Indimicins A–E, Bisindole Alkaloids from the Deep-Sea-Derived Streptomyces sp SCSIO 03032

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Indimicins A−E, Bisindole Alkaloids from the Deep-Sea-Derived *Streptomyces* sp. SCSIO 03032

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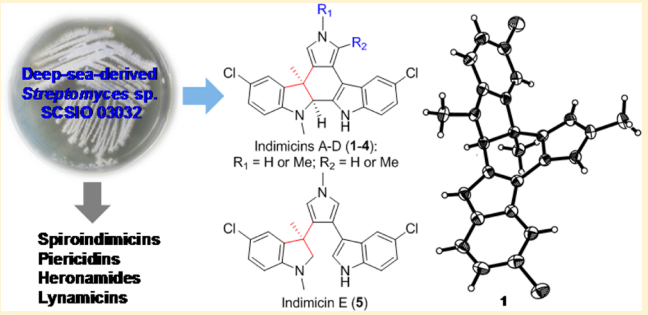
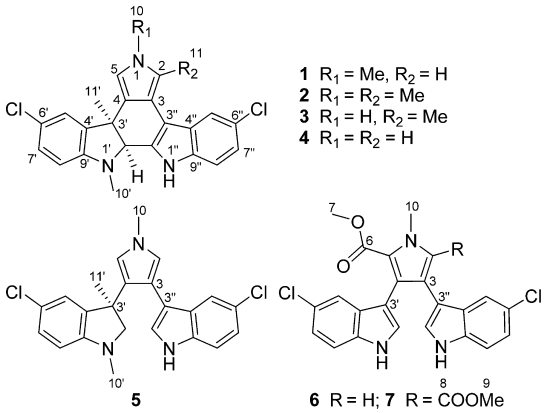
Supporting Information

ABSTRACT: Five new bisindole alkaloids, indimicins A−E (**1**−**5**), bearing a unique 1′,3′-dimethyl-2′-hydroindole moiety, were isolated from the marine-derived *Streptomyces* sp. SCSIO 03032, along with two new compounds, lynamicins F and G (**6** and **7**). Their planar structures were elucidated by detailed interpretation of their MS and NMR spectroscopic data, and the absolute configurations were determined by X-ray crystallographic analysis (for **1**), comparison of CD spectra (for **2**−**4**), and quantum chemical calculations (for **5**). Indimicin B (**2**) exhibited moderate cytotoxic activity toward the MCF-7 cell line.



Bisindole alkaloids are a large family of natural products with diverse biological activities that have attracted pharmaceutical interest.[1] Several analogues of bisindole alkaloids have been used as protein kinase inhibitors and DNA-topoisomerase I inhibitors in cancer clinical trials.[2] Although hundreds of family members of bisindole alkaloids that are derived from the fusion of two molecules of L-tryptophan have been reported,[1] their chemical space is continuing to be expanded by isolation from natural sources,[3] chemical synthesis,[4] biosynthesis and metabolic engineering,[5] and the culture-independent approach of metagenomic screening.[6] In accordance with the emerging importance of marine actinomycetes in offering opportunities for the discovery of novel natural products,[7] new bisindole alkaloids with unique bioactivities have been reported from marine-derived actinomycetes.[8] Recently, we also reported the isolation of spiroindimicins, new bisindole alkaloids with unprecedented [5,6] or [5,5] *spiro*-rings, from the deep-sea-derived actinomycete *Streptomyces* sp. SCSIO 03032,[9] which is also capable of producing lynamicins,[9] α-pyridone antibiotic piericidins,[10] and polyketide macrolactam heronamides.[11] A careful investigation of the production profile of *Streptomyces* sp. SCSIO 03032 reveals the presence of several minor components with characteristic UV spectra of bisindole alkaloids. Herein we report the isolation and biological activity of five new bisindole analogues, indimicins A−E (**1**−**5**), bearing a unique 1′,3′-dimethyl-2′-hydroindole, together with two new bisindole alkaloids, lynamicins F (**6**) and G (**7**).



