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Isocoumarin Derivatives and Benzofurans from a Sponge-Derived *Penicillium* sp. Fungus

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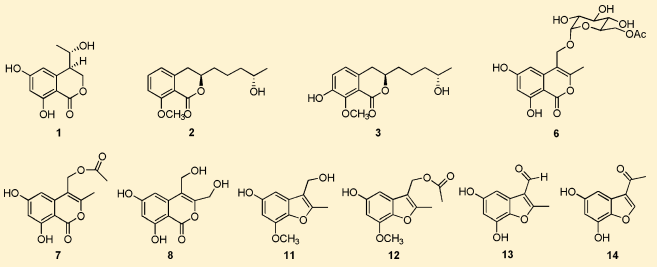
Supporting Information

ABSTRACT: Ten new fungal metabolites, including three hydroisocoumarins, penicimarins A–C (**1**–**3**), three isocoumarins, penicimarins D–F (**6**–**8**), and four benzofurans, penicifurans A–D (**11**–**14**), together with four known isocoumarin derivatives (**4**, **5**, **9**, **10**), were obtained from the sponge-derived fungus *Penicillium* sp. MWZ14-4, collected from the South China Sea. Their planar structures and relative configurations were elucidated by detailed analysis of spectroscopic data and by comparison with related known compounds. The absolute configurations of **1**–**4** were assigned by the modified Mosher's method and TDDFT ECD calculations together with comparison of their CD spectra. Compound **1** represents a rare naturally occurring isocoumarin derivative with 4-substitution, but no substituent at the 3-position. These compounds were evaluated for antibacterial activities and cytotoxic activities *in vitro*. Among them, penicifuran A (**11**) exhibited inhibitory activity against *Staphylococcus albus* with an MIC value of 3.13 μM.



Marine microorganisms, especially marine fungi, have proven to be a rich source of structurally novel and biologically active secondary metabolites, which have become significant resources for drug discovery.[1,2] Marine-derived fungi in the genus *Penicillium* produce various bioactive metabolites, such as cytotoxic citrinadin A,[3] antioxidant terrestrols,[4] antifungal and antibacterial scalusamide A,[5] and antileukemic sorbicillactone A,[6] and have drawn the attention of both pharmaceutical and natural product chemists. During our ongoing search for bioactive metabolites from marine fungi in the South China Sea,[7−10] a sponge-derived fungus, *Penicillium* sp. MWZ14-4, attracted our attention because an EtOAc extract of the fungal culture exhibited antibacterial activity. Bioassay-guided fractionation of the active extract led to the isolation of 10 new compounds including three hydroisocoumarins, penicimarins A–C (**1**–**3**), three isocoumarins, penicimarins D–F (**6**–**8**), and four benzofurans, penicifurans A–D (**11**–**14**), together with four known isocoumarin derivatives, aspergillumarin B (**4**),[11] aspergillumarin A (**5**),[11] sescandelin B (**9**),[12] and 5,6,8-trihydroxy-4-(1′-hydroxyethyl)isocoumarin (**10**).[12] Herein we report the isolation, structure elucidation, and biological activities of these compounds.

RESULTS AND DISCUSSION

Penicimarin A (**1**) was isolated as a colorless, amorphous powder with the molecular formula assigned as $C_{11}H_{12}O_5$ on the basis of its HREIMS data, indicating six degrees of unsaturation. Detailed inspection of the ^{1}H NMR and ^{13}C NMR data (Table 1) revealed that **1** belongs to the isocoumarin class. Analysis of the ^{1}H and ^{13}C NMR data (Table 1) indicated that **1** has a tetrasubstituted benzene ring. In addition, the ^{1}H NMR spectrum (Table 1) displayed signals for one set of nonequivalent oxymethylene protons (δ_H 4.72, dd, J = 10.8, 1.5 Hz, H-3a; 4.43, dd, J = 10.8, 3.0 Hz, H-3b), one oxymethine (δ_H 3.70, dq, J = 7.8, 6.6 Hz, H-9), one methine (δ_H 2.62, ddd, J = 7.8, 3.0, 1.5 Hz, H-4), and one methyl group (δ_H 1.04, d, J = 6.6 Hz, H_3-10). The ^{13}C NMR spectrum (Table 1) showed resonances for one lactone carbonyl (δ_C 169.1), one oxymethylene (δ_C 68.3), one oxymethine (δ_C 65.8), one methine (δ_C 44.6), and one methyl (δ_C 21.4). The HMBC correlations from the methyl protons (H_3-10) to C-9 and C-4, from the oxymethine (H-9) to C-3, C-4, and C-4a, and other HMBC correlations (Figure 1) established a hydroisocoumarin derivative with a 1-hydroxyethyl unit at C-4. The presence of a

