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Potent Antifouling Resorcylic Acid Lactones from the Gorgonian-Derived Fungus *Cochliobolus lunatus*

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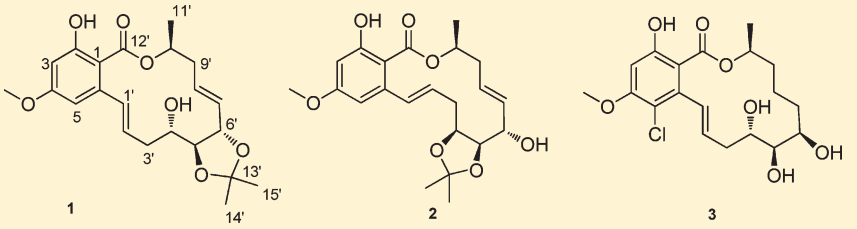
Supporting Information

ABSTRACT:



Three new 14-membered resorcylic acid lactones, two with a rare natural acetonide group and one with a 5-chloro-substituted lactone, named cochliomycins A−C (**1**−**3**), together with four known analogues, zeaenol (**4**), LL-Z1640-1 (**5**), LL-Z1640-2 (**6**), and paecilomycin F (**7**), were isolated from the culture broth of *Cochliobolus lunatus*, a fungus obtained from the gorgonian *Dichotella gemmacea* collected in the South China Sea. Their structures and the relative configurations of **1**−**3** were elucidated using comprehensive spectroscopic methods including NOESY spectra and chemical conversions. A transetherification reaction was also observed in which cochliomycin B (**2**) in a solution of $CDCl_3$ slowly rearranged to give cochliomycin A (**1**) at room temperature. These resorcylic acid lactones were evaluated against the larval settlement of barnacle *Balanus amphitrite*, and antifouling activity was detected for the first time for this class of metabolites. The antibacterial and cytotoxic activities of these compounds were also examined.

A series of 14-membered resorcylic acid lactones, such as hypothemycin,[1,2] radicicol A,[3] aigialomycins A−E,[4] and paecilomycins A−F,[5] have been reported as fungal polyketide metabolites. These compounds have received much attention because of their multiple potent biological activities including antifungal,[6] cytotoxic,[4,7] antimalarial,[7] and nematicidal activities.[8] As part of our ongoing investigation on new natural antibacterial, cytotoxic, and nontoxic antifouling agents from marine fungi in the South China Sea, the fungus *Cochliobolus lunatus* isolated from the gorgonian *Dichotella gemmacea* attracted our attention because the EtOAc extract of the fungal culture showed the presence of resorcylates with characteristic UV absorption spectra[4] (λ_{max} 197, 236, 272, 315 nm). Two new 14-membered resorcylic acid lactones with a rare natural acetonide group, cochliomycins A and B (**1** and **2**), and one new 5-chloro-substituted lactone, cochliomycin C (**3**), together with four known analogues, namely, zeaenol (**4**),[9] LL-Z1640-1 (**5**),[10] LL-Z1640-2 (**6**),[10] and paecilomycin F (**7**),[5] were obtained from the culture broth of the fungus *C. lunatus* by using column chromatography and semipreparative HPLC guided by the characteristic UV absorption spectra of resorcylates. This study successfully identified potential new sources of antifouling compounds. Herein we report the isolation, structure elucidation, relative configurations, and biological activities of these compounds.

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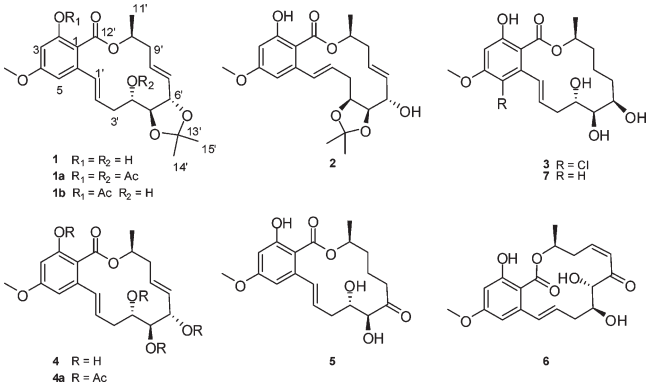