RESULTS AND DISCUSSION

The deep-sea-derived strain *Streptomyces* sp. SCSIO 03032 has been previously shown to produce spiroindimicins A−D,[9] lynamycins A and D,[9] and piericidins,[10] when cultivated in the modified A1BFe + C medium.[9,12] An investigation of metabolite

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Table 1. ^{1}H NMR (500 MHz) Data for Indimicins A–E (1–5) and Lynamicins F and G (6 and 7) in $CDCl_3$[a]

	1	2	3	4	5	6	7
no.	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)	δ_H, mult. (J in Hz)	δ_H, mult. (J in Hz)
1			7.64, br s	8.04, br s			
2	6.84, d (2.0)			6.99, dd (1.5, 1.5)	6.66, d (1.8)	7.13, s	
5	6.36, d (2.0)	6.27, s	6.34, d (2.0)	6.53, dd (1.5, 1.5)	6.58, d (1.8)		
7						3.52, s	3.51, s
9							3.51, s
10	3.60, s	3.45, s			3.69, s	4.07, s	4.25, s
11		2.60, s	2.67, s				
1′						8.06, br s	7.87, br s
2′	4.02, s	3.93, s	3.96, s	4.05, s	3.08, d (8.8)	6.97, d (2.5)	6.69, d (2.5)
					3.31, d (8.8)		
5′	7.33, d (2.0)	7.33, d (2.0)	7.30, d (2.0)	7.33, d (2.0)	6.91, d (1.5)	7.30, d (2.0)	7.24, d (2.0)
7′	7.09, dd (8.5, 2.0)	7.10, dd (8.5, 2.0)	7.09, dd (8.5, 1.5)	7.07, dd (8.5, 2.0)	7.02, dd (8.2, 1.5)	7.08, dd (8.0, 2.0)	7.03, dd (8.5, 2.0)
8′	6.43, d (8.5)	6.45, d (8.0)	6.44, d (8.5)	6.42, d (8.5)	6.26, d (8.2)	7.23, d (8.5)	7.11, d (8.5)
10′	2.77, s	2.78, s	2.79, s	2.75, s	2.32, s		
11′	1.50, s	1.44, s	1.46, s	1.50, s	1.54, s		
1″	8.18, br s	8.22, br s	8.30, br s	8.34, s	7.93, s	7.89, br s	7.87, br s
2″					6.09, s	6.61, d (2.5)	6.69, d (2.5)
5″	7.71, d (2.0)	7.98, br s	7.99, br s	7.72, d (1.5)	7.47, br s	7.50, d (1.5)	7.24, d (2.0)
7″	7.18, dd (8.5, 2.0)	7.18, dd (8.5, 1.5)	7.18, dd (8.5, 1.0)	7.18, dd (8.5, 1.0)	7.10, br d (8.6)	7.08, dd (8.0, 2.0)	7.03, dd (8.5, 2.0)
8″	7.31, d (8.5)	7.33, d (8.0)	7.31, d (8.0)	7.31, d (8.5)	7.22, d (8.6)	7.18, d (8.5)	7.11, d (8.5)

[a]Data were recorded on a Bruker Avance 500 NMR spectrometer in $CDCl_3$ with TMS as an internal standard.

profiles of *Streptomyces* sp. SCSIO 03032 in different media led to the isolation of heronamides D–F from cultures in the modified ISP3 medium.[11] We observed several minor components with characteristic UV spectra of bisindole alkaloids in the modified A1BFe + C medium. Subsequently, the XAD-16 resin-assisted extraction of a 12 L fermentation of *Streptomyces* sp. SCSIO 03032 in the modified A1BFe + C medium afforded seven new bisindole analogues, indimicins A–E (**1**–**5**) and lynamicins F (**6**) and G (**7**).

Indimicin A (**1**) was obtained as a colorless crystal. The molecular formula of **1** was established as $C_{23}H_{19}Cl_2N_3$ by HRESIMS, indicating 15 degrees of unsaturation. The IR absorption of compound **1** at 3371 cm^{-1} indicated the presence of an NH group. The ^{1}H, ^{13}C, and HSQC NMR data of compound **1** (Tables 1 and 2) presented three singlet methyls, eight sp^2 methines, one sp^3 methine, 10 sp^2 quaternary carbons, and one sp^3 quaternary carbon. The COSY analysis of compound **1** revealed two typical ABX spin systems with signals [δ_H 7.33 (1H, d, J = 2.0 Hz), 7.09 (1H, dd, J = 8.5, 2.0 Hz), 6.43 (1H, d, J = 8.5 Hz) and δ_H 7.71 (1H, d, J = 2.0 Hz), 7.18 (1H, dd, J = 8.5, 2.0 Hz), 7.31 (1H, d, J = 8.5 Hz)] indicating the presence of two 1,2,4-trisubstituted benzene rings in **1**. The NMR data of **1** were similar to data for the previously reported spiroindimicins A–D and lynamicins A–E,[9,13] indicating that **1** also belonged to the bisindole alkaloid family containing a pyrrole unit. Careful analysis of COSY and HMBC correlations assigned a 6″-chloro-2″,3″-disubstitued-1″-*H*-indole, in which the chlorine was located at the sp^2 quaternary carbon (δ_C 125.8, C-6″) by ^{13}C NMR, and a 1-methyl-3,4-disubstituted pyrrole ring in **1** (Figure 1). Unlike lynamicins A–D, an unusual 6′-chloro-1′,3′-dimethyl-2′-hydroindole was present in **1**, which was deduced by key HMBC correlations (Figure 1) from H-10′ (δ_H 2.77) to C-2′ (δ_C 72.8) and C-9′ (δ_C 134.9), from H-2′ (δ_H 4.02) to C-3′ (δ_C 45.5), and from H-11′ (δ_H 1.50) to C-2′, C-3′, and C-4′ (δ_C 139.3). Moreover, a six-membered ring was formed in **1** by connecting C-2′ and C-2″, which was deduced by HMBC correlations from H-2′ to C-2″ (δ_C 130.3), C-3″ (δ_C 109.2), and C-4 (δ_C 123.1) (Figure 1). Thus, the planar structure of **1** was established, and the *cis*-configuration of H-2′ and H_3-11′ in **1** was determined by a NOESY correlation between H-2′ and H_3-11′ (Figure 1). The structure of **1** was confirmed by a single-crystal X-ray diffraction analysis with Cu Kα radiation, which also established the absolute configuration of **1** as 2′*S* and 3′*R* (Figure 1) with a convincing absolute structure parameter of −0.014 (12) (Table S1).[14]

