*)- and (*R*)-Mosher esters of **1** at C-9′.[13] The ^{1}H NMR spectrum of each MTPA ester showed two sets of peaks with almost identical intensities, which were the two diastereomeric esters formed from each of the starting (*R*)- and (*S*)-acid chlorides. It may be suggested that the rotamer ratio of 9′*R*-**1** and 9′*S*-**1** was nearly 1:1.[14]

The molecular formula of spiropreussione B (**2**) was determined to be $C_{29}H_{16}O_6$ by HREIMS (m/z 460.0949, $[M]^+$; Δ −0.2 mmu). Detailed analysis of the ^{1}H, ^{13}C, and HSQC NMR data revealed the presence of one methine carbon, two carbonyl carbons, two

quaternary carbons (oxygenated δ_C 88.2 and doubly oxygenated δ_C 106.6), and 24 aromatic/olefinic carbons (13 of which were protonated). The ^{1}H and ^{13}C NMR spectrum again showed a typical pattern and spin system for the 1,8-dioxynaphthalene fragment and the acetal unit at δ_C 106.6 (C-6′). The HMBC correlations of olefinic protons H-2′ and H-3′ with C-1′, C-4′, and C-5′, as well as H-5′ with C-1′ and C-4′, revealed the C-1′−C-5′ subunit to be a cyclopentenone. The correlations of H-5′ with the aromatic/olefinic carbons C-5″ and C-6″ and with the acetal carbon C-6′ indicated that C-4′−C-6′ and C-5″−C-6″ form a cyclopentene that condensed to a cyclopentenone unit and that C-6′ is a spiro center between the cyclopentene and 1,8-dioxynaphthalene fragments. A singlet in the ^{1}H NMR spectrum at δ_H 15.93 (OH-9″) indicated a strongly chelated hydrogen bond derived from an enolized β-dicarbonyl function, and this was confirmed by two signals at δ_C 111.3 (C-9″a) and 183.1 (C-1″) of the acetylacetone fragment in the ^{13}C NMR spectrum. Of two pairs of aromatic protons, H-2″ and H-3″ (δ_H 7.10 and 8.02, d, J = 9.0 Hz), and H-7″ and H-8″ (δ_H 8.70 and 7.22, d, J = 9.0 Hz), H-3″ and H-7″ correlated to C-1″ and C-9″, respectively, while H-2″ and H-8″, and H-3″ and H-7″ correlated to C-9″a and C-9″b, respectively, suggesting the presence of a naphthalenone fragment in **2**. The correlations of H-2″ with C-3″a and H-8″ with C-6″a confirmed the integrity of the naphthalenone unit. The chemical shift of C-3″a (δ_C 129.0) suggested a benzene ring unit condensed to the naphthalenone. The correlations of singlet aromatic proton H-4″ with C-3″, C-6″, and C-9″b revealed that C-4″ and C-6″ are attached to the naphthalenone unit at C-3″a and C-6″a, respectively. The correlations of H-4″ with C-4′ indicated the connection of C-5″ to C-4″, leading to the determination of a 9-hydroxyphenalenone moiety, and confirmed the proximity of the 9-hydroxyphenalenone and cyclopentenone fragments. On the basis of the data discussed above, the planar structure for spiropreussione B was established as depicted in **2**.