RESULTS AND DISCUSSION

Cochliomycin A (**1**) was obtained as a white, amorphous powder. Its molecular formula of $C_{22}H_{28}O_7$ (nine degrees of unsaturation) was determined by high-resolution EI mass spectrometry. This molecular formula was also corroborated by ^{1}H and ^{13}C NMR spectroscopic data (Tables 1 and 2). The ^{13}C NMR and DEPT spectroscopic data of **1** revealed the presence of 22 carbon signals including one ester carbonyl (δ_C 170.7), 10 olefinic carbons, four oxygenated carbons, one methoxy group, three methyl groups, two methylene groups, and one quaternary carbon (δ_C 108.4). The nine degrees of unsaturation inherent in the molecular formula of **1**, coupled with the NMR data showing the presence of one carbonyl and 10 olefinic carbons (six degrees of unsaturation), indicated that compound **1** must contain three rings. In the ^{1}H NMR spectrum of **1**, one hydrogen-bonded hydroxy group signal at δ_H 11.49 (s), two *meta*-coupled aromatic protons signals at δ_H 6.39 (d, J = 2.4 Hz) and 6.46 (d, J = 2.4 Hz), one methoxy group, one doublet methyl group (δ_H 1.44, d, J = 6.6 Hz), and two singlet methyl groups were observed. Acetylation of **1** with Ac_2O/pyridine gave the diacetyl derivative (**1a**) and the monoacetyl derivative (**1b**), which supported the presence of two OH groups in **1**. Detailed analysis of the ^{1}H and ^{13}C NMR spectra revealed that **1** is a resorcylic acid lactone, which is similar to zeaenol (**4**).[9] The contiguous sequence of correlations from H-1′ to H-11′ in the COSY spectrum confirmed the presence of an aliphatic subunit similar to that seen in **4**. The HMBC correlations from H-1′ to C-1, C-5, and C-6, from H-2′ to C-6, and the correlation between the chelated hydroxy group and C-12′ established that the connection points of the aliphatic ring and the benzene ring were at C-6 and C-1′. Furthermore, the observed HMBC correlations between the remaining two methyl proton signals at δ_H 1.43 and 1.36 and one quaternary carbon, which was a ketal according to its chemical shift (δ_C 108.4), established the presence of one acetonide group in **1**. This acetonide group was also confirmed by the existence of the fragment ion peak at m/z 347.0 $[M - CH_3COCH_3 + H]^{\bullet+}$ in the ESIMS spectrum. The two positions of the ether linkages were located at C-5′ and C-6′ on the basis of the two relatively downfield oxygenated carbons (C-5′, δ_C 81.4 and C-6′, δ_C 75.2) together with the HMBC correlation from H-6′ to C-13′. In addition, the *trans*, *trans* geometries at C-1′−C-2′ and C-7′−C-8′ double bonds were assigned by the coupling constants ($J_{1',2'}$ = 15.6 Hz and $J_{7',8'}$ = 15.0 Hz). On the basis of these results, the complete planar assignment for compound **1** was confirmed.

The relative configurations of the stereocenters in cochliomycin A (**1**) were assigned on the basis of 1D NOE measurements, 2D NOESY experiments, and the formation of an acetonide from **4**. In the NOESY spectrum of **1**, a correlation between H-5′ and the acetonide methyl at δ_H 1.36 was evident, while H-6′ correlated to the acetonide methyl at δ_H 1.43. Also, in the selective NOE experiments, the irradiation of H-5′ at δ_H 3.89

