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Antibacterial Anthraquinone Derivatives from a Sea Anemone-Derived Fungus *Nigrospora* sp.

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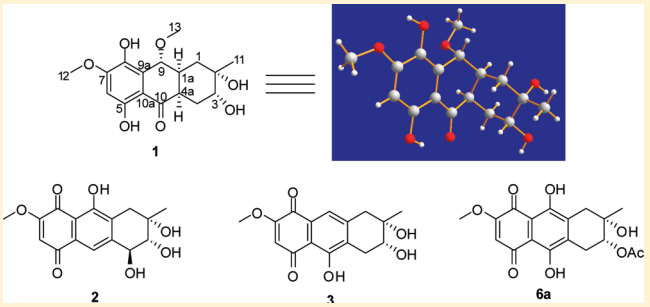
Supporting Information

ABSTRACT: Chemical investigation of a marine-derived fungus *Nigrospora* sp., isolated from an unidentified sea anemone, yielded two new hydroanthraquinone analogues, 4a-*epi*-9α-methoxydihydrodeoxybostrycin (**1**) and 10-deoxybostrycin (**2**), together with seven known anthraquinone derivatives (**3**–**9**). The structures of the two new compounds were established through extensive NMR spectroscopy as well as a single-crystal X-ray diffraction analysis using Cu Kα radiation. The antibacterial activities of compounds **1**–**9** and 10 acetyl derivatives (**6a**, **7a**, **8a**–**8g**, **9a**) were evaluated *in vitro*. Compound **6a**, the acetylated derivative of **6**, exhibited promising activity against *Bacillus cereus* with an MIC value of 48.8 nM, which was stronger than that of the positive control ciprofloxacin (MIC = 1250 nM). Analysis of the antibacterial screening data for the metabolites and their acetyl derivatives revealed the key structural features required for this activity.



Marine-derived fungi have proven to be rich sources of structurally novel and biologically active secondary metabolites that have become interesting and significant resources for drug discovery. A literature survey revealed natural products from marine fungi isolated from sponges, soft corals, algae, mangrove trees, and sediments.[1] However, chemical studies on marine fungi derived from sea anemones were relatively rare, and only a few metabolites were reported.[2,3] As part of our ongoing investigation on new natural antibacterial and cytotoxic products from marine fungi in the South China Sea,[4−6] a sea-anemone-derived fungus, *Nigrospora* sp., attracted our attention because the EtOAc extract of the fungal fermentation broth exhibited significant antibacterial activity against a panel of pathogenic bacteria. Chemical investigation of the bioactive extract led to the discovery of two new hydroanthraquinone derivatives, 4a-*epi*-9α-methoxydihydrodeoxybostrycin (**1**) and 10-deoxybostrycin (**2**), together with seven known anthraquinone analogues, nigrosporin B (**3**),[7] 9α-hydroxydihydrodesoxybostrycin (**4**),[8] 9α-hydroxyhalorosellinia A (**5**),[8] 4-deoxybostrycin (**6**),[9,10] bostrycin (**7**),[9,11] 3,5,8-trihydroxy-7-methoxy-2-methylanthracene-9,10-dione (**8**),[12] and austrocortirubin (**9**).[12] Compound **1** is the first example of a hydroanthraquinone derivative possessing a 9-OMe group. Compounds **3**–**9** were previously isolated from other *Nigrospora* spp.[7−9,12] Herein, we report the isolation, structural characterization, antibacterial activity, and structure−activity relationship of these compounds.

RESULTS AND DISCUSSION

The marine-derived fungus ZJ-2010006, isolated from a sea anemone, was identified on the basis of molecular characteristics combined with morphologic traits as a *Nigrospora* sp. All compounds were isolated using chromatographic techniques including column chromatography and semipreparative HPLC, and their structures were elucidated by spectroscopic data (IR, UV, NMR, and MS).

Compound **1** was obtained as colorless crystals. Its molecular formula of $C_{17}H_{22}O_7$ (seven degrees of unsaturation) was determined by HREIMS. Careful comparison of the ^{1}H NMR and ^{13}C NMR data of **1** (Table 1) with those of **4** showed a close structural relationship between the two compounds. The most obvious differences were the presence of a singlet methoxy group signal at δ_H 3.38 and the relatively small coupling constant J = 7.2 Hz between H-4a (δ_H 3.13, ddd, J = 7.2, 5.3, 2.5 Hz) and H-1a (δ_H 2.85, dddd, 13.6, 7.2, 3.7, 2.9 Hz) in **1**, instead of the large coupling constant J = 13.6 Hz between H-4a (δ_H 2.44, ddd, J = 13.6, 12.0, 3.8 Hz) and H-1a

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Table 1. ^{1}H and ^{13}C NMR Data for 1[a] and 2[b]

	1		2	
position	δ_C	δ_H (J/Hz)	δ_C	δ_H (J/Hz)
1	38.1, CH_2	1.56, ddd (13.6, 3.7, 2.0, H-1eq)	37.4, CH_2	2.88, d (18.2)
		1.00, t (13.6, H-1ax)		2.58, d (18.2)
1a	35.4, CH	2.85, dddd (13.6, 7.2, 3.7, 2.9)[c]	130.0, C	
2	70.1, C		70.8, C	
3	71.3, CH	3.20, dd (11.6, 4.6)	77.1, CH	3.33, dd (7.2, 5.6)[c]
4	28.7, CH_2	2.56, ddd (12.6, 4.6, 2.5, H-4eq)	71.5, CH	4.51, t (7.2)
		1.71, ddd (12.6, 11.6, 5.3, H-4ax)		
4a	41.1, CH	3.13, ddd (7.2, 5.3, 2.5)	149.8, C	
5	159.0, C		183.3, C	
6	99.2, CH	6.47, s	111.0, CH	6.28, s
7	155.4, C		160.9, C	
8	137.8, C		183.9, C	
9	72.8, CH	4.63, d (2.9)	159.3, C	
9a	123.8, C		112.1, C	
10	203.9, C		117.9, CH	7.68, s
10a	108.0, C		129.5, C	
11	26.7, CH_3	1.10, s	27.1, CH_3	1.29, s
12	55.7, CH_3	3.95, s	57.2, CH_3	3.86, s
13	56.1, CH_3	3.38, s		
2-OH		3.09, s		4.46, s
3-OH		3.64, br s		4.92, d (5.6)
4-OH				5.70, d (7.2)
5-OH		12.87, s		
8-OH		7.50, s		
9-OH				12.03, s

[a]Measured at 400 MHz (^{1}H) and 100 MHz (^{13}C), acetone-d_6. [b]Measured at 600 MHz (^{1}H) and 150 MHz (^{13}C), DMSO-d_6. [c]Signals are overlapped.