The molecular formula of spiropreussomerin A (**3**) was determined to be $C_{20}H_{14}O_5$ from the HREIMS (*m*/*z* 334.0845, $[M]^+$; Δ −0.4 mmu). Detailed analysis of ^{1}H, ^{13}C, and HSQC NMR data revealed the presence of three oxymethine carbons, one doubly oxygenated quaternary carbon (δ_C 99.1, C-1′), and 16 aromatic/olefinic carbons (nine of which were protonated). These data accounted for all but two exchangeable protons. The ^{1}H and ^{13}C NMR spectrum again showed typical three-spin systems, notably for a 1,8-dioxynaphthalene fragment and an acetal carbon at δ_C 99.1. In addition, a three-spin system with signals at δ_H 3.64 (d, J = 4.2 Hz, H-2′), δ_H 3.53 (dd, J = 2.4, 4.2 Hz, H-3′), and δ_H 5.49 (d, J = 2.4 Hz, H-4′) was observed in the ^{1}H NMR spectrum and confirmed by the COSY correlations of H-2′ with H-3′, H-3′ with H-2′ and H-4′, and H-4′ with H-3′ (Table 2). The chemical shifts in the ^{1}H and ^{13}C NMR spectra (C-2′: δ_C 51.7, C-3′: δ_C 54.5, C-4′: δ_C 61.6) indicated epoxidation to occur between C-2′ and C-3′ and an oxygenated substituent at C-4′. The correlations of H-5′ (δ_H 7.25, d, J = 7.8 Hz) to C-7′ and C-8′a, H-6′ (δ_H 6.95, d, J = 7.8 Hz) to C-4′a and C-8′, and H-8′ (δ_H 7.25, s) to C-4′a, C-6′, and C-1′, as well as H-2′ to C-1′ and C-8′a, H-3′ to C-4′a, and H-4′ to C-8′a, revealed that the C-1′−C-4′ and the C-5′−C-8′ subunits were attached at C-4′a and C-8′a, forming a decalin fragment, with C-1′ as a spiro center between the decalin and 1,8-dioxynaphthalene moieties. The chemical shift of C-7′ (δ_C 157.2) suggested further oxygenation in **3**. The molecular formula requires C-4′ and C-7′ to bear free hydroxy groups. In light of these data, the structure of spiropreussomerin A was determined as depicted in **3**. This new compound has a similar molecular arrangement in palmarumycin C_{11} with variations occurring only in the hydroxy substitution at C-5′.[2] The relative configuration of **3** was proposed by analogy with palmarumycin C_{11}.[2]

Spiropreussione A (**1**) showed activity against *Staphylococcus aureus* (CMCC B26003) in a standard disk assay, affording a zone of inhibition of 16.4 ± 0.3 mm (n = 3) at 5 μg/disk. Its minimum inhibitory concentration (MIC), tested by agar dilution using NCCLS 2002 criteria, was 25 μM. However, compound **1** did not display antimicrobial activity against *Candida albicans* (ATCC 10231) at 50 μg/disk. Compound **1** also exhibited in vitro cytotoxicity, as determined by a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method, against the A2780 human ovarian carcinoma cell line and the BEL-7404 human liver carcinoma cell line, with IC_{50} values of 2.4 and 3.0 μM, respectively. Compound **1** was inactive (IC_{50} >10 μM) against the HCT-8 (colon carcinoma), BGC-823 (gastric carcinoma), and A-549 (lung adenocarcinoma) human cancer cell lines. Neither spiropreussione B (**2**) nor spiropreussomerin A (**3**) displayed antimicrobial activity and/or cytotoxicity in the above-mentioned assays.

Experimental Section

General Experimental Procedures. Melting points were determined on a X-4 micro melting point apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. UV spectra were recorded on a Philips Pye Unicam Pu 8800 UV−vis spectrophotometer. IR spectra were taken with a Perkin-Elmer 983G IR spectrometer. NMR spectra, including COSY, HMQC, and HMBC experiments, were measured in $CDCl_3$ on a Varian Unity INOVA-600 spectrometer at 600 MHz (^{1}H) or 150 MHz (^{13}C), using tetramethylsilane (TMS) as an internal chemical shift reference. HREIMS were measured using a Micromass ZabSpec mass spectrometer.

Fungal Material. The endophytic fungus *Preussia* sp. employed in this study was isolated by X. M. Tan from a mature stem of *A. sinensis* collected form Guangxi Medicinal Arboretum, Naning, Guangxi Province, People's Republic of China, in July 2004. The fungus was identified by K. X. Hu and assigned the accession number CGMCC No. 2022 in the China General Microbiology Culture Collection at the Institute of Microbiology, Chinese Academy of Sciences, and CPCC 810274 in the China Pharmaceutical Culture Collection at the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences.

The isolate was subcultured on PDA plates at 24 °C for 14 days. The agar plugs (0.5 × 0.5 cm) were used to inoculate 250 mL Erlenmeyer flasks, each containing 100 mL of wheat bran liquid media (2.0% glucose, 0.3% potassium dihydrogen phosphate, 0.15% magnesium sulfate heptahydrate, and 3.0% wheat bran, which was boiled for 30 min and strained, and the final pH of the medium was adjusted to 6.0 before sterilization). Flask cultures were incubated at 24 °C on a rotary shaker at 120 rpm for 8 days.