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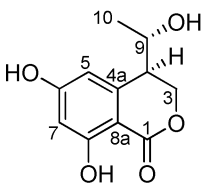
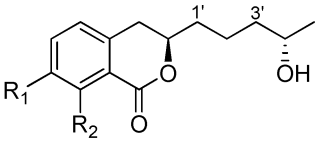
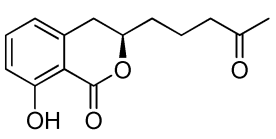
Chart 1



1



2: R_1=H, R_2=OCH_3
3: R_1=OH, R_2=OCH_3
4: R_1=H, R_2=OH



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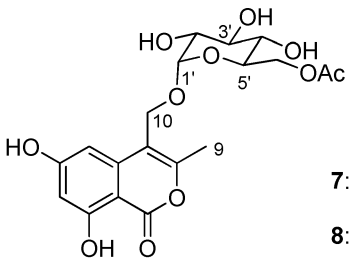
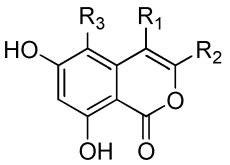
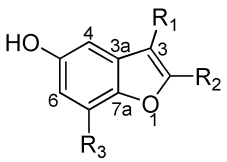


6



7: R_1=$^{10}CH_2OCO^{11}Me^{12}$, R_2=$^{9}CH_3$, R_3=H
8: R_1=$^{10}CH_2OH$, R_2=$^{9}CH_2OH$, R_3=H
9: R_1=$^{10}CH_2OH$, R_2=$^{9}CH_3$, R_3=H
10: R_1=CH(OH)CH_3, R_2=H, R_3=OH

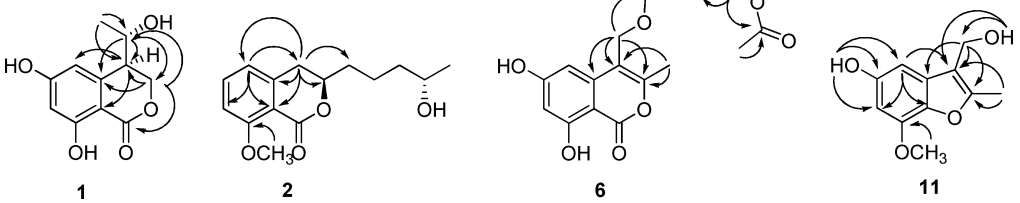


11: R_1=$^{9}CH_2OH$, R_2=$^{8}CH_3$, R_3=OCH_3
12: R_1=$^{9}CH_2OCO^{10}Me^{11}$, R_2=$^{8}CH_3$, R_3=OCH_3
13: R_1=^{8}CHO, R_2=$^{9}CH_3$, R_3=OH
14: R_1=$^{8}CO^{9}Me$, R_2=H, R_3=OH

Table 1. ^{1}H NMR Data (600 MHz, δ in ppm, J in Hz) and ^{13}C NMR Data (150 MHz, δ in ppm) for 1−4