(δ_H 2.24, dddd, J = 13.6, 11.8, 10.4, 2.8 Hz) in **4**. Also C-1a and C-4a were shifted upfield (δ_C 35.4 and 41.1 CH in **1** vs 41.8 and 47.0 CH in **4**) and established the location of H-4a at an equatorial position in **1**, not at the axial position as in **4**. The location of the methoxy group (δ_C 56.1, C-13) at C-9 was established by the HMBC correlation between H-13 and C-9. It should be noted that the small coupling constant J = 2.9 Hz between H-9 (δ_H 4.63, d, J = 2.9 Hz) and H-1a (δ_H 2.85, dddd, J = 13.6, 7.2, 3.7, 2.9 Hz) showed that the dihedral angle between these two protons should be about 70° according to the Karplus formula, indicating that these two protons may be placed either on the same or on opposite faces of the molecule. Taking into account the large coupling constant J_{9-1a} = 9.9 Hz of **4** possessing the *trans* configuration and the small coupling constant J_{9-1a} = 3.5 Hz of fusaranthraquinone[13] with the *cis* configuration, it seemed that **1** should have a *cis* configuration. However, in the selective NOE experiments, the irradiation of H-9 resulted in no obvious enhancement for H-1a, suggesting that H-9 and H-1a might be *trans* oriented. Fortunately, by slow crystallization from MeOH, single crystals of **1** suitable for X-ray diffraction analysis using Cu Kα radiation were obtained, allowing the structure of **1** to be unambiguously established with H-9 and H-1a in a *trans* relationship (Figure 1). According to the crystal data, the dihedral angle between H-9 and H-1a was calculated as ca. 73°, which was consistent with the value

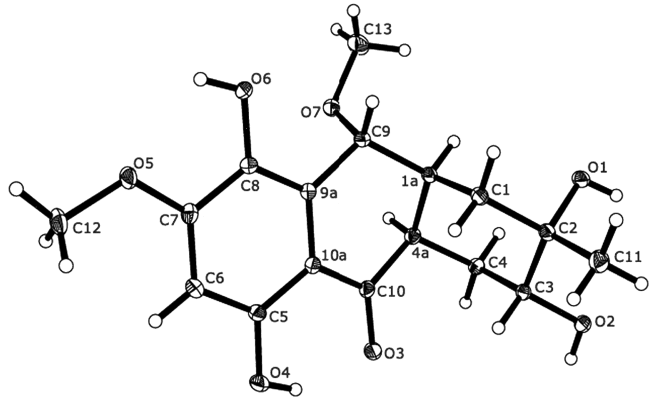

Figure 1. Perspective ORTEP drawing for **1**.

deduced by the Karplus formula. It should be mentioned that the unexpectedly small coupling constant between H-9 and H-1a may be partly affected by the relatively bigger 9-OMe group. Furthermore, the absolute configuration of **1** was determined as 2*S*,3*R*,9*R*,1a*S*,4a*R* by Flack's method.[14] This is the first report of an anthraquinone derivative possessing a 9-OMe group. Compound **1** was named 4a-*epi*-9α-methoxydihydrodeoxybostrycin.

Compound **2** was obtained as a yellow, amorphous powder. Its molecular formula was established as $C_{16}H_{16}O_7$ with nine degrees of unsaturation based on the HRESIMS data, thus revealing the loss of one oxygen atom compared with that of bostrycin (**7**).[9,11] The ^{1}H and ^{13}C NMR spectroscopic features (Table 1) suggested that compound **2** was very similar to **7**. The only significant difference in the ^{1}H NMR spectrum of **2** in comparison with that of **7** was the aromatic proton signal for H-10 at δ_H 7.68 (1H, s) in **2** instead of a hydrogen-bonded hydroxy group at δ_H 13.38 (1H, s) in **7**. This was also confirmed by the presence of a methine carbon at C-10 (δ_C 117.9) in **2** instead of a quaternary carbon at δ_C 160.0 in **7** in the ^{13}C NMR spectrum. This coincided with the loss of one oxygen atom in the molecular formula in **2** compared with that of **7**. Furthermore, the position of the aromatic proton was confirmed at C-10 on the basis of the HMBC correlations from H-10 (δ_H 7.68) to C-1a (δ_C 130.0), C-4 (δ_C 71.5), C-5 (δ_C 183.3), and C-9a (δ_C 112.1). The 1D NOE data also revealed that the relative configurations of all asymmetric carbons in **2** were identical to those of **7**. Moreover, on the basis of the absolute configuration and a shared biogenesis with **1**, the absolute configurations of **2** could be tentatively assigned as 2*S*,3*R*,4*S*. Compound **2** is therefore 10-deoxybostrycin.