Table 1. ^{1}H NMR (600 MHz) Data (δ) for 1−3[a]

position	1	2	3
3	6.39, d (2.4)	6.39, d (2.4)	6.48, s
5	6.46, d (2.4)	6.40, d (2.4)	
1′	7.16, dd (15.6, 2.4)	7.00, dd (15.6, 2.4)	6.62, m
2′	5.72, ddd (15.6, 10.2, 3.0)	5.56, ddd (15.6, 9.0, 3.6)	5.56, m
3′	2.75, m	2.76, m	2.84, m
	2.25, m	2.59, m	2.54, m
4′	4.20, ddd (12.0, 4.8, 2.4)	4.36, ddd (12.0, 4.8, 3.6)	4.20, m
5′	3.89, dd (8.4, 2.4)	3.85, dd (9.0, 4.8)	3.46, s
6′	4.56, t (8.4)	4.12, t (9.0)	4.16, m
7′	5.52, ddt (15.0, 9.6, 1.2)	5.46, m	1.87, m
			1.42, m
8′	5.99, ddd (15.0, 8.4, 4.8)	6.07, ddd (15.6, 9.0, 4.8)	1.81, m
			1.62, m
9′	2.50, m	2.50, m	1.51, m
	2.42, m	2.44, m	1.28, m
10′	5.44, m	5.44, m	5.14, m
11′	1.44, d (6.6)	1.44, d (6.6)	1.34, d (6.6)
14′	1.36, s	1.42, s	
15′	1.43, s	1.52, s	
2-OH	11.49, s	11.50, s	12.20, s
4-OCH_3	3.81, s	3.81, s	3.92, s

[a] Solvent: $CDCl_3$ for **1**−**3**.

Table 2. ^{13}C NMR (150 MHz) Data (δ) for 1−3[a]

position	1	2	3
1	104.3, C	104.6, C	105.4, C
2	164.7, C	164.8, C	163.7, C
3	100.0, CH	100.1, CH	100.0, CH
4	163.9, C	164.1, C	160.3, C
5	107.1, CH	107.7, CH	114.5, C
6	142.0, C	142.6, C	139.9, C
1′	134.0, CH	134.5, CH	128.3, CH
2′	126.4, CH	126.3, CH	131.0, CH
3′	36.0, CH_2	31.4, CH_2	38.1 CH_2
4′	68.7, CH	77.4, CH	76.4, CH
5′	81.4, CH	79.5, CH	69.0, CH
6′	75.2, CH	69.7, CH	66.7, CH
7′	132.6, CH	132.9, CH	30.8, CH_2
8′	129.5, CH	130.5, CH	35.0, CH_2
9′	37.8, CH_2	38.3, CH_2	21.1, CH_2
10′	70.5, CH	70.6, CH	73.8, CH
11′	19.2, CH_3	18.8, CH_3	21.4, CH_3
12′	170.7, C	170.6, C	171.0, C
13′	108.4, C	107.9, C	
14′	26.9, CH_3	28.5, CH_3	
15′	26.9, CH_3	25.9, CH_3	
4-OCH_3	55.4, CH_3	55.5, CH_3	55.7, CH_3

[a] Solvent: $CDCl_3$ for **1**−**3**.

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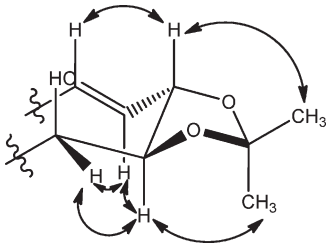

Figure 1. Key NOE correlations for **1**.

resulted in the enhancement of the signal for H-4′, but no enhancement for H-6′, and therefore, H-5′ and H-6′ should be placed on the opposite side of the acetonide ring (*trans*-fused acetonide). The relative configurations from C-4′ to C-8′ in **1** are shown in Figure 1. Treatment of zeaenol (**4**) with 2,2-dimethoxypropane in the presence of TsOH afforded compound **1** as the major acetonide product together with a minor product (**2**) as reported for the reaction of the very similar analogue aigialomycin B,[4] which further confirmed the structure of **1**. Because the relative and absolute configurations of **4** have been previously established,[5] the chemical conversion from **4** allows the determination of the 4′*S*,5′*S*,6′*S*,10′*S* configuration for **1**.