	1[a]		2[b]		3[b]		4[b]	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	169.1, C		162.9, C		162.9, C		170.1, C	
3	68.3, CH_2	4.72, dd (10.8, 1.5)	77.9, CH	4.38, m	78.8, CH	4.41, m	79.8, CH	4.58, m
		4.43, dd (10.8, 3.0)						
4	44.6, CH	2.62, ddd (7.8, 3.0, 1.5)	34.6, CH_2	2.90, dd (15.6, 11.7)	33.7, CH_2	2.84, dd (16.2, 11.1)	33.0, CH_2	2.95, dd (16.2, 10.2)
				2.83, dd (15.6, 2.4)		2.79, dd (16.2, 3.0)		2.91, dd (16.2, 4.2)
4a	143.4, C		142.1, C		132.2, C		139.5, C	
5	108.3, CH	6.29, d (2.1)	119.3, CH	6.79, d (7.2)	123.1, CH	6.86, d (8.4)	118.1, CH	6.68, d (7.2)
6	164.4, C		134.5, CH	7.44, dd (8.4, 7.2)	120.6, CH	7.12, d (8.4)	136.3, CH	7.40, dd (8.4, 7.2)
7	101.3, CH	6.21, d (2.1)	111.0, CH	6.90, d (8.4)	149.0, C		116.3, CH	6.88, d (8.4)
8	163.2, C		161.3, C		148.6, C		162.3, C	
8a	100.2, C		114.0, C		117.5, C		108.6, C	
9	65.8, CH	3.70, dq (7.8, 6.6)						
10	21.4, CH_3	1.04, d (6.6)						
1′			34.8, CH_2	1.86, 1.69, m	34.8, CH_2	1.85, 1.69, m	34.9, CH_2	1.90, 1.74, m
2′			21.4, CH_2	1.58, m	21.4, CH_2	1.57, m	21.4, CH_2	1.61, m
3′			39.0, CH_2	1.49, m	39.0, CH_2	1.49, m	38.8, CH_2	1.51, m
4′			68.0, CH	3.81, m	68.0, CH	3.82, m	68.0, CH	3.83, m
5′			23.7, CH_3	1.19, d (6.0)	23.7, CH_3	1.19, d (6.6)	23.8, CH_3	1.21, d (6.6)
8-OH		11.08, brs						11.00, s
8-OCH_3			56.3, OCH_3	3.93, s	62.4, OCH_3	3.95, s		

[a]DMSO-d_6. [b]$CDCl_3$.



1 **2** **6** **11**

Figure 1. Key HMBC correlations for compounds **1**, **2**, **6** and **11**.

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1-hydroxyethyl unit was reinforced by acetylation of **1** with Ac_2O–pyridine. The chemical shift for the triacetyl derivative **1a** assigned to the oxymethine proton (δ_H 5.03) was shifted significantly downfield ($\Delta\delta$ 1.33 ppm) relative to the original oxymethine proton (δ_H 3.70) in **1**. Furthermore, in the selective 1D NOE experiment of **1**, the irradiation of H_3-10 resulted in the signal intensity of H-5, which further confirmed the complete planar structure assignment for **1**.

The relative and absolute configurations of **1** were determined by quantum chemical calculation of its ECD spectrum combined with the modified Mosher's method. First, conformational analyses of 4*S*,9*R*-**1** and 4*R*,9*R*-**1** were carried out via Monte Carlo searching with the MMFF94 force field in the SPARTAN 08 software package.[13] The results showed eight lowest energy conformers for 4*S*,9*R*-**1** (4*S*9*R*C1–4*S*9*R*C8; see Supporting Information Figure S93-1) and four for 4*R*,9*R*-**1** (4*R*9*R*C1–4*R*9*R*C4; see Supporting Information Figure S93-2), whose relative energies were within 2 kcal/mol. Subsequently, the conformers were reoptimized using DFT at the B3LYP/6-31G(d) level in the gas phase by the GAUSSIAN 09 program.[14] The B3LYP/6-31G(d) harmonic vibrational frequencies were further calculated to confirm their stability. The energies, oscillator strengths, and rotational strengths of the first 20 electronic excitations were calculated using TDDFT methodology at the B3LYP/6-311++G(2d,2p) level in a vacuum. The ECD spectra were simulated by the overlapping Gaussian function (σ = 0.2 or 0.3 eV)[15] in which the first nine excitations for 4*S*,9*R*-**1** and three excitations for 4*R*,9*R*-**1** were adopted, respectively. To get the final spectra for each compound, the simulated spectra of the six lowest energy conformations were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy (ΔG). The theoretical ECD spectra for 4*R*,9*S*-**1** and 4*S*,9*S*-**1** were obtained by directly reversing the spectra of 4*S*,9*R*-**1** and 4*R*,9*R*-**1**, respectively (Figures 2 and 3). The difference observed in