Extraction and Isolation. The 50 L cultures were filtered, and the dried mycelium (55 °C, 350 g) was extracted three times with EtOH (ca. 4 L each). The extracts were filtered and concentrated at reduced pressure to afford 162 g of a crude extract. The residue was suspended with water and then extracted sequentially with petroleum ether and EtOAc. The petroleum ether-soluble part (13 g) was subjected to column chromatography on silica gel eluting with a stepwise gradient of 100% petroleum ether to 1:1 petroleum ether−EtOAc. The 9:1 petroleum ether−EtOAc fraction was purified by Sephadex LH-20 open column chromatography with 1:1 $CHCl_3$−MeOH to afford spiropreussione A (**1**) as yellow needle crystals (64 mg). The EtOAc-soluble part (4.1 g) was subject to gradient elution of column chromatography using $CHCl_3$ to 1:1 $CHCl_3$−MeOH. The $CHCl_3$ fraction, which was purified by recrystallization from MeOH, afforded spiropreussomerin A (**3**) as white needle crystals (1 mg). The 20:1 $CHCl_3$−MeOH fraction was purified by passage over Sephadex LH-20 with 1:1 $CHCl_3$−MeOH to afford spiropreussione B (**2**) as a yellow, amorphous solid (4.5 mg).

Spiropreussione A (1): yellow needle crystals (MeOH); mp 153−154 °C; $[\alpha]_D$ −6.41 (*c* 0.78, MeOH); UV (MeOH) λ_{max} (log ε) 226 (3.91) nm; IR (KBr) ν_{max} 3510, 3346, 1749, 1705 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HREIMS *m*/*z* 320.0690 (calcd for $C_{19}H_{12}O_5$, 320.0685).

Preparation of MTPA Esters of 1. The (*R*)- and (*S*)-MTPA esters of compound **1** were prepared by reacting 0.03 mmol of racemic compound **1** with 0.075 mmol of (*S*)- and (*R*)-MTPA chloride, respectively, in 2 mL of CH_2Cl_2 containing 0.075 mmol of 4-dimethylaminopyridine and 60 mmol of triethylamine. Each reaction mixture was stirred at room temperature for 2 h. The crude product was purified by column chromatography on silica gel with petroleum ether−EtOAc (3:1).[16]

Spiropreussione B (2): yellow, amorphous solid ($CHCl_3$); $[\alpha]_D$ +6.06 (*c* 0.50, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 227 (4.12) nm; IR (KBr) ν_{max} 3446, 3056, 1716, 1637 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HREIMS *m*/*z* 460.0949 (calcd for $C_{29}H_{16}O_6$, 460.0947).

Spiropreussomerin A (3): white needle crystals ($CHCl_3$); mp 241−243 °C; ^1H and ^{13}C NMR, see Table 1; HREIMS *m*/*z* 334.0845 (calcd for $C_{20}H_{14}O_5$, 334.0841).

Biological Testing. Antimicrobial bioassays were conducted according to a literature procedure.[17] The bacterial strain *Staphylococcus aureus* (CMCC B26003) was grown on Mueller-Hinton agar, and the yeast *Candida albicans* (ATCC 10231) was grown on Sabouraud dextrose agar. Targeted microbes were prepared from broth culture, and the final colony-forming units (cfu) of *S. aureus* (in MHB medium) and *C. albicans* (in SDB medium) were 10^6 and 10^5 cfu/mL, respectively. In a standard disk assay, test compounds were absorbed onto individual paper disks (6 mm diameter) at 5 and 50 μg/disk and placed on the surface of MH and SD agar, respectively. Ampicillin sodium (10 μg/disk, 38.2 ± 0.1 mm) and fluconazole (25 μg/disk, 31.3 ± 0.2 mm) were used as positive controls. In the agar dilution method, compound **1** was assayed at 11 concentrations (800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 μM) to determine the MIC. The microbes and assay plates were incubated at 37 °C for 24 h for bacteria and at 25 °C for 48 h for yeast.

Cytotoxicity bioassays were carried out according to the method described in ref 18 with five human cancer cell lines (A2780, A-549, BEL-7404, BGC-823, and HCT-8). Paclitaxel was used as positive control and exhibited IC_{50} values of 7.9 nM (A2780), 2.3 nM (A-549), 12.3 nM (BEL-7404), 3.3 nM (BGC-823), and 3.6 nM (HCT-8).