Indimicin B (**2**) was isolated as a white powder. The molecular formula of **2** was assigned as $C_{24}H_{21}Cl_2N_3$ by HRESIMS. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **2** were highly similar to those of **1**. Compound **2** was different from **1** by having an additional methyl moiety, which was located at C-2 in **2** by HMBC correlations from H_3-11 (δ_H 2.60, 3H, s) to C-2 (δ_C 122.0) and and C-3 (δ_C 112.5). The planar structure of **2** was further confirmed by 2D NMR analysis (Figure 2).

Indimicin C (**3**) was obtained as a colorless solid. The molecular formula of **3** was assigned as $C_{23}H_{19}Cl_2N_3$ by HRESIMS. The ^{1}H and ^{13}C NMR spectroscopic data of **3** and **2** were highly similar (Tables 1 and 2). Given the observed COSY correlation between NH-1 (δ_H 7.64 1H, br s) and H-5 (δ_H 6.34 1H, d, J = 2.0 Hz) in **3**, compound **3** was deduced to be different from **2** by the absence of the methyl group at N-1 in **2**. The planar structure of **3** was confirmed by detailed 2D NMR data analysis (Figure 2).

Indimicin D (**4**) was isolated as a white, amorphous powder. The molecular formula of **4** was assigned as $C_{22}H_{17}Cl_2N_3$ by its HRESIMS. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) revealed that **1** and **4** were almost identical, except that **4** lacked the N-1 methyl present in **1**. Further analysis of the 2D NMR data confirmed the planar structure of **4** (Figure 2).

Compounds **2**–**4** have two stereogenic centers at C-2′ and C-3′, the same as compound **1**. The *cis*-configurations of H_3-11′ and H-2′ in compound **2**–**4** were determined by NOESY correlations between H_3-11′ and H-2′ (Figure 2). To determine the absolute configurations of **2**–**4**, their CD spectra were compared with that of **1**. The almost identical Cotton effects of **1**–**4** (Figure 2) suggested that compounds **2**–**4** should have the 2′*S*, 3′*R* configuration, the same as that of **1**.

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Table 2. ^{13}C NMR (125 MHz) Data for Indimicins A–E (1–5) and Lynamicins F and G (6 and 7) in $CDCl_3$[a]