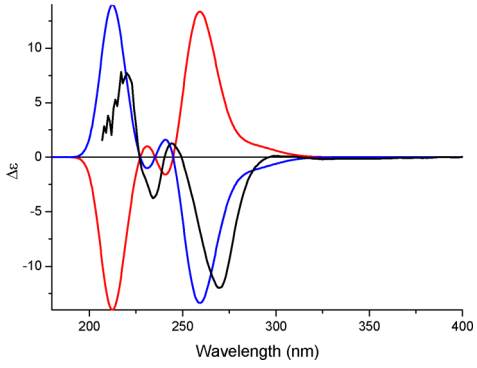

Figure 2. B3LYP/6-311++G(2d,2p)//B3LYP/6-31G(d)-calculated ECD spectra of 4*S*,9*R*-**1** (red) and 4*R*,9*S*-**1** (blue) and the experimental ECD spectrum of **1** (black) (σ = 0.3 eV).

the second Cotton effect around 245 nm for 4*R*,9*R*-**1** and 4*S*,9*S*-**1** (Figure 3) may be attributed to overestimation of the corresponding positive rotatory strengths in the calculations. For compound **1** the experimental first negative (270 nm), second small positive (244 nm), third small negative (234 nm), and fourth positive (220 nm) Cotton effects compared well with the calculated ECD curve for 4*R*,9*S*-**1**, which showed four corresponding Cotton effects around 259, 241, 231, and 212 nm (Figure 2). Therefore, qualitative analysis of the result allowed the assignment of the absolute configuration of **1** as

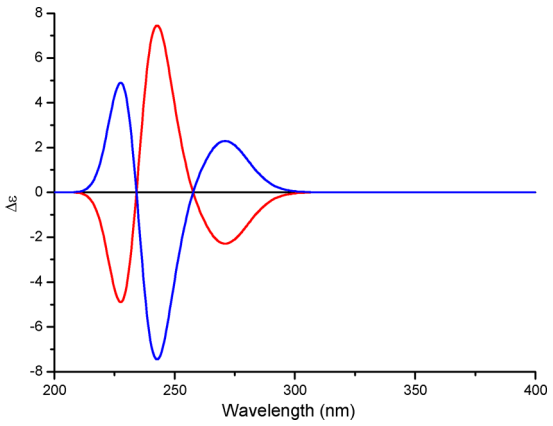

Figure 3. B3LYP/6-311++G(2d,2p)//B3LYP/6-31G(d)-calculated ECD spectra of 4*R*,9*R*-**1** (red) and 4*S*,9*S*-**1** (blue) (σ = 0.2 eV).

4*R*,9*S*-**1**. The absolute configuration at C-9 was further confirmed by the modified Mosher's method.[16] Compound **1** was treated with (*R*)- and (*S*)-MTPA chloride, to afford the (*S*)- and (*R*)-MTPA esters, **1s** and **1r**, respectively. The differences in chemical shift values ($\Delta\delta = \delta_S - \delta_R$) for the diastereomeric esters **1s** and **1r** were calculated in order to assign the absolute configuration at C-9 (Figure 4). Calculations for all of the relevant signals confirmed the *S* absolute configuration at C-9 in **1**.

Penicimarin B (**2**) was obtained as a colorless oil. The molecular formula was determined as $C_{15}H_{20}O_4$ by HRESIMS with six degrees of unsaturation. In the ^{1}H NMR spectrum (Table 1), the proton signals and the coupling constants at δ_H 7.44 (dd, *J* = 8.4, 7.2 Hz), 6.90 (d, *J* = 8.4 Hz), and 6.79 (d, *J* = 7.2 Hz) indicated the presence of a 1,2,3-trisubstituted benzene system. The ^{1}H NMR and ^{13}C NMR spectra included four methylenes, two methines, one methoxy, and one methyl. The ^{13}C NMR spectrum also displayed signals for six aromatic carbons and a carbonyl carbon. These spectroscopic features suggested that **2** was very similar to aspergillumarin B (**4**),[11] with the main differences being the additional methoxy signal (δ_H 3.93, s in **2**) and the absence of a chelated hydroxy proton signal (δ_H 11.01, s in **4**) in the ^{1}H NMR spectra. Thus, it could be deduced that the 8-OH in **4** was replaced by a methoxy in **2**. The 8-OMe was further confirmed by an HMBC correlation from the methoxy to C-8 and the correlation from H-5, one of the *ortho*-coupled aromatic protons, to C-4 (Figure 1). On the basis of these results, the planar structure of **2** was elucidated.