Consequently, as the relative configurations of the known analogues (**3**−**7**) have been established, the absolute configurations of these compounds could also be tentatively proposed as 2*S*,3*R* for nigrosporin B (**3**), 2*S*,3*R*,9*R*,1a*S*,4a*S* for 9α-hydroxydihydrodesoxybostrycin (**4**), 2*S*,3*R*,4*S*,9*R*,1a*S*,4a*R* for 9α-hydroxyhalorosellinia A (**5**), 2*S*,3*R* for 4-deoxybostrycin (**6**), and 2*S*,3*R* for bostrycin (**7**), respectively, which were consistent with the earlier assignments of these compounds.[7,8,10,11]

It was reported that compound **3** showed antibacterial activity against *Bacillus subtilis* at 100 ppm,[7] compounds **4** and **5** displayed inhibition against *B. subtilis* at 100 μg/disk,[15] compounds **6** and **7** inhibited *B. subtilis*, *Staphylococcus aureus*, and *Escherichia coli* with IC_{50} values of 3.13 μg/mL,[9] while compound **9** did not exhibit inhibitory activity against *S. aureus*.[13] In our current research, the minimum inhibitory

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Chart 1

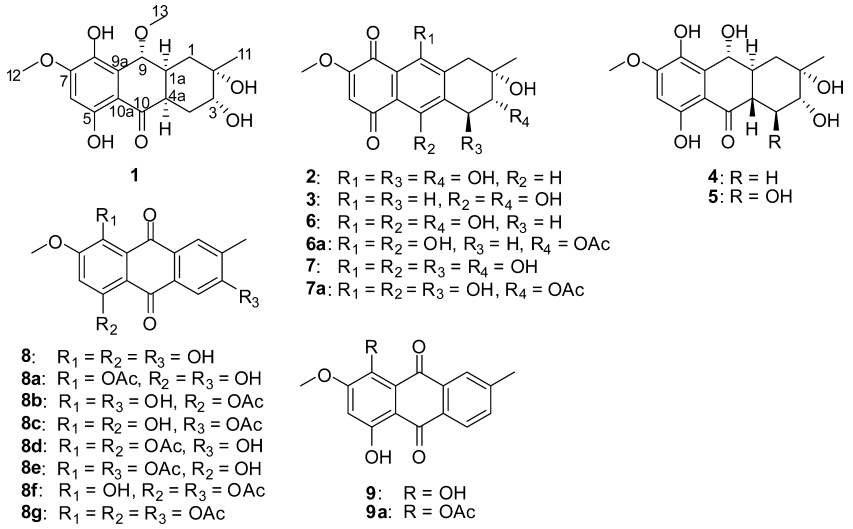

Table 2. Antibacterial and Cytotoxic Activities of the Tested Compounds

	MIC (μM)									IC_{50} (μM)
compd	*B. subtilis*	*B. cereus*	*M. luteus*	*S. albus*	*S. aureus*	*M. tetragenus*	*E. coli*	*V. anguillarum*	*V. parahemolyticus*	A549
1	>100	50.0	>100	>100	1.56	0.780	0.780	25.0	25.0	>50.0
2	0.625	10.0	20.0	5.00	2.50	1.25	2.50	2.50	1.25	4.56
3	0.312	0.312	10.0	2.50	1.25	0.625	1.25	1.25	0.625	3.32
4	12.5	12.5	25.0	>100	25.0	1.56	6.25	1.56	12.5	>50.0
5	12.5	12.5	>100	>100	0.780	3.12	1.56	3.12	3.12	41.5
6	3.12	3.12	200	3.12	3.12	3.12	1.56	3.12	3.12	NT[c]
6a	0.780	0.0488	100	200	>200	0.195	>200	0.0975	0.780	5.25
7	3.12	3.12	100	3.12	1.56	3.12	1.56	0.390	3.12	NT
7a	>200	3.12	>200	>200	>200	3.12	>200	>200	>200	2.72
8	>200	>200	>200	>200	>200	>200	>200	>200	>200	>50.0
8a	>200	>200	>200	100	100	>200	>200	>200	>200	NT
8b	>200	>200	>200	>200	>200	>200	>200	>200	>200	NT
8c	12.5	50.0	>200	>200	>200	>200	>200	>200	>200	NT
8d	5.00	37.5	>150	>150	>150	9.40	>150	4.70	75.0	NT
8e	>200	>200	200	>200	>200	200	>200	>200	>200	NT
8f	50.0	>200	>200	50.0	>200	>200	>200	>200	>200	NT
8g	35.0	140	140	70.0	>140	>140	>140	140	>140	>50.0
9	>200	>200	>200	>200	>200	>200	>200	>200	>200	>50.0
9a	>200	>200	>200	>200	>200	>200	>200	>200	>200	NT
ciprofloxacin[a]	0.312	1.25	2.50	0.312	0.156	0.0390	0.156	0.0780	0.0780	
mitomycin[b]										3.00

[a]Ciprofloxacin was used as a positive control. [b]Mitomycin was used as a positive control. [c]Not tested.

concentration (MIC, μM) values of compounds **1**–**9** and 10 acetyl derivatives of compounds **6**–**9** (**6a**, **7a**, **8a**–**8g**, **9a**) against a panel of pathogenic bacteria, including Gram-positive *B. subtilis*, *B. cereus*, *Micrococcus luteus*, *M. tetragenus*, *S. albus*, and *S. aureus* and Gram-negative *E. coli*, *Vibrio anguillarum*, and *V. parahemolyticus*, were determined *in vitro* using a standard screening protocol (Table 2). The results indicated the isolated hydroanthraquinone analogues (**1**–**7**) together with their acetyl derivatives (**6a** and **7a**) had widely varying antibacterial effects. The hydroanthraquinones **2** and **3** and the acetyl derivative **6a** were found to be the most active. Compound **2** showed strong antibacterial activity against *B. subtilis* with an MIC value of 625 nM. Compound **3** showed pronounced antibacterial activity against *B. subtilis* and *B. cereus* with the same MIC values of 312 nM, which were equivalent or stronger than those of the positive control ciprofloxacin, with MIC values of 312 and 1250 nM, respectively. Additionally, the derivative **6a** exhibited promising inhibitory activity against *B. cereus*, with an MIC value of 48.8 nM, which was approximately 25-fold more potent than that of ciprofloxacin (MIC = 1250 nM).