	1	2	3	4	5	6	7
no.	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type	δ_C, type
2	114.8, CH	122.0, C	111.9, C	110.7, CH	120.1, CH	127.3, CH	126.2, C
3	114.3, C	112.5, C	120.7, C	114.2, C	114.1, C	117.6, C	123.4, C
4	123.1, C	122.0, C	124.5, C	123.4, C	128.4, C	122.7, C	123.4, C
5	118.7, CH	118.2, CH	113.2, CH	114.8, CH	123.4, CH	121.3, C	126.2, C
6						162.1, C	162.0, C
7						50.8, CH_3	51.3, CH_3
8							162.0, C
9							51.3, CH_3
10	36.3, CH_3	33.9, CH_3			36.3, CH_3		35.5, CH_3
11		12.9, CH_3	15.1, CH_3				
2′	72.8, CH	73.5, CH	73.3, CH	72.7, CH	69.3, CH_2	124.9, CH	125.0, CH
3′	45.5, C	44.9, C	45.1, C	45.5, C	43.7, C	111.1, C	109.8, C
4′	139.3, C	139.5, C	139.5, C	139.3, C	142.3, C	129.3, C	129.3, C
5′	122.3, CH	122.4, CH	122.4, CH	122.5, CH	123.6, CH	119.7, CH	119.2, CH
6′	123.7, C	123.9, C	123.9,C	123.7, C	122.1, C	125.5, C	125.4, C
7′	127.1, CH	127.0, CH	127.1, CH	127.1, CH	127.1, CH	121.9, CH	121.9, CH
8′	109.0, CH	109.3, CH	109.4, CH	108.9, CH	107.6, CH	111.8, CH	111.8, CH
9′	150.1, C	150.1, C	150.1, C	150.1, C	150.6, C	134.0, C	133.7, C
10′	34.9, CH_3	35.1, CH_3	35.1, CH_3	34.9, CH_3	35.6, CH_3		
11′	27.1, CH_3	26.0, CH_3	26.1, CH_3	27.2, CH_3	27.5, CH_3		
2″	130.3, C	130.3, C	130.5, C	130.7, C	125.4, CH	123.4, CH	125.0, CH
3″	109.2, C	110.1, C	110.0, C	108.9, C	111.5, C	110.5, C	109.8, C
4″	125.2, C	125.2, C	125.3, C	125.2, C	129.8, C	127.9, C	129.3, C
5″	119.6, CH	120.9, CH	120.8, CH	119.6, CH	119.3, CH	119.2, CH	119.2, CH
6″	125.8, C	125.4, C	125.5, C	125.8, C	125.4, C	125.3, C	125.4, C
7′′	122.4, CH	122.2, CH	122.3, CH	122.4, CH	122.1, CH	122.1, CH	121.9, CH
8″	112.2, CH	112.2, CH	112.2, CH	112.3, CH	111.7, CH	111.9, CH	111.8, CH
9″	134.9, C	135.2, C	135.1, C	135.0, C	133.7, C	134.0, C	133.7, C

[a]Data were recorded on a Bruker Avance 125 NMR spectrometer in $CDCl_3$ with TMS as an internal standard.

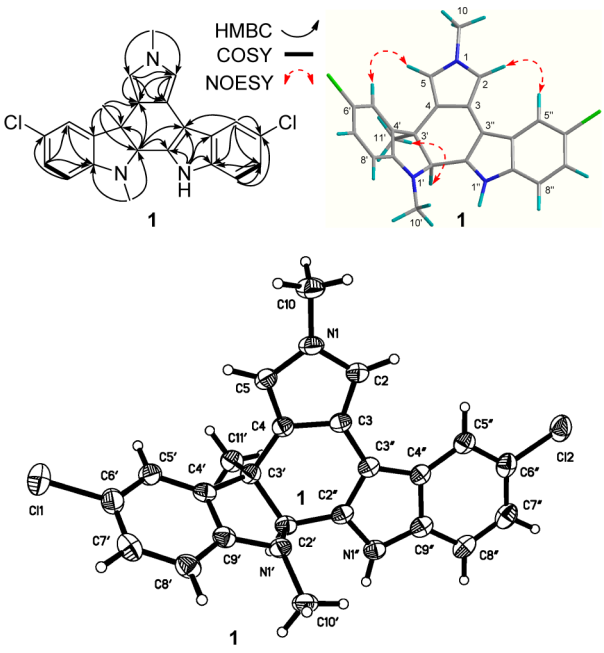

Figure 1. Key HMBC, COSY, and selected NOE correlations for 1 and the X-ray crystallographic structure of 1.

Indimicin E (5) was isolated as an off-white solid. The molecular formula of 5 was established as $C_{23}H_{21}Cl_2N_3$ (14 degrees of unsaturation) by HRESIMS. Analysis of the ^1H, ^{13}C, and HSQC NMR data of 5 (Tables 1 and 2) revealed the close similarity of 5 and 1 by the presence of nearly identical structural features including a 6″-chloro-3″-substitued-1″-*H*-indole, an unusual 6′-chloro-1′,3′-dimethyl-2′-hydroindole, and a 1-methyl-2,3-disubstitued pyrrole ring. By careful comparisons, the resonances for a methine and an aromatic quaternary carbon in 1 were absent in 5; instead, a methylene [δ_H 3.08, 3.31 (H-2′); δ_C 69.3] and a methine [δ_H 6.09 (H-2″); δ_C 125.4] were present in 5. The methylene was located at C-2′ in 5 by HMBC correlations from H_3-10′ (δ_H 2.32) to C-2′ (δ_C 69.3) and from H_2-2′ (δ_H 3.03, 3.31) to C-3′ (δ_C 43.7). The location of the methine at C-2″ in 5 was supported by a COSY correlation between H-2″ (δ_H 6.09 1H, br s) and NH-1″ (δ_H 7.93 1H, br s). On the basis of these observations, the planar structure of 5 was established. The absolute configuration of 5 at C-3′ was determined by comparison of the experimental CD spectrum of 5 with the calculated ECD spectra for (3′*R*)-5 and (3′*S*)-5 (Figure 3). The random conformational search by the SYBYL 8.0 software package using the TRIPOS force field and the following geometry optimizations with DFT at the B3LYP/6-31G (d) level yielded seven conformers for (3′*R*)-5 (Figure S6) and five conformers for (3′*S*)-5 (Figure S7). The stable conformers were submitted to CD calculations by time-dependent (TD) DFT calculations (B3LYP/6-31G(d)) with Gaussian 09.[15] The overall predicted CD spectra of (3′*R*)-5 and (3′*S*)-5 were compared with the experimental one. The Cotton effects (CEs) of 5a were in good accordance with the experimental CEs of 5 in the region of 200–400 nm, both showing negative CEs in the 200–240 and 270–350 nm regions and