In order to determine the absolute configuration of C-4′ at the side chain, the modified Mosher's method was applied.[16] When reacted with (*R*)- and (*S*)-MTPA chloride, **2** gave the corresponding (*S*)- and (*R*)-MTPA esters, **2s** and **2r**, respectively. The observed chemical shift differences $\Delta\delta_{H(S-R)}$ (Figure 4) clearly defined the *S* configuration at C-4′. The absolute configuration of C-3 was determined by CD spectroscopy. The negative circular dichroism at 259 nm (Figure 5) suggested the *R* configuration at C-3, by comparison with data for dihydroisocoumarins described in the literature.[17] Thus, the absolute configuration of **2** was established as 3*R*, 4′*S*.

Penicimarin C (**3**) was also isolated as a colorless oil. Its molecular formula of $C_{15}H_{20}O_5$ was determined by HRESIMS, thus revealing the addition of one oxygen atom compared with that of **2**. The ^{1}H NMR data (Table 1) resembled those of **2** except for the absence of one aromatic proton signal. The signals at δ_H 7.12 (d, *J* = 8.4 Hz) and 6.86 (d, *J* = 8.4 Hz)

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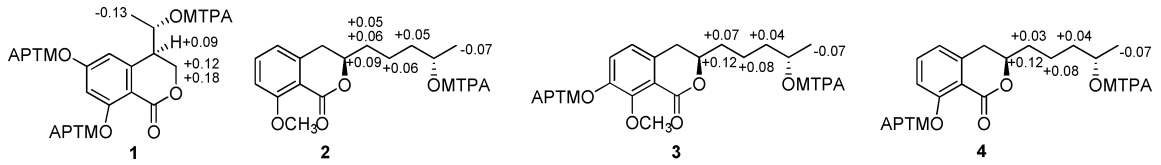

Figure 4. $\Delta\delta$ ($\delta_S - \delta_R$) values (in ppm) for the MTPA esters of **1**–**4**.

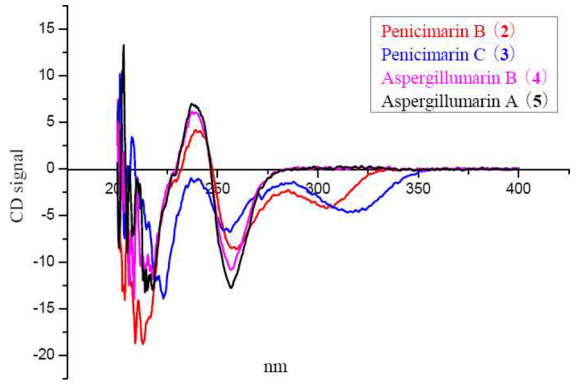

Figure 5. CD spectra of compounds **2**–**5**.

indicated the presence of a tetrasubstituted benzene system in **3**, instead of the trisubstituted benzene in **2**. The ^{13}C NMR spectra revealed that a downfield aromatic quaternary carbon (δ_C 149.0, C-7) in **3** replaced an aromatic methine carbon (δ_C 111.0, C-7) in **2**. The downfield shift suggested the position of the additional hydroxy group was at C-7 in **3**. Additionally, the HMBC spectrum showed a correlation from H-5, one of the two *ortho*-coupled aromatic protons, to C-4, indicating the substituted hydroxy and the methoxy should be located at C-7 and C-8. The HMBC correlation from the methoxy to C-8 and the correlation from H-5 to C-7 confirmed the locations of 8-OMe and 7-OH. The absolute configuration at C-4′, bearing a secondary OH group, was determined as *S* by the modified Mosher's method (Figure 4).[16] The absolute configuration of C-3 was also determined to be *R* by CD (Figure 5).[17]

Penicimarin D (**6**) was isolated as a colorless, amorphous powder, with the molecular formula $C_{19}H_{22}O_{11}$. Analysis of the ^{1}H and ^{13}C NMR spectroscopic data (Table 2) of **6** revealed similarities to those of **9**, except for the presence of a hexose residue, with an anomeric carbon at δ_H 4.78 (d, J = 3.6 Hz)/δ_C