It should be noted that the introduction of the hydroxy groups at C-4/C-9/C-10 in **2** and **3** did not appreciably change the MIC values over **6** and **7**, indicating that adding the 4-OH/9-OH/10-OH groups had little effect on the antibacterial activity. Moreover, there is no obvious alteration of the antibacterial activity between **2** and **3** and between **4** and **5** against most bacteria. This further confirmed that the 4-OH plays no apparent role in the antibacterial activity.

Interestingly, switching from a hydroxy group at C-3 in **6** to an acetoxy group in **6a** significantly increased antibacterial

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activity between 4-fold and 64-fold against *B. subtilis*, *B. cereus*, *M. tetragenus*, *V. anguillarum*, and *V. parahemolyticus*. This suggests that the acetoxy group at C-3 has a positive contribution to the antibacterial activity. The improved activity may be attributed to the acetoxy group, which can help this compound traverse the phospholipid bilayer of bacterial cells.[16] However, this modification decreased the inhibitory activity against *S. albus*, *S. aureus*, and *E. coli*. The above results clearly indicate that although the mechanisms of this type of compound against the bacteria are presently unknown, these compounds may have different interactions with molecular targets in different bacteria. A similar observation was made for compounds **7** and **7a**, where **7a**, with a 3-OAc group, displayed weaker activities against most strains, but showed equal activities against *B. cereus* and *M. tetragenus* to those of **7**. In addition, upon comparing the activity of **8** with its seven acetyl derivatives **8a**−**8g**, only **8d** exhibited stronger inhibitory effects against *B. subtilis*, *B. cereus*, *M. tetragenus*, *V. anguillarum*, and *V. parahemolyticus* than those of **8**. The results for the other acetyl derivatives indicated that only acetylation of both hydrogen-bonded hydroxy groups 5-OH and 8-OH could improve the antibacterial activity.

Furthermore, compounds **1**−**7**, with a cycloaliphatic ring, exhibited stronger antibacterial activity than **8** and **9**, which possess an aromatic C ring, obviously indicating that this cycloaliphatic ring is very important for antibacterial activity. However, the presence of an aromatic B ring may be responsible for enhanced antibacterial activity, which is confirmed by the stronger activity of **2**, **3**, **6**, and **7** compared to **1**, **4**, and **5** (Figure 2). All of the tested compounds showed

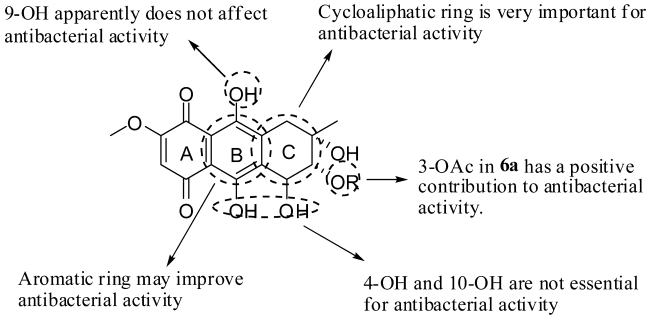

Figure 2. Summarized antibacterial structure−activity relationships for hydroanthraquinone derivatives.

extremely weak inhibitory effects against *M. luteus* compared with the other Gram-positive bacterial strains, which suggested that *M. luteus* may be a drug-resistant strain to this type of compound.

The cytotoxic activities of compounds **1**−**5**, **8**, **9**, **6a**, **7a**, and **8g** were also evaluated against the human lung cancer cell line A549 (Table 2). The hydroanthraquinones **2** and **3**, and the acetylated derivatives **6a** and **7a**, showed strong activities against A549 cells, with IC_{50} values of 4.56, 3.32, 5.25, and 2.72 μM, respectively.

In conclusion, two new (**1** and **2**) and seven known (**3**−**9**) anthraquinone derivatives were isolated from a sea anemone-derived fungus *Nigrospora* sp., and 10 acetyl derivatives (**6a**, **7a**, **8a**−**8g**, **9a**) were also prepared. The antibacterial activities of the isolated compounds and their acetyl derivatives were evaluated *in vitro*, and their structure−activity relationships were also examined. Nigrosporin B (**3**) showed pronounced antibacterial activity against *B. subtilis* and *B. cereus*, with MIC values of 312 nM, which were equal to or stronger than that of ciprofloxacin. Additionally, the acetylated derivative **6a** exhibited strong activity against *B. cereus* and *V. anguillarum*, with MIC values of 48.8, and 97.5 nM, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on an X-4 micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were recorded on an UV-2501PC spectrophotometer. IR spectra were recorded on a Bruker EQUINOX 55 spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 (400 and 100 MHz) or Varian Mercury-Plus 300 (300 and 75 MHz) NMR spectrometer in $CDCl_3$, DMSO-d_6, or acetone-d_6. Chemical shifts δ are reported in ppm, using TMS as internal standard, and coupling constants (J) are in Hz. EIMS spectra were measured on a Thermo DSQ EI-mass spectrometer and HREIMS on a Thermo MAT95XP high-resolution mass spectrometer. ESIMS spectra were obtained from a Micromass Q-TOF spectrometer. Single-crystal data were measured on an Agilent Gemini Ultra diffractometer (Cu Kα radiation). Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200−300 mesh), octadecylsilyl silica gel (Unicorn; 45−60 μm), and Sephadex LH-20 (GE Healthcare) were used for column chromatography. Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co.; G60, F-254) were used for thin layer chromatography. Semipreparative HPLC was performed on an LC1620 system using a semipreparative C_{18} (Kromasil 7 μm, 10 × 250 mm) column coupled with an LC-UV 1620 ultraviolet detector, at a flow rate of 2.0 mL/min and a gradient between MeOH/H_2O (5:5) and MeOH/H_2O (8:2). The antibacterial activities were observed with a Multiskan Mk3 (Thermo Labsystems) at 630 nm. A vertical heating pressure steam sterilizer LDZX-75KBS was used for the sterilization of culture medium. The fungi were cultivated in a biochemical incubator (Zhejiang Scientific Instruments, model SPX-250B-Z) or SHIPING constant temperature culture vibrator at 25 °C with a speed of 160 rpm. During the DNA extraction process, a Sigma 1-14 (Sigma Laborzentrifugen) was used to mix the solution.