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Supporting Information Available: Spectroscopic data for **1**−**3**. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Krohn, K.; Michel, A.; Flörke, U.; Aust, H. J.; Draeger, S.; Schulz, B. *Liebigs Ann. Chem.* **1994**, *11*, 1093–1097.
(2) Krohn, K.; Michel, A.; Flörke, U.; Aust, H. J.; Draeger, S.; Schulz, B. *Liebigs Ann. Chem.* **1994**, *11*, 1099–1108.
(3) Hu, H. J.; Guo, H. J.; Li, E. W.; Liu, X. Z.; Zhou, Y. G.; Che, Y. S. *J. Nat. Prod.* **2006**, *69*, 1672–1675.
(4) Prajoubklang, A.; Sirithunyalug, B.; Charoenchai, P.; Suvannakad, R.; Sriubolmas, N.; Piyamongkol, S.; Kongsearee, P.; Kittakoop, P. *Chem. Biodiversity* **2005**, *2*, 1358–1367.
(5) Weber, H. A.; Gloer, J. B. *J. Org. Chem.* **1991**, *56*, 4355–4360.
(6) Singh, S. B.; Zink, D. L.; Liesch, J. M.; Ball, R. G.; Goetz, M. A.; Bolessa, E. A.; Giacobbe, R. A.; Silverman, K. C.; Bills, G. F.; Pelaez, F.; Cascales, C.; Gibbs, J. B.; Lingham, R. B. *J. Org. Chem.* **1994**, *59*, 6296–6302.
(7) Vilella, D.; Sánchez, M.; Platas, G.; Salazar, O.; Genilloud, O.; Royo, I.; Cascales, C.; Martín, I.; Díez, T.; Silverman, K. C.; Lingham, R. B.; Singh, S. B.; Jayasuriya, H.; Peláz, F. *J. Ind. Microbiol. Biotechnol.* **2000**, *25*, 315–327.
(8) Martínez-Luis, S.; Della-Togna, G.; Coley, P. D.; Kursar, T. A.; Gerwick, W. H.; Cubilla-Rios, L. *J. Nat. Prod.* **2008**, *71*, 2011–2014.
(9) Macías-Rubalcava, M. L.; Hernández-Bautista, B. E.; Jiménez-Estrada, M.; González, M. C.; Glenn, A. E.; Hanlin, R. T.; Hernández-Ortega, S.; Saucedo-García, A.; Muria-González, J. M.; Anaya, A. L. *Phytochemistry* **2008**, *69*, 1185–1196.
(10) Dong, J. Y.; Song, H. C.; Li, J. H.; Tang, Y. S.; Sun, R.; Wang, L.; Zhou, Y. P.; Wang, L. M.; Shen, K. Z.; Wang, C. R.; Zhang, K. Q. *J. Nat. Prod.* **2008**, *71*, 952–956.
(11) McDonald, L. A.; Abbanat, D. R.; Barbieri, L. R.; Bernan, V. S.; Discafani, C. M.; Greenstein, M.; Janota, K.; Korshalla, J. D.; Lassota, P.; Tischler, M.; Carter, G. T. *Tetrahedron Lett.* **1999**, *40*, 2489–2492.
(12) Van der Sar, S. A.; Blunt, J. W.; Munro, M. H. G. *Org. Lett.* **2006**, *8*, 2059–2061.
(13) Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17–117.
(14) Kang, C. Q.; Guo, H. Q.; Qiu, X. P.; Bai, X. L.; Yao, H. B.; Gao, L. X. *Magn. Reson. Chem.* **2006**, *44*, 20–24.
(15) Murphy, A. C.; Devenish, S. R. A.; Muscroft-Taylor, A. C.; Blunt, J. W.; Munro, M. H. G. *Org. Biomol. Chem.* **2008**, *6*, 3854–3862.
(16) Yang, C. H.; Chen, J. W.; Li, Q.; Peng, K.; Pan, X. F.; Cui, Y. X. *Chem. J. Chin. Univ.* **2006**, *27*, 1295–1297.
(17) Wicklow, D. T.; Joshi, B. K.; Gamble, W. R.; Gloer, J. B.; Dowd, P. F. *Appl. Environ. Microbiol.* **1998**, *64*, 4482–4484.
(18) Sladowski, D.; Steer, S. T.; Chothier, R. H.; Balls, M. *J. Immunol. Methods* **1993**, *157*, 203–207.

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