Cochliomycin B (**2**) had the same molecular formula as **1** on the basis of the HREIMS data, and its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) were very similar to those of **1**. The only significant differences between the spectra of **2** and **1** were the chemical shifts of C-4′ (δ_C 77.4 in **2** vs δ_C 68.7 in **1**) and C-6′ (δ_C 69.7 in **2** vs δ_C 75.2 in **1**), suggesting that the acetonide group in **2** was formed by the ether linkages at C-4′ and C-5′ rather than C-5′ and C-6′ in **1**. This structure was also confirmed by the acetonide products of **4**, which included **2** as a minor product and **1** as the major product. Thus, the configurations of **2** were assigned as 4′*S*,5′*R*,6′*S*,10′*S*. Interestingly, a fortuitous observation revealed that cochliomycin B (**2**) slowly rearranged to cochliomycin A (**1**) in a solution of $CDCl_3$. Thus, compound **1** can arise from **2** via a transetherification reaction in which the ether migrates from O-4′ to O-6′.

Cochliomycin C (**3**) was isolated as a trace macrocyclic lactone that gave a $[M + Na]^+$ ion at *m*/*z* 423.1182 in the HRESIMS spectrum, consistent with a molecular formula of $C_{19}H_{25}ClO_7$. Analysis of its ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) indicated that **3** was also a resorcylic acid lactone derivative, with a structure very similar to that of paecilomycin F (**7**), except for differences at the benzene ring. Detailed comparison of the ^{1}H NMR spectrum of **3** with that of **7** showed that the H-5 signal in **3** was missing, and the chemical shift of H-3 (6.40, s) in **7** was changed to δ_H 6.48 (s) in **3**, suggesting that H-5 had been replaced by chlorine. The HMBC correlations from H-3 to C-2 and from 2-OH to C-3 further confirmed the chlorine substituent at C-5. Thus, cochliomycin C (**3**) was assigned as the 5-chloro analogue of paecilomycin F (**7**). On the basis of the absolute configuration of paecilomycin F (**7**) and a shared biogenesis with **1** and **2**, the configuration of cochliomycin C (**3**) should be assigned as 4′*S*,5′*R*,6′*R*,10′*S*.

The structures of compounds **4** and **7** were identified as zeaenol[9] and paecilomycin F[5] by the comparison of their spectroscopic data with those in the literature, and the structures of compounds **5** and **6** were elucidated as LL-Z1640-1[9,10] and LL-Z1640-2[10] by comprehensive spectroscopic methods, especially 2D NMR and comparison of data with those in the literature.[9,11]

Table 3. Antifouling Activity

compound	EC_{50} (μg/mL)	LC_{50}/EC_{50}
1	1.2	>16.7
1a	15.4	>6.49
1b	12.5	>8.00
4	5.0	>20.0
4a	>50	ND[a]
5	5.3	>18.9
7	17.9	>5.59

[a] ND: not determined.

The most structurally unique part of compounds **1** and **2** is the acetonide group, which has never been described before in any natural product 14-membered resorcylic acid lactone. During the extraction or purification processes, compounds **1** and **2** were isolated without using acetone, and they were also clearly detected in freshly prepared ethyl acetate extracts. Indeed, even though compound **4** was dissolved in acetone and left at 40 °C for 1 week, compounds **1** and **2** were not detected in solution. Therefore, it seems very unlikely that the acetonide moiety in these two compounds comes from an artificial origin, and **1** and **2** should be considered as true natural products possessing an acetonide group. Interestingly, a possible artifact coumarin compound possessing the acetonide group, *threo*-murrangatin acetonide,[12] has also been recently isolated and confirmed as a natural product from the leaves of *Galipea panamensis*.[13]