Isolation of the Fungal Material. The fungal strain *Nigrospora* sp. (ZJ-2010006) was isolated from a piece of fresh tissue from the inner part of an unidentified sea anemone (GX-WZ-20100026), collected from the Weizhou coral reef in the South China Sea in April 2010. 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. Following surface sterilization with 75% EtOH for 30 s, the sea anemone was rinsed three times in sterile water. To distinguish the remaining epiphytic fungi from endophytic fungi, an imprint of the sea anemone surface on PDA was made. Small tissue samples from the inner part of the sea anemone were aseptically cut and pressed onto PDA plates containing an antibiotic to suppress bacterial growth (composition of isolation medium: potatoes 200 g/L, glucose 20 g/L, agar 15 g/L, and chloramphenicol 0.2 g/L in seawater, pH 7.4−7.8). After incubation at 25 °C, the fungal strain under investigation was found to grow exclusively out of the sea anemone tissue, but not on the agar plates taken from the imprint of the anemone surface. A pure strain of *Nigrospora* sp. (ZJ-2010006) was isolated from the growing cultures by repeated reinoculation on PDA plates.

Identification of Fungal Cultures. The fungus was identified as *Nigrospora* sp. according to morphologic traits and a molecular biological protocol by DNA amplification and sequencing as described below. About 100 mg of fresh fungal mycelium was collected in a microcentrifuge tube (1.5 mL) to extract genomic DNA from the fungus using a fungal DNA kit (50) (E.Z.N.A., Omega) according to the manufacturer's protocol. The PCR reactions were performed in a final volume of 50 μL, which was composed of template DNA (2 μL), 5 μL of 10× buffer, 1 μL of dNTP, 0.5 μL of ITS1F, 0.5 μL of ITS4 (20 μmol/mL each), 0.25 μL of Taq polymerase, and appropriate ultrapure water under the following conditions: (1) initial denaturation

at 94 °C for 5 min; (2) denaturation at 94 °C for 40 s; (3) annealing at 52 °C for 40 s; (4) extension at 72 °C for 1 min; (5) final extension at 72 °C for 10 min. Steps 2−4 were repeated 30 times. Then, 5 μL of the amplification products was loaded onto an agarose gel (1.2% agarose in 0.5× TAE, 5 μL of ethidium bromide 1% m/v solution per 100 mL of gel). After electrophoresis at 100 V for 35 min, the band due to the PCR product (approximate size 526 bp) was isolated from the gel slice using a gel extraction kit (E.Z.N.A., Omega) according to the manufacturer's protocol. The PCR product was then submitted for sequencing (Invitrogen, Shanghai) with the primer ITS4. The fungus was identified as a *Nigrospora* sp. whose 526 base pair ITS sequence had 99% sequence identity to that of *Nigrospora* sp. P19E2 (JN207298). The sequence data have been submitted to GenBank, accession number HM565952.

Fermentation, Extraction, and Isolation. The fungal strain was cultivated in 70 L of liquid medium (composition of medium: 200 g/L cooked and sliced potatoes, 20 g/L glucose in artificial seawater, in 1 L Erlenmeyer flasks each containing 400 mL of culture broth) at 27 °C without shaking for 4 weeks. Then the fermentation broth (70 L) was extracted three times with an equal volume of EtOAc. The combined EtOAc layer was evaporated to dryness under reduced pressure to give an EtOAc extract (28.6 g), which was subjected to silica gel column chromatography (petroleum ether, EtOAc v/v, gradient) to afford five fractions (Fr. 1−Fr. 5). Fr. 2 was subjected to silica gel column chromatography ($CHCl_3$/MeOH) and then ODS column chromatography (MeOH/H_2O, v/v, 3:2). Further purification was carried out using HPLC on a ODS semipreparative C_{18} column (Kromasil 7 μm, 10 × 250 mm, 2 mL/min) eluted with 75% MeOH/H_2O to obtain **1** (9.0 mg) and 55% MeOH/H_2O for **2** (4.2 mg). The fungal mycelia were extracted three times with $CHCl_3$/MeOH (v/v, 1:1); then the organic extracts were concentrated *in vacuo* to obtain an extract (46.0 g), which was subjected to silica gel column chromatography eluting with a gradient of petroleum ether to EtOAc to yield six fractions (Fr. 1−Fr. 6). Fr. 2 and Fr. 3 were further purified by column chromatography on silica gel, Sephadex LH-20, and semipreparative HPLC, to yield **3** (8.0 mg), **4** (20.0 mg), **5** (9.0 mg), **6** (26.0 mg), **7** (23.0 mg), **8** (81.0 mg), and **9** (176.0 mg), respectively.

4a-*epi*-9α-Methoxydihydrodeoxybostrycin (1): colorless crystals; mp 214 °C; $[\alpha]^{24}_D$ −10.3 (*c* 0.070, MeOH); UV (MeOH) λ_{max} (log ε) 211 (4.28), 244 (4.17), 284 (4.10), 358 (3.98) nm; IR (KBr) ν_{max} 3448, 1712, 1624 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; EIMS *m/z* 338 $[M]^{\bullet+}$; HREIMS *m/z* 338.1358 $[M]^{\bullet+}$ (calcd for $C_{17}H_{22}O_7$, 338.1360).

10-Deoxybostrycin (2): yellow, amorphous powder; mp 212 °C; $[\alpha]^{24}_D$ 59.6 (*c* 0.025, acetone); UV (MeOH) λ_{max} (log ε) 219 (4.66), 247 (4.33), 281 (4.21), 358 (3.90), 431 (3.69) nm; IR (KBr) ν_{max} 3380, 2918, 1606 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; ESIMS *m/z* 321.1 $[M + H]^+$; HRESIMS *m/z* 321.0979 $[M + H]^+$ (calcd for $C_{16}H_{17}O_7$, 321.0974).