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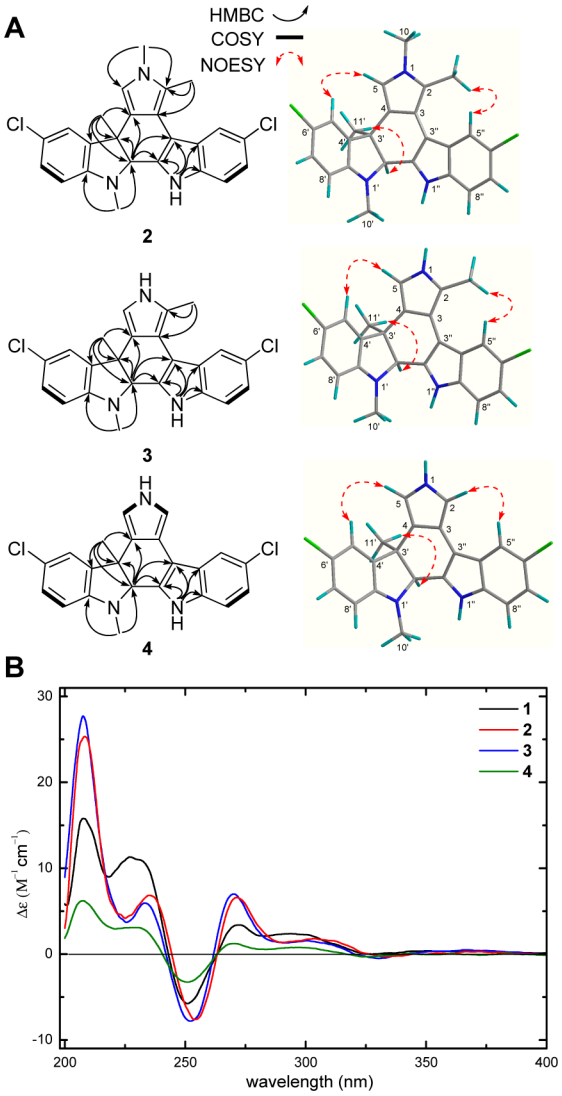

Figure 2. (A) Key HMBC, COSY, and selected NOE correlations for **2**–**4**. (B) Experimental CD spectra of **1**–**4** in MeOH.

positive CEs in the 240–260 nm region (Figure 3). Therefore, compound **5** was deduced to have the 3′*R* configuration.

Lynamicin F (**6**) was obtained as an off-white solid. The molecular formula of **6**, $C_{23}H_{16}Cl_2N_3O_2$, was derived from HRESIMS, which indicated 16 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectroscopic data of **6** (Tables 1 and 2) were highly similar to those of lynamicin A.[13] Detailed analysis of the 2D NMR data (HSQC, COSY, HMBC) (Figure 4) revealed that **6** was only different from lynamicin A by having an additional methyl group, which was located at N-1 by HMBC correlations from H_3-10 to C-2/C-3. Lynamicin G (**7**) was isolated as an off-white and optically active solid. The molecular formula of **7** was assigned as $C_{23}H_{16}Cl_2N_3O_2$ by HRESIMS. The comparison of the ^{1}H and ^{13}C NMR spectral data of **7** with those of lynamicn D[13] indicated an additional *N*-methyl group [δ_H 4.25 (3H, s, H-10); δ_C 35.5] in **7** (Tables 1 and 2). The HMBC correlations from the *N*-methyl protons H_3-10 to C-2 and C-5 confirmed the location of the methyl group at N-1 (Figure 4).