The isolated resorcylic acid lactones as well as their derivatives were evaluated against the larval settlement of the barnacle *Balanus amphitrite* according to literature procedures.[14] Four of the seven tested compounds completely inhibited the larval settlement of *B. amphitrite* at a concentration of 20.0 μg/mL. Compound **1** showed a significant inhibitory effect on larval settlement even at a concentration of 5.0 μg/mL (none of the larvae settled), but the compound was found to have some toxic effects on the larvae at this concentration, as paralysis of larvae was observed in the treatments. At a concentration lower than 2.5 μg/mL, no toxic effect of compound **1** on the larvae was observed. Further investigation revealed that **1** and its derivatives **1a** and **1b** as well as **4**, **5**, and **7** had potent antifouling activities at nontoxic concentrations with EC_{50} values of 1.2, 15.4, 12.5, 5.0, 5.3, and 17.9 μg/mL, respectively (Table 3), which were lower than the standard requirement of an EC_{50} of 25 μg/mL established by the U.S. Navy program as an efficacy level for natural antifouling agents. This is the first report of antifouling activities for this class of metabolites.

The above results indicated that **1** and **4** showed better potent antifouling activity against the larval settlement of barnacle *B. amphitrite* with EC_{50} values equal to or less than 5.0 μg/mL. The introduction of the acetonide moiety in **1** improved the EC_{50} value (1.2 μg/mL) approximately 3-fold over **4** (5.0 μg/mL), indicating that the acetonide functionality might contribute to the antifouling activity. Moreover, the antifouling activity of **1a** (EC_{50} of 15.4 μg/mL), more than 3 times that of **4a** (EC_{50} >50 μg/mL), also confirmed this. In addition, the acetylation products **1a** and **1b** exhibited antifouling activity with **1** > **1b** > **1a**, while **4** also showed stronger antifouling activity than its acetylation product **4a**. This implied that the hydroxy groups probably also have an effect on antifouling activity. Furthermore, the therapeutic ratio (LC_{50}/EC_{50}) is a way of expressing the effectiveness of the compound in relation to its toxicity, and the

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desired target ratio should be much greater than 1 for use in an antifouling coating. Compounds **1**, **4**, and **5** all have high therapeutic ratios (Table 3), suggesting that they might be useful as environmentally benign antifouling agents.

The antibacterial activities of compounds **1**, **4**, **7**, and **4a** were also determined against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Micrococcus tetragenus* by the method of Fromtling et al.[15] Only compound **1**, possessing the acetonide moiety, exhibited moderate antibacterial activity against *S. aureus*, with an inhibition zone of 11 mm in diameter at a concentration of 50 μg/mL.

Moreover, compound **5** showed moderate cytotoxicity[16] against A549 and HepG2 tumor cell lines with IC_{50} values of 44.5 and 98.6 μM, respectively. However, the above antifouling compounds (**1**, **4**, **7**, **1a**, and **1b**) were all found to be relatively noncytotoxic (IC_{50} > 50 μg/mL) against these two tumor cell lines. Thus, these compounds merit further investigation as models for the discovery of new antifouling molecules. No activities were evaluated for compounds **2**, **3**, and **6** because of their low yields.

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 recorded on a Beckman DU 640 UV spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL Eclips-600 spectrometer at 600 MHz for ^{1}H and 150 MHz for ^{13}C in $CDCl_3$ or acetone-d_6. Chemical shifts δ are reported in ppm, using TMS as internal standard, and coupling constants (J) are in Hz. ESIMS and HRESIMS spectra were measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. HREIMS were measured on a Thermo MAT95XP high-resolution mass spectrometer, and EIMS spectra on a Thermo DSQ EI-mass spectrometer. Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200−300 mesh), octadecylsilyl silica gel (Unicorn; 45−60 μm), and Sephadex LH-20 (Amersham Biosciences) 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. Semipreparative HPLC was performed on a Waters 1525 system using a semipreparative C18 (Kromasil 7 μm, 10 × 250 mm) column coupled with a Waters 2996 photodiode array detector, at a flow rate of 2.0 mL/min.

Fungal Material. The fungal strain *Cochliobolus lunatus* was isolated from a piece of tissue from the inner part of the freshly collected gorgonian coral *D. gemmacea* (GX-WZ-20080034), which was collected from the Weizhou coral reef in the South China Sea in September 2008. The strain was deposited in 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 access code ZJ-2008002. The fungal strain was cultivated in 15 L of liquid medium (10.0 g of glucose, 2.0 g of yeast extract, 2.0 g of peptone in 1 L of seawater, in 1 L Erlenmeyer flasks each containing 400 mL of culture broth) at 27 °C without shaking for 4 weeks.