Nigrosporin B (3): red crystals; $[\alpha]^{24}_D$ −12.9 (*c* 0.085, MeOH); [lit. $[\alpha]^{28}_D$ −16.7 (*c* 0.02, $CHCl_3$)].[7]

9α-Hydroxydihydrodesoxybostrycin (4): colorless crystals; $[\alpha]^{24}_D$ −99.2 (*c* 0.065, MeOH); [lit. $[\alpha]^{25}_D$ −5.6 (*c* 0.16, MeOH)].[8]

9α-Hydroxyhalorosellinia A (5): colorless crystals; $[\alpha]^{24}_D$ −147.8 (*c* 0.04, MeOH); [lit. $[\alpha]^{28}_D$ −95.8 (*c* 0.15, MeOH)].[8]

4-Deoxybostrycin (6): red crystals; $[\alpha]^{24}_D$ 2.3 (*c* 0.14, MeOH).

Bostrycin (7): red crystals; $[\alpha]^{24}_D$ −37.5 (*c* 0.195, MeOH); [lit. $[\alpha]^{24}_D$ −275 (Me_2SO)].[11]

X-ray Crystallographic Analysis of 1. Colorless crystals of **1** were obtained from MeOH. The crystal data were recorded at 293 K on an Agilent Gemini Ultra diffractometer with Cu Kα radiation (λ = 1.54718 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 as riding atoms with the relative isotropic parameters. The crystallographic data for **1** have been deposited at the Cambridge Crystallographic Data Centre with the deposition number 852962. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, UK [fax: +44(0)-1233-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Crystal data for **1**: $C_{17}H_{22}O_7$, M_r = 338.35, orthorhombic, space group $P222_1$ with *a* = 7.06090(10) Å, *b* = 8.67570(10) Å, *c* = 25.6838(4) Å, α = β = γ = 90°, *V* = 1573.34(4) $Å^3$, *Z* = 4, D_x = 1.428 mg/m^3, μ(Cu Kα) = 0.933 mm^{-1}, and *F*(000) = 720. Crystal dimensions: 0.44 × 0.43 × 0.29 mm^3. Independent reflections: 2829 (R_{int} = 0.0262). The final R_1 values were 0.0271, Flack parameter = 0.05(14), wR2 = 0.0740 ($I > 2\sigma(I)$).

Acetylation of Compounds 6−9. Compound **6** (10 mg) was dissolved in 1.0 mL of pyridine and 2.0 mL of acetone, 1.0 mL of acetic anhydride was then added, and the solution was allowed to stir at room temperature for 4 h. The solvent and excess reagents were removed *in vacuo*. The crude mixture was subjected to silica gel column chromatography (7:3 petroleum ether/EtOAc) to obtain compound **6a** (10.6 mg). Acetylation of compounds **7** (9.0 mg), **8** (60.0 mg), and **9** (3.0 mg) under the same reaction conditions gave compounds **7a** (9.5 mg), **8a** (9.0 mg), **8b** (4.0 mg), **8c** (15.0 mg), **8d** (4.0 mg), **8e** (6.0 mg), **8f** (8.0 mg), **8g** (16.0 mg),[10] and **9a** (3.0 mg), respectively.

The structures of the acetylated derivatives were identified by EIMS and NMR spectra in comparison with the reaction substrates, and the positions of acetoxy groups were further confirmed by the key HMBC correlations (see Supporting Information).

3-Acetoxy-4-deoxybostrycin (6a): red crystals; mp 115 °C; $[\alpha]^{24}_D$ −167 (*c* 0.080, MeOH); ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 13.08 (1H, s, 10-OH), 12.73 (1H, s, 9-OH), 6.16 (1H, s, H-6), 5.07 (1H, dd, *J* = 7.5, 5.5 Hz, H-3), 3.92 (3H, s, H-12), 3.17 (1H, dd, *J* = 19.0, 5.5 Hz, H-4β), 3.06 (1H, d, *J* = 18.7 Hz, H-1β), 2.94 (1H, dd, *J* = 19.0, 7.5 Hz, H-4α), 2.82 (1H, d, *J* = 18.7 Hz, H-1α), 2.14 (3H, s, C-3-OAc), 1.36 (3H, s, H-11); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm) 184.2 (C, C-5), 177.7 (C, C-8), 170.5 (C, C-3-OAc), 161.3 (C, C-9), 160.6 (C, C-7), 159.5 (C, C-10), 137.4 (C, C-4a), 135.2 (C, C-1a), 109.6 (CH, C-6), 109.6 (C, C-9a), 107.7 (C, C-10a), 73.6 (CH, C-3), 69.3 (C, C-2), 56.7 (CH_3, C-12), 35.6 (CH_2, C-1), 27.1 (CH_2, C-4), 25.5 (CH_3, C-11), 21.1 (CH_3, C-3-OAc); ESIMS *m/z* 361.0 $[M - H]^-$; HREIMS *m/z* 362.0993 $[M]^{\bullet+}$ (calcd for $C_{18}H_{18}O_8$, 362.0996).

3-Acetoxybostrycin (7a): red, amorphous powder; mp 198 °C; $[\alpha]^{24}_D$ −170 (*c* 0.085, MeOH); ^1H NMR (400 MHz, $CDCl_3$, δ, ppm, *J*/Hz) 13.49 (1H, s, 10-OH), 12.61 (1H, s, 9-OH), 6.20 (1H, s, H-6), 5.24 (1H, d, *J* = 7.2 Hz, H-3), 5.16 (1H, d, *J* = 7.2 Hz, H-4), 3.96 (3H, s, H-12), 3.15 (1H, d, *J* = 18.8 Hz, H-1α), 2.82 (1H, d, *J* = 18.8 Hz, H-1β), 2.22 (3H, s, C-3-OAc), 1.39 (3H, s, H-11); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm) 184.8 (C, C-5), 178.2 (C, C-8), 170.5 (C, C-3-OAc), 160.9 (C, C-7), 160.8 (C, C-9), 159.5 (C, C-10), 138.4 (C, C-4a), 135.2 (C, C-1a), 110.4 (C, C-9a), 109.7 (CH, C-6), 108.6 (C, C-10a), 78.1 (CH, C-3), 70.8 (CH_2, C-2), 68.7 (CH, C-4), 56.9 (CH_3, C-12), 36.0 (CH_2, C-1), 26.0 (CH_3, C-11), 21.0 (CH_3, C-3-OAc); EIMS *m/z* 378 $[M]^{\bullet+}$, 360 $[M - H_2O]^+$, 342 $[M - 2H_2O]^+$; HREIMS *m/z* 378.0945 $[M]^{\bullet+}$ (calcd for $C_{18}H_{18}O_9$, 378.0945).