Indimicins A–E (**1**–**5**) and lynamicins F (**6**) and G (**7**) showed no antimicrobial activities with minimal inhibition values of >128 μg/mL against five indicator strains, including *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC

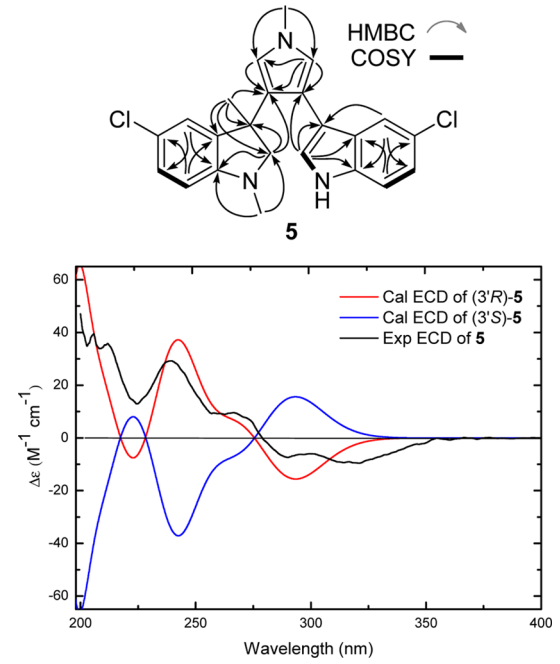

Figure 3. Key HMBC and COSY correlations for **5** and comparison of the experimental ECD spectrum of **5** in MeOH and the calculated ECD spectra of (3′*R*)-**5** and (3′*S*)-**5**.

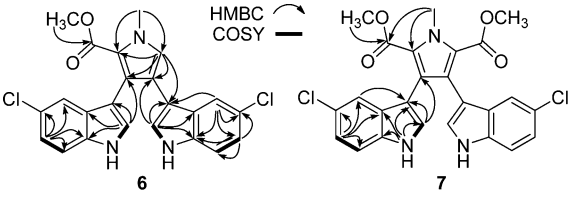

Figure 4. Key HMBC and COSY correlations for **6** and **7**.

29213, *Bacillus thuringensis* SCSIO BT01, *Bacillus subtilis* SCSIO BS01, and *Candida albicans* ATCC10231. The *in vitro* cytotoxicities of compounds **1**–**7** were evaluated against four cancer cell lines, including SF268, MCF7, NCI-H460, and HepG2. Indimicin B (**2**) was active against the MCF-7 cell line (IC_{50} 10.0 μM), but inactive against the other cell lines (IC_{50} > 10.0 μM, Table S2). Compounds **1** and **3**–**7** were inactive toward these cancer cell lines (Table S2).

In summary, we have isolated seven new bisindole alkaloids (**1**–**7**) from the deep-sea-derived *Streptomyces* sp. SCSIO 03032. Indimicins A–E (**1**–**5**) bear a unique 1′,3′-dimethyl-2′-hydroindole moiety. The *C*-methylation at the quaternary carbon C-3′, leading to the dearomatization of the indolocarbazole scaffold in **1**–**4**, or the *C*-methylation at C-2 of the pyrrole moiety in **2** and **3** is rarely found in the L-tryptophan-derived bisindole alkaloids. These new structures expand the chemical variety of indole alkaloids and provide opportunities to investigate their biosynthetic origins at genetic and biochemical levels.

EXPERIMENTAL SECTION

General Experimental Procedures. The melting point was determined with a DSC 204F1 apparatus (Netzsch Inc.). Optical rotations were measured on a 341 polarimeter (PerkinElmer, Inc.). UV spectra were recorded on a U-2900 spectrophotometer (Hitachi), ECD spectra were recorded on a JASCO J-810 spectropolarimeter (JASCO), and IR spectra were measured on a Nicolet*6700 FT-IR spectrometer (Thermo Scientific). ^{1}H, ^{13}C, and 2D NMR spectra were recorded on a

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Bruker AV-500 MHz NMR spectrometer (Bruker Biospin GmbH) with TMS as an internal standard. Low-resolution mass spectrometric data were determined using an amaZon SL ion trap mass spectrometer. HRESIMS data were measured using a MaXis 4G UHR-TOFMS spectrometer (Bruker Daltonics Inc.). Sephadex LH-20 (GE Healthcare Bio-Sciences AB) was used. Medium-pressure liquid chromatography (MPLC) was performed on an automatic flash chromatograph (CHEETAHTM MP 200, Bonna-Agela Technologies Co., Ltd.) with the monitoring wavelength at 220 nm and the collecting wavelength at 254 nm. Semipreparative HPLC was performed on a Hitachi HPLC station (Hitachi-L2130) with a diode array detector (Hitachi L-2455) using a Phenomenex ODS column (250 mm × 10.0 mm, 5 μm; Phenomenex). Preparative TLC was conducted with precoated glass plates (silica gel GF254, 10–40 nm).