Table 2. ^{1}H NMR Data (600 MHz, δ in ppm, J in Hz) and ^{13}C NMR Data (150 MHz, δ in ppm) for 6–9

	6[a]		7[a]		8[a]		9[a]	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	164.8, C		164.5, C		165.1, C		165.8, C	
3	154.3, C		155.1, C		153.9, C		152.5, C	
4	109.6, C		108.6, C		114.5, C		113.2, C	
4a	139.5, C		138.9, C		139.4, C		139.7, C	
5	101.4, CH	6.59, d (1.8)	100.7, CH	6.44, d (2.4)	102.4, CH	6.72, d (2.1)	101.5, CH	6.63, d (2.1)
6	165.9, C		165.9, C		165.8, C		165.1, C	
7	101.3, CH	6.34, d (1.8)	101.5, CH	6.35, d (2.4)	101.9, CH	6.39, d (2.1)	101.2, CH	6.33, d (2.1)
8	162.9, C		163.1, C		163.0, C		163.0, C	
8a	98.0, C		97.9, C		98.7, C		98.2, C	
9	16.7, CH_3	2.33, s	16.8, CH_3	2.35, s	57.5, CH_2	4.38, s	16.6, CH_3	2.30, s
10	61.7, CH_2	4.59, d (12.3)	58.7, CH_2	5.06, s	55.2, CH_2	4.49, s	55.9, CH_2	4.41, s
		4.46, d (12.3)						
11			170.3, C					
12			20.6, CH_3	2.02, s				
1′	97.8, CH	4.78, d (3.6)						
2′	71.5, CH	3.24, ddd (9.6, 6.0, 3.6)						
3′	72.9, CH	3.35, ddd (9.6, 9.6, 4.8)						
4′	70.3, CH	3.06, ddd (9.6, 9.6, 5.4)						
5′	70.1, CH	3.57, ddd (9.6, 6.9, 1.8)						
6′	63.9, CH_2	4.26, dd (12.0, 1.8)						
		4.00, dd (12.0, 6.9)						
7′	170.4, C							
8′	20.6, CH_3	2.03, s						
6-OH		10.88, brs		10.95, brs		10.95, brs		10.90, brs
8-OH		11.11, s		11.06, brs		11.19, s		11.16, s
9-OH						5.48, brs		
10-OH						5.06, brs		
2′-OH		4.72, d (6.0)						
3′-OH		4.90, d (4.8)						
4′-OH		5.17, d (5.4)						

[a]DMSO-d_6.

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Table 3. ^{1}H NMR Data (600 MHz, δ in ppm, J in Hz) and ^{13}C NMR Data (150 MHz, δ in ppm) for 11−14

	11[a]		12[b]		13[a]		14[a]		7-methoxy-2,3-dimethylbenzofuran-5-ol[c]	
position	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
2	151.8, C		156.1, C		167.8, C		154.0, CH	8.81, s	151.5, C	
3	115.3, C		111.9, C		117.5, C		122.0, C		110.0, C	
3a	130.3, C		131.5, C		126.1, C		125.8, C		132.1, C	
4	96.12, CH	6.48, d (3.3)	96.8, CH	6.48, d (2.1)	96.4, CH	6.81, d (2.1)	97.0, CH	6.90, d (2.1)	97.0, CH	6.39, d (1.8)
5	153.7, C		155.2, C		155.5, C		155.4, C		137.8, C	
6	96.05, CH	6.29, d (3.3)	97.6, CH	6.34, d (2.1)	100.5, CH	6.28, d (2.1)	100.9, CH	6.32, d (2.1)	95.7, CH	6.30, d (1.8)
7	144.4, C		146.4, C		142.3, C		142.8, C		144.8, C	
7a	136.4, C		139.0, C		136.6, C		138.7, C		151.8, C	
8	11.9, CH_3	2.35, s	12.0, CH_3	2.43, s	186.3, CH	10.11, s	193.2, C		11.8, CH_3	2.33, s
9	53.5, CH_2	4.44, d (7.8)	57.7, CH_2	5.14, s	12.7, CH_3	2.72, s	27.9, CH_3	2.47, s	8.0, CH_3	2.04, s
10			172.8, C							
11			20.8, CH_3	2.03, s						
5-OH		9.04, s				9.18, s		9.18, brs		4.92, brs
7-OH						10.11, s		10.15, brs		
7-OCH_3	55.5, OCH_3	3.83, s	56.5, OCH_3	3.91, s					56.0, OCH_3	3.92, s
9-OH		4.84, t (7.8)								