8-Acetoxy-3,5-dihydroxy-7-methoxy-2-methylanthracene-9,10-dione (8a): yellow, amorphous powder; mp 230 °C; ^1H NMR (400 MHz, DMSO-d_6, δ, ppm) 13.45 (1H, s, 5-OH), 11.10 (1H, brs, 3-OH), 7.88 (1H, s, H-1), 7.54 (1H, s, H-4), 7.04 (1H, s, H-6), 3.92 (3H, s, H-12), 2.35 (3H, s, C-8-OAc), 2.26 (3H, s, H-11); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) 186.4 (C, C-10), 179.8 (C, C-9), 168.4 (C, C-8-OAc), 162.6 (C, C-5), 161.3 (C, C-3), 159.4 (C, C-7), 133.3 (C, C-8), 132.3 (C, C-2), 132.2 (C, C-1a), 129.9 (CH, C-1), 125.5 (C, C-4a), 123.6 (C, C-9a), 110.4 (CH, C-4), 108.3 (C, C-10a), 105.6 (CH, C-6), 57.0 (CH_3, C-12), 20.5 (CH_3, C-8-OAc), 16.1 (CH_3, C-11); EIMS *m/z* 342 $[M]^{\bullet+}$.

5-Acetoxy-3,8-dihydroxy-7-methoxy-2-methylanthracene-9,10-dione (8b): yellow, amorphous powder; mp 207 °C; ^1H NMR (400 MHz, DMSO-d_6, δ, ppm) 13.71 (1H, s, 8-OH), 7.98 (1H, s, H-1), 7.43 (1H, s, H-4), 7.18 (1H, s, H-6), 3.94 (3H, s, H-12), 2.37 (3H, s, C-5-OAc), 2.27 (3H, s, H-11); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) 187.5 (C, C-9), 179.6 (C, C-10), 168.7 (C, C-5-OAc), 163.1 (C, C-3), 154.6 (C, C-7), 151.1 (C, C-8), 144.4 (C, C-5), 134.6 (C, C-2), 131.7 (C, C-1a), 129.6 (CH, C-1), 123.4 (C, C-4a), 115.0 (C, C-10a),

114.5 (C, C-9a), 113.1 (CH, C-6), 111.5 (CH, C-4), 56.7 (CH_3, C-12), 21.0 (CH_3, C-5-OAc), 16.1 (CH_3, C-11); EIMS *m/z*: 342 $[M]^{\bullet+}$.

3-Acetoxy-5,8-dihydroxy-7-methoxy-2-methylanthracene-9,10-dione (8c): orange, amorphous powder; mp 221 °C; ^{1}H NMR (400 MHz, $CDCl_3$, *δ*, ppm) 13.45 (1H, s, 5-OH), 13.42 (1H, s, 8-OH), 8.21 (1H, s, H-1), 7.96 (1H, s, H-4), 6.68 (1H, s, H-6), 4.00 (3H, s, H-12), 2.39 (3H, s, C-3-OAc), 2.36 (3H, s, H-11); ^{13}C NMR (100 MHz, $CDCl_3$, *δ*, ppm) 186.7 (C, C-9), 183.6 (C, C-10), 168.3 (C, C-3-OAc), 160.8 (C, C-3), 157.7 (C, C-5), 154.5 (C, C-7), 150.3 (C, C-8), 137.6 (C, C-1a), 133.8 (C, C-2), 130.9 (C, C-4a), 130.2 (CH, C-1), 120.5 (CH, C-4), 112.4 (C, C-9a), 106.8 (CH, C-6), 106.2 (C, C-10a), 56.7 (CH_3, C-12), 20.8 (CH_3, C-11), 16.8 (CH_3, C-3-OAc); EIMS *m/z* 342 $[M]^{\bullet+}$; HREIMS *m/z* 342.0735 $[M]^{\bullet+}$ (calcd for $C_{18}H_{14}O_7$, 342.0734).

5,8-Diacetoxy-3-hydroxy-7-methoxy-2-methylanthracene-9,10-dione (8d): pale yellow crystals; mp 198 °C; ^{1}H NMR (400 MHz, acetone-d_6, *δ*, ppm) 10.14 (1H, brs, 3-OH), 7.89 (1H, s, H-1), 7.51 (1H, s, H-4), 7.27 (1H, s, H-6), 4.00 (3H, s, H-12), 2.38 (3H, s, C-5-OAc), 2.38 (3H, s, C-8-OAc), 2.33 (3H, s, H-11); ^{13}C NMR (100 MHz, acetone-d_6, *δ*, ppm) 181.3 (C, C-9), 180.9 (C, C-10), 169.0 (C, C-8-OAc), 168.7 (C, C-5-OAc), 162.1 (C, C-3), 158.6 (C, C-7), 150.8 (C, C-5), 134.9 (C, C-8), 132.7 (C, C-2), 130.6 (CH, C-1), 127.4 (C, C-1a), 127.0 (C, C-4a), 118.8 (C, C-9a), 113.6 (CH, C-4), 111.7 (CH, C-6), 100.8 (C, C-10a), 57.4 (CH_3, C-12), 21.2 (CH_3, C-8-OAc), 20.8 (CH_3, C-5-OAc), 16.4 (CH_3, C-11); EIMS *m/z* 384 $[M]^{\bullet+}$.