Identification of Fungal Cultures. Fungal cultures were identified according to a molecular biological protocol by DNA amplification and sequencing of the ITS region as described in the literature.[17] The sequence data have been submitted to GenBank, accession number HQ215514. The fungal strain was identified as *Cochliobolus lunatus*.

Extraction and Isolation. The fungal cultures were filtered through cheesecloth, and the filtrate (15 L) was extracted with EtOAc (15 L × 3). The organic extracts were concentrated *in vacuo* to yield an oily residue (1.2 g). This extract was chromatographed on a silica gel column using a stepwise gradient of petroleum ether−EtOAc and then subjected to Sephadex LH-20 chromatography eluting with mixtures of petroleum ether/$CHCl_3$/MeOH (2:1:1) and $CHCl_3$/MeOH (1:1). Final purification by semipreparative HPLC using a C18 (Kromasil 7 μm, 10 × 25 mm) column at a flow rate of 2.0 mL/min (7:3 MeOH/H_2O; UV detection at 272 nm) yielded compounds **1** (15.2 mg), **2** (2.5 mg), **3** (0.6 mg), **4** (42.0 mg), **5** (2.5 mg), **6** (2.6 mg), and **7** (8.2 mg).

Cochliomycin A (1): white, amorphous powder; mp 67.4−68.1 °C; $[\alpha]^{24}_D$ +10.5 (c 0.43, MeOH); UV (CH_3OH) λ_{max} (log ε) 197 (4.21), 236 (4.35), 272 (4.05), 315 (3.72) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; EIMS m/z 404 $[M]^{\bullet+}$, 386, 371, 346 $[M - CH_3COCH_3]^{\bullet+}$, 328, 311, 267, 233, 219, 189, 177; ESIMS m/z 405.1 $[M + H]^+$, 347.0 $[M - CH_3COCH_3 + H]^+$; HREIMS m/z 404.1829 (calcd for $C_{22}H_{28}O_7$, 404.1830).

Cochliomycin B (2): white, amorphous powder; $[\alpha]^{24}_D$ +7.3 (c 0.05, MeOH); UV (CH_3OH) λ_{max} (log ε) 197 (4.21), 236 (4.36), 272 (4.02), 315 (3.70) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; EIMS m/z 404 $[M]^{\bullet+}$, 389, 346, 328, 307, 284, 249, 231, 203, 190, 177; ESIMS m/z 403.2 $[M - H]^-$; HREIMS m/z 404.1827 (calcd for $C_{22}H_{28}O_7$, 404.1830).

Cochliomycin C (3): white, amorphous powder; $[\alpha]^{24}_D$ −18 (c 0.04, MeOH); UV (CH_3OH) λ_{max} (log ε) 198 (4.26), 234 (4.36), 271 (4.02), 314 (3.70) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; ESIMS m/z 423.1 $[M + Na]^+$; HRESIMS m/z 423.1182 (calcd for $C_{19}H_{25}ClNaO_7$, 423.1187).

Zeaenol (4): white, amorphous powder; $[\alpha]^{24}_D$ −60.4 (c 0.26, MeOH); [lit. $[\alpha]^{24}_D$ −92 (c 0.52, MeOH)].[9]

LL-Z1640-1 (5): white, amorphous powder; mp 144.0−144.7 °C; UV (CH_3OH) λ_{max} (log ε) 199 (4.22), 233 (4.47), 273 (4.09), 314 (3.78) nm; ESIMS m/z 365.1 $[M + H]^+$, 387.2 $[M + Na]^+$.

LL-Z1640-2 (6): white, amorphous powder; mp 167.5−168.1 °C; UV (CH_3OH) λ_{max} (log ε) 199 (4.22), 233 (4.49), 270 (4.11), 311 (3.79) nm; ESIMS m/z 363.0 $[M + H]^+$, 385.2 $[M + Na]^+$.