[a]DMSO-d_6. [b]CD_3OD. [c]$CDCl_3$. ^{1}H NMR data (500 MHz, δ in ppm, J in Hz) and ^{13}C NMR data (125 MHz, δ in ppm) for 7-methoxy-2,3-dimethylbenzofuran-5-ol from ref 19.

97.8. Assignment of the relative configuration of the sugar moiety in **6** was accomplished by analyses of coupling constants. The J values of 3.6, 9.6, 9.6, and 9.6 Hz for $J_{H\text{-}1',H\text{-}2'}$, $J_{H\text{-}2',H\text{-}3'}$, $J_{H\text{-}3',H\text{-}4'}$, and $J_{H\text{-}4',H\text{-}5'}$ indicated equatorial, axial, axial, and axial orientations of H-1′, H-2′, H-3′, and H-4′, respectively. Comparison of the NMR data of the sugar unit in **6** with those of the isocoumarin glucoside halorosellin A[18] confirmed the sugar unit is an α-glucopyranose in **6**. The additional signals of an acetyl group (δ_H 2.03, s; δ_C 170.4) in the ^{1}H and ^{13}C NMR spectra, together with the HMBC correlation (Figure 1) from H-6′ to the carbonyl carbon, suggested that the hydroxy group at C-6′ was acetylated. The HMBC correlation from H-10 to C-1′ established the linkage between the α-glucopyranose and the isocoumarin unit.

Penicimarin E (**7**) was isolated as a colorless, amorphous powder with a molecular formula of $C_{13}H_{12}O_6$. Careful comparison of the ^{1}H and ^{13}C NMR spectra of **7** (Table 2) with those of **9** showed a close structural relationship. The obvious differences in the ^{1}H NMR spectra were the presence of a singlet methyl signal at δ_H 2.02 in **7** and the significantly downfield shift of H-10 (δ_H 5.06 in **7** vs 4.41 in **9** in DMSO-d_6). The presence of a carbonyl group (δ_C 170.3) in the ^{13}C NMR spectrum was also observed. These spectroscopic features indicated that the hydroxy group at C-10 was acetylated in **7**. The location of the acetoxy group at C-10 was confirmed by an HMBC correlation from H-10 to the carbonyl carbon. Therefore, **7** was a 10-acetylated derivative of **9**.

Penicimarin F (**8**) was isolated as a colorless, amorphous powder with the molecular formula $C_{11}H_{10}O_6$. The spectroscopic data (Table 2) of **8** also closely corresponded with those of **9** except for the replacement of the methyl signal (δ_H 2.30, s) located at C-3 in **9** with a hydroxymethyl signal (δ_H 4.38, s, 2H and 5.48, brs, 1H) in **8**. The location of the hydroxymethyl at C-3 was confirmed by HMBC correlations from H-9 to C-3 and C-4.

Penicifuran A (**11**) was isolated as a pale brown, semicrystalline solid with the molecular formula $C_{11}H_{12}O_4$ by HREIMS, indicating six degrees of unsaturation. The ^{1}H and ^{13}C NMR spectra (Table 3) were similar to those of 7-methoxy-2,3-dimethylbenzofuran-5-ol obtained from the culture of the fungus *Malbranchea cinnamomea* HKI0286.[19] The only difference between these two compounds was the replacement of the methyl signal located at C-3 in 7-methoxy-2,3-dimethylbenzofuran-5-ol with a hydroxymethyl signal (δ_H 4.44, d, J = 7.8 Hz, 2H and 4.84, t, J = 7.8 Hz, 1H) in **11**. The HMBC spectrum (Figure 1) displayed the correlations from the hydroxy group (δ_H 4.84) to C-3 and C-9 and from H-9 to C-2, C-3, and C-3a, confirming the location of the hydroxymethyl at C-3. In addition, the other hydroxy group (δ_H 9.04) showed correlations with C-4, C-5, and C-6, confirming its location at C-5. This suggested that the assignments for C-5 (δ_C 137.8) and C-7a (δ_C 151.8) of 7-methoxy-2,3-dimethylbenzofuran-5-ol in the literature[19] should be exchanged.