Screening and Fermentation. The isolation of strain *Streptomyces* sp. SCSIO 03032 has been previously described.[9] *Streptomyces* sp. SCSIO 03032 was grown and maintained on ISP4-agar containing 3% natural sea salt. The natural sea salt was obtained from the Guangdong Province Salt Industry Group Co Ltd. A few loops of cells of *Streptomyces* sp. SCSIO 03032 were inoculated into 50 mL of seed medium (modified A1BFe + C starch 1.0%, yeast extract 0.4%, peptone 0.2%, $CaCO_3$ 0.2%, natural sea salt 3%, pH 7.2–7.4) in a 250 mL Erlenmeyer flask. The cultivation was carried out on a rotary shaker (200 rpm) at 28 °C for 4 days. A 20 mL (5 vol %) portion of the sterilized polystyrene resin (Amberlite XAD-16) was added into the production medium (400 mL/2L), and the fermentation was extended for another day.

Extraction and Isolation. The mycelia and polystyrene resin were separated by filtration through a metal sieve (40 mesh). The mycelia were extracted three times, each with 12 L of acetone, and the acetone was removed under vacuum. The resins were washed twice with H_2O and transferred to a glass column. The glass column was eluted with 2 L of acetone. The acetone fractions were concentrated under vacuum to afford an aqueous residue, which was extracted four times with 1.5 L of EtOAc. The EtOAc extracts were combined and concentrated under vacuum to yield a dry extract (12.0 g). The extract was subjected to column chromatography (CC) over silica gel (300–400 mesh), eluting with a gradient of $CHCl_3$/MeOH (100:0 → 0:100), to give four fractions (Fr.1–Fr.4). Fr.2 was purified by C_{18} reversed-phase MPLC (40 × 2.5 cm i.d.), eluting with a linear gradient of H_2O/MeOH (0–100%, 20 mL min^{-1}, 200 min), to give three fractions, Fr.2-1–Fr.2-4. Fr.2-3 was subjected to Sephadex LH-20, eluting with $CHCl_3$/MeOH (1:1), to give five fractions (Fr.2-3-A, Fr.2-3-B, Fr.2-3-C, Fr.2-3-D, Fr.2-3-E). Compounds **1** (10.6 mg), **3** (7.2 mg), and **5** (3.5 mg) were obtained from Fr.2-3-C by preparative TLC (pTLC) in the solvent system with petroleum benzin/EtOAc (P/E, 80/20, v/v). Compound **2** (4.3 mg) was obtained from Fr.2-3-B by pTLC (P/E, 80/20). Compound **4** (3.6 mg) was purified from Fr.2-3-D by pTLC (P/E, 75/25). Fr.2-2 was further purified by Sephadex LH-20 and semipreparative HPLC (H_2O/MeCN, 30/70) to get compounds **6** (2.5 mg) and **7** (3.0 mg). Semipreparative HPLC was performed on an HPLC (Hitachi-L2130, diode array detector, Hitachi L-2455) using a Phenomenex ODS column (250 mm × 10.0 mm i.d., 5 μm; Phenomenex).

*Indimicin A (**1**):* colorless crystal; mp 254 °C $[\alpha]_D^{20}$ +125 (*c* 0.97, MeOH); UV (MeOH) λ_{max} (log ε) 252 nm (4.55), 207 nm (4.75); ECD (*c* 3.9 × 10^{-5} M, MeOH) λ_{max} ($\Delta\varepsilon$) 251 (−4.43), 227 (8.67), 217 (12.11) nm; IR (KBr) ν_{max} 3371, 2291, 1603, 1477 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 408.1051 $[M - H]^-$ (calcd for $C_{23}H_{20}Cl_2N_3$, 408.1029).

*Indimicin B (**2**):* white powder; $[\alpha]_D^{20}$ +190 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 239 nm (4.61), 207 nm (4.81); ECD (*c* 2.4 × 10^{-5} M, MeOH) λ_{max} ($\Delta\varepsilon$) 252 (−7.81), 233 (5.94), 207 (34.23) nm; IR (KBr) ν_{max} 3339, 2922, 1600, 1476 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 422.1191 $[M - H]^-$ (calcd for $C_{23}H_{20}Cl_2N_3$, 422.1185).

*Indimicin C (**3**):* colorless solid; $[\alpha]_D^{20}$ +250 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ε) 238 nm (4.29), 204 nm (4.51); ECD (*c* 2.4 × 10^{-5} M, MeOH) λ_{max} ($\Delta\varepsilon$) 254 (−9.73), 235 (8.72), 208 (32.37) nm; IR (KBr) ν_{max} 3410, 2921, 1603, 1479 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 408.1039 $[M - H]^-$ (calcd for $C_{23}H_{20}Cl_2N_3$, 408.10295).