Paecilomycin F (7): white, amorphous powder; $[\alpha]^{24}_D$ −99 (c 0.08, MeOH); [lit. $[\alpha]^{24}_D$ −106.4 (c 0.28, MeOH)].[5]

Acetylation of Compounds 1 and 4. Compound **1** (9.6 mg) was dissolved in 1.0 mL of pyridine, 1.0 mL of acetic anhydride was then added, and the solution was allowed to stir at room temperature for 24 h. The solvent and excess reagents were removed with a high-vacuum pump, and the crude mixture was subjected to preparative HPLC to obtain compounds **1a** (5.6 mg) and **1b** (2.1 mg). Acetylation of compound **4** (10.0 mg) under the same reaction conditions gave compound **4a** (9.5 mg).

Compound 1a: white, amorphous powder; UV (CH_3OH) λ_{max} (log ε) 197.0 (4.18), 234.5 (4.23) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) 7.06 (1H, dd, 15.0, 2.4, H-1′), 6.77 (1H, d, 2.4, H-5), 6.55 (1H, d, 2.4, H-3), 6.02 (1H, dt, 15.6, 5.4, H-8′), 5.85 (1H, ddd, 15.0, 10.8, 3.6, H-2′), 5.50 (1H, m, H-7′), 5.43 (1H, m, H-10′), 5.38 (1H, m, H-4′), 4.60 (1H, t, 7.8, H-6′), 3.96 (1H, dd, 7.8, 2.4, H-5′), 3.82 (3H, s, 4-OCH_3), 2.63 (1H, m), 2.52 (2H, m), 2.32 (1H, m), 2.28 (3H, s, −OAc), 2.12 (3H, s, −OAc), 1.38 (3H, d, 6.0, H-11′), 1.36 (3H, s, $-CH_3$), 1.35 (3H, s, $-CH_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) 170.3 (C), 169.5 (C), 165.1 (C), 161.8 (C), 151.4 (C), 139.6 (C), 133.2 (CH), 131.1 (CH), 130.6 (CH), 127.3 (CH), 116.9 (C), 110.2 (CH), 108.9 (C), 108.2 (CH), 79.6 (CH), 76.7 (CH), 70.1 (CH), 69.7 (CH), 55.7 (OCH_3), 37.2 (CH_2), 35.0 (CH_2), 27.2 (CH_3), 26.8 (CH_3), 21.2 (CH_3), 21.0 (CH_3), 20.0 (CH_3); ESIMS m/z 511.2 $[M + H]^+$.

Compound 1b: white, amorphous powder; UV (CH_3OH) λ_{max} (log ε) 195.8 (4.19), 233.3 (4.23) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) 7.04 (1H, dd, 15.0, 1.8, H-1′), 6.78 (1H, d, 2.4, H-5), 6.54 (1H, d, 2.4, H-3), 5.98 (1H, dt, 15.6, 5.4, H-8′), 5.81 (1H, ddd, 15.0, 10.8, 3.6, H-2′), 5.50 (1H, m, H-7′), 5.42 (1H, m, H-10′), 4.54 (1H, t, 8.4, H-6′), 4.15 (1H, m, H-4′), 3.87 (1H, dd, 7.8, 2.4, H-5′), 3.82 (3H, s, 4-OCH_3), 2.66 (1H, m), 2.56−2.46 (3H, m), 2.28 (3H, s, −OAc), 1.43 (3H, s, $-CH_3$),

1.38 (3H, d, 6.0, H-11′), 1.36 (3H, s, −CH_3); ^{13}C NMR ($CDCl_3$, 150 MHz) 169.5 (C), 165.1 (C), 161.7 (C), 151.4 (C), 139.9 (C), 132.9 (CH), 131.2 (CH), 130.7 (CH), 128.1 (CH), 116.9 (C), 110.3 (CH), 108.0 (C), 107.9 (CH), 81.1 (CH), 75.8 (CH), 69.7 (CH), 68.6 (CH), 55.6 (OCH_3), 37.1 (CH_2), 36.1 (CH_2), 27.0 ($2CH_3$), 20.9 (CH_3), 19.9 (CH_3); ESIMS m/z 469.1 $[M + H]^+$.