Penicifuran B (**12**), with the molecular formula $C_{13}H_{14}O_5$ (seven degrees of unsaturation) from HREIMS data, was also obtained as a pale brown, semicrystalline solid. The ^{1}H and ^{13}C NMR spectroscopic data (Table 3) were very similar to those of **11**. The differences in the ^{1}H and ^{13}C NMR spectra were the presence of an acetyl group (δ_H 2.03, s; δ_C 20.8/δ_C 172.8) and the downfield shift of H-9. These spectroscopic features indicated that the hydroxy group at C-9 was acetylated in **12**. The location of the acetoxy group at C-9 was confirmed by the HMBC correlation from H-9 to the carbonyl carbon. Thus, **12** was a 9-acetylated derivative of **11**.

Penicifuran C (**13**) was isolated as a pale brown, amorphous powder with the molecular formula assigned as $C_{10}H_8O_4$ by HREIMS. Comparison of the ^{1}H and ^{13}C NMR data (Table 3) of **13** with those of **11** revealed the replacement of a hydroxymethyl (δ_H 4.44, d, J = 7.8 Hz, 2H and 4.84, t, J = 7.8 Hz, 1H; δ_C 53.5) and a methoxy (δ_H 3.83, s, 3H; δ_C 55.5) in

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11 with an aldehyde group (δ_H 10.11, s, 1H; δ_C 186.3) and a hydroxy (δ_H 10.11, s, 1H) in **13**, respectively. The HMBC spectrum displayed correlations from the aldehyde proton to C-3 and C-3a, confirming the position of the aldehyde group at C-3. HMBC correlations from the δ_H 9.18 hydroxy to C-4, C-5, and C-6 and those from the other hydroxy (δ_H 10.11) to C-6, C-7, and C-7a confirmed the positions of the two hydroxy groups at C-5 and C-7, respectively.

Penicifuran D (**14**) was obtained as a pale brown, semicrystalline solid. The molecular formula was determined as $C_{10}H_8O_4$ on the basis of HREIMS data. Comparison of the ^{1}H NMR spectrum of **14** (Table 3) with that of **13** showed similar structures between them. The significant differences in the ^{1}H NMR spectrum of **14** were the presence of an olefinic proton signal for H-2, downfield shifted to δ_H 8.81, and the absence of an exchangeable proton (δ_H 10.11 in **13**). The primary differences in the ^{13}C NMR spectra were that the methyl carbon signal downfield shifted from δ_C 12.7 (C-9) in **13** to δ_C 27.9 (C-9) in **14** and that the ketone carbon (δ_C 193.2) and olefinic methine carbon (δ_C 154.0) in **14** replaced the aldehyde carbon and olefinic quaternary carbon in **13**. These results revealed the aldehyde group located at C-3 in **13** was replaced by an acetyl group in **14** and that the substituent methyl at C-2 was not present. In addition, the HMBC correlations from H_3-9 to C-3 and C-8 implied that the acetyl group was located at C-3.

The known compounds aspergillumarin B (**4**),[11] aspergillumarin A (**5**),[11] sescandelin-B (**9**),[12] and 5,6,8-trihydroxy-4-(1′-hydroxyethyl)isocoumarin (**10**)[12] were determined by comparison of their spectroscopic data with those in the literature. The *R* configuration at C-3 in **4** was determined by comparison of the CD spectrum (Figure 5) with those of **2**, **3**, and reported compounds in the literature.[17] The absolute configuration of C-4′ in **4** was determined as *S* by the modified Mosher's method (Figure 4).[16] Comparison of the CD spectrum (Figure 5) as well as the specific rotation of **5** ($[\alpha]^{25}_D$ −92.1) with those reported for similar compounds[17,20] suggested that C-3 in **5** has the *R* configuration. Additionally, the Dess−Martin oxidation of **4** led to **5**, further confirming the *R* configuration at C-3 in **4**.

The antibacterial activities of all isolated compounds were evaluated with seven terrestrial pathogenic bacteria, *Escherichia coli*, *Staphylococcus aureus