3,8-Diacetoxy-5-hydroxy-7-methoxy-2-methylanthracene-9,10-dione (8e): yellow, amorphous powder; mp 190 °C; ^{1}H NMR (400 MHz, acetone-d_6, *δ*, ppm) 13.47 (1H, s, 5-OH), 8.08 (1H, s, H-1), 7.91 (1H, s, H-4), 6.77 (1H, s, H-6), 3.93 (3H, s, H-12), 2.47 (3H, s, C-8-OAc), 2.39 (3H, s, C-3-OAc), 2.33 (3H, s, H-11); ^{13}C NMR (100 MHz, acetone-d_6, *δ*, ppm) 186.0 (C, C-10), 180.9 (C, C-9), 169.2 (C, C-8-OAc), 168.3 (C, C-3-OAc), 163.7 (C, C-5), 159.8 (C, C-7), 154.0 (C, C-3), 138.0 (C, C-2), 134.3 (C, C-8), 132.4 (C, C-1a), 131.7 (C, C-4a), 130.6 (CH, C-1), 124.1 (C, C-9a), 120.1 (CH, C-4), 109.1 (C, C-10a), 105.7 (CH, C-6), 56.7 (CH_3, C-12), 20.8 (CH_3, C-3-OAc), 20.8 (CH_3, C-8-OAc), 16.8 (CH_3, C-11); EIMS *m/z* 384 $[M]^{\bullet+}$.

3,5-Diacetoxy-8-hydroxy-7-methoxy-2-methylanthracene-9,10-dione (8f): yellow, amorphous powder; mp 202 °C; ^{1}H NMR (400 MHz, $CDCl_3$, *δ*, ppm) 13.57 (1H, s, 8-OH), 8.17 (1H, s, H-1), 7.87 (1H, s, H-4), 6.81 (1H, s, H-6), 4.01 (3H, s, H-12), 2.46 (3H, s, C-5-OAc), 2.37 (3H, s, C-3-OAc), 2.35 (3H, s, H-11); ^{13}C NMR (100 MHz, $CDCl_3$, *δ*, ppm) 188.3 (C, C-9), 179.1 (C, C-10), 169.6 (C, C-5-OAc), 168.2 (C, C-3-OAc), 154.9 (C, C-7), 154.8 (C, C-3), 153.2 (C, C-8), 144.9 (C, C-5), 137.3 (C, C-2), 134.4 (C, C-1a), 130.1 (C, C-4a), 130.0 (CH, C-1), 121.0 (CH, C-4), 115.5 (C, C-9a), 115.2 (C, C-10a), 112.6 (CH, C-6), 56.6 (CH_3, C-12), 21.2 (CH_3, C-5-OAc), 20.7 (CH_3, C-3-OAc), 16.7 (CH_3, C-11); EIMS *m/z* 384 $[M]^{\bullet+}$; HREIMS *m/z* 384.0840 $[M]^{\bullet+}$ (calcd for $C_{20}H_{16}O_8$, 384.0840).

8-Acetoxyaustrocortirubin (9a): pale yellow, amorphous powder; mp 183 °C; ^{1}H NMR (400 MHz, $CDCl_3$, *δ*, ppm, *J*/Hz) 13.59 (1H, s, 5-OH), 8.15 (1H, d, *J* = 7.9 Hz, H-4), 7.98 (1H, d, *J* = 1.1 Hz, H-1), 7.56 (1H, dd, *J* = 7.9, 1.1 Hz, H-3), 6.76 (1H, s, H-6), 3.92 (3H, s, H-12), 2.50 (3H, s, H-11), 2.46 (3H, s, C-5-OAc); ^{13}C NMR (100 MHz, $CDCl_3$, *δ*, ppm) 186.8 (C, C-10), 181.8 (C, C-9), 169.3 (C, C-5-OAc), 163.6 (C, C-5), 159.5 (C, C-7), 145.6 (C, C-2), 134.9 (CH, C-3), 134.1 (C, C-8), 134.0 (C, C-1a), 130.5 (C, C-4a), 127.6 (CH, C-1), 126.7 (CH, C-4), 124.3 (C, C-9a), 109.0 (C, C-10a), 105.7 (CH, C-6), 56.6 (CH_3, C-12), 21.9 (CH_3, C-11), 20.8 (CH_3, C-5-OAc); EIMS *m/z* 326 $[M]^{\bullet+}$, 284 $[M - OAc + H]^+$; HREIMS *m/z* 326.0783 $[M]^{\bullet+}$ (calcd for $C_{18}H_{14}O_6$, 326.0785).

Biological Assays. The antibacterial activities against nine bacterial strains, Gram-positive *B. subtilis* (ATCC 6633), *B. cereus* (ACCC 11077), *M. luteus* (ATCC 49732), *M. tetragenus* (ATCC 13623), *S. albus* (ATCC 8799), *S. aureus* (ATCC 27154), Gram-negative *E. coli* (ATCC 25922), *V. anguillarum* (ATCC 19019), and *V. parahemolyticus* (ATCC 17802), were determined by a serial dilution technique using 96-well microtiter plates.[17] The compounds were dissolved in DMSO to give a stock solution. Bacterial species were cultured overnight at 37 °C in LB broth and diluted to 10^6 cfu/mL when used. LB broth was used as a blank control, and DMSO was used as a negative control, while ciprofloxacin was used as a positive control. The plates were incubated at 37 °C for 24 h. The results were observed with a Multiskan Mk3 (Thermo Labsystems) at 630 nm. The cytotoxic activities were evaluated against human lung cancer cell line A549 by the MTT method as described previously.[18] Mitomycin was used as a positive control.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, HMQC, HMBC, and MS spectra of the new compounds (**1**, **2**, **6a**, **7a**, **8a**–**8f**, **9a**); key HMBC correlations of compounds **1**, **2**, **6a**, **7a**, **8a**, **8e**, and **9a**; CIF file and X-ray crystallographic data for **1**. 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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