Compound 4a: white, amorphous powder; UV (CH_3OH) λ_{max} (log ε) 195.8 (4.21), 226.3 (4.23) nm; ^{1}H NMR ($CDCl_3$, 600 MHz) 6.86 (1H, m), 6.73 (1H, d, 2.4, H-5), 6.55 (1H, d, 2.4, H-3), 6.03 (1H, m), 5.93 (1H, m), 5.63 (1H, m), 5.44 (1H, t, 7.8, H-6′), 5.30 (1H, m), 5.18 (1H, m), 5.06 (1H, m), 3.81 (3H, s, 4-OCH_3), 2.60−2.41 (4H, m), 2.27 (3H, s, −OAc), 2.05 (3H, s, −OAc), 2.03 (3H, s, −OAc), 2.02 (3H, s, −OAc), 1.39 (3H, d, 6.0, H-11′); ^{13}C NMR ($CDCl_3$, 150 MHz) 170.3 (C), 169.9 (C), 169.8 (C), 169.2 (C), 165.3 (C), 161.3 (C), 150.7 (C), 142.0 (C), 139.1 (CH), 133.7 (CH), 131.4 (CH), 126.5 (CH), 117.2 (C), 110.5 (CH), 107.7 (CH), 77.0 (CH), 73.5 (CH), 72.9 (CH), 71.9 (CH), 55.6 (OCH_3), 37.5 ($2CH_2$), 21.0 (CH_3), 20.9 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 19.9 (CH_3); ESIMS m/z 555.1 $[M + Na]^+$.

Acetonide Formation from Compound 4. A mixture of **4** (12.0 mg), 2,2-dimethoxypropane (1.5 mL), and *p*-TsOH (4.0 mg) was stirred at room temperature for 6 h. Saturated aqueous $NaHCO_3$ (5 mL) was then added, and the reaction mixture was extracted with EtOAc (5 mL × 3). The organic solvents were removed with a high-vacuum pump, and the crude mixture was subjected to preparative HPLC to obtain compounds **1** (8.0 mg) and **2** (1.1 mg).

Antifouling Bioassay. The antilarval-attachment activity was determined using cyprids of the barnacle *Balanus amphitrite* Darwin. Adults of *B. amphitrite* exposed to air for more than 6 h were collected from the intertidal zone in Hong Kong and then were placed in a container filled with 0.22 μm of filtered seawater (FSW) to release nauplii. The collected nauplii were reared to cyprid stage according to the method described by Thiyagarajan et al.[14] When kept at 26−28 °C and fed with *Chaetoceros gracilis*, larvae developed to cyprids on the fourth day. Fresh cyprids were used in the tests. Larval settlement assays were performed using 24-well polystyrene plates (Becton Dickinson 353047). The tested compounds **1**, **1a**, **1b**, **4**, **4a**, **5**, and **7** were first dissolved in a small amount of DMSO and then diluted with filtered FSW to achieve final concentrations at 50, 20, 10, 5, 2.5, 1.25, and 0.625 μg/mL. About 15−20 competent larvae were gently transferred into each well with 1 mL of testing solution in three replicates, and wells containing larvae in FSW with DMSO only served as a positive control. Then the plates were incubated for 24−48 h at 23 °C. The effects of the test samples against biofouling were determined by examining the plates under a dissecting microscope to check for (1) settled larvae and (2) nonsettled larvae, as well as (3) any possible toxic effects of the treatments, such as death or paralysis of larvae, which were also recorded. The number of settled or metamorphosed larvae was expressed as a percentage of the total number of larvae added into each well. The EC_{50} (inhibits 50% of settlement of cyprids in comparison with the control) was calculated by using the Probit software program with the mean of three repeated experiments using different batches of larvae.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. ^{1}H, ^{13}C, COSY, HMQC, HMBC, and MS spectra for **1**, **2**, and **3** are available free of charge via Internet at http://pubs.acs.org.

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