*Indimicin D (**4**):* white, amorphous powder; $[\alpha]_D^{20}$ +94.2 (*c* 0.47, MeOH); UV (MeOH) λ_{max} (log ε) 239 nm (4.17), 204 nm (4.39); ECD (*c* 2.6 × 10^{-5} M, MeOH) λ_{max} ($\Delta\varepsilon$) 251 (−3.89), 228 (3.67), 207 (7.38) nm; IR (KBr) ν_{max} 3278, 2924, 1604, 1481 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 392.0712 $[M - H]^-$ (calcd for $C_{23}H_{20}Cl_2N_3$, 392.0727).

*Indimicin E (**5**):* off-white solid; $[\alpha]_D^{20}$ +33 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 203 nm (4.50); ECD (*c* 2.4 × 10^{-5} M, MeOH) λ_{max} ($\Delta\varepsilon$) 318 (−4.24), 239 (12.98), 206 (13.80) nm; IR (KBr) ν_{max} 3418, 2929, 1722, 1462 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Tables 1 and 2; HRESIMS *m/z* 410.1207 $[M - H]^-$ (calcd for $C_{23}H_{20}Cl_2N_3$, 410.1185).

*Lynamicin F (**6**):* off-white solid; UV (MeOH) λ_{max} (log ε) 290 nm (3.93); 232 nm (4.61); 213 nm (4.56); IR (KBr) ν_{max} 3413, 1684, 1260 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Tables 1 and 2; ESIMS *m/z* 436 $[M - H]^-$ and 873 $[2M - H]^-$; HRESIMS *m/z* 436.0595 $[M - H]^-$ (calcd for $C_{23}H_{15}Cl_2N_3O_2$, 436.0620).

*Lynamicins G (**7**):* off-white and optically inactive solid; UV (MeOH) λ_{max} (ε) 260 nm (4.16); 229 nm (4.66); IR (KBr) ν_{max} 3418, 1712, 1223 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Tables 1 and 2; ESIMS *m/z* 436 $[M - H]^-$ and 873 $[2M - H]^-$; HRESIMS *m/z* $[M - H]^-$ 436.0595 (calcd for $C_{23}H_{15}Cl_2N_3O_2$, 436.0620).

Quantum Chemical Circular Dichroism (CD) Calculation. Quantum chemical CD calculation methods were used to support or establish the C-3′ absolute configuration of compound **5**. The compounds were charged using the Gasteiger-Huckel method, and the preliminary conformational search was performed with the SYBYL 8.0 software package using the TRIPOS force field. The geometry optimizations were then performed by using DFT at the B3LYP/6-31G(d) level as implemented in the Gaussian 09 program package.[15] The stable conformers obtained were subsequently submitted to CD calculations by TDDFT calculations (B3LYP/6-31G(d)) with Gaussian 09.

X-ray Crystallographic Analysis. An optically active light white crystal (mp 254 °C) of **1** was obtained in $CHCl_3$/MeOH. The crystal data were recorded with an Oxford Xcalibur Onyx Nova single-crystal diffractometer with Cu Kα radiation (λ = 1.54184 Å). The structures were solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques.[16] Crystallographic data have been deposited in the Cambridge Crystallographic Data Center with the deposition number CCDC 994677. A copy of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, U.K. (fax, +44(0)-1233-336033; e-mail, deposit@ccdc.cam.ac.uk).

Crystal data of **1**: colorless (block), orthorhombic, space group *P*1 21 1, *a* = 9.5701(4) Å, *b* = 7.9131(3) Å, *c* = 13.6352(6) Å, *V* = 979.76(7) $Å^3$, *Z* = 2, μ(Cu Kα) = 3.078, *T* = 150(2), and *F*(000) = 424, crystal size 0.41 × 0.38 × 0.07 mm^3; 10 943 reflections measured, of which 3483 were unique (R_{int} (*R* factor for symmetry-equivalent intensities) = 0.0338), were used in all calculations (Table S1).

Biological Assays. Antimicrobial activities were measured against the five indicator strains *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Bacillus thuringensis* SCSIO BT01, *Bacillus subtilis* SCSIO BS01, and *Candida albicans* ATCC10231 by the broth microdilution method.[17] *In vitro* cytotoxic activities were evaluated against four tumor cell lines, MCF7 (human breast adenocarcinoma cell line), NCI-H460 (human non-small-cell lung cancer cell line), HepG2 (human hepatocellular liver carcinoma cell line), and SF268 (human glioma cell line), by the SRB assays according to a previously described protocol.[18]

■ ASSOCIATED CONTENT

Supporting Information

Supplementary methods, 1D and 2D NMR, UV, IR, and MS spectroscopic data of compounds **1**–**7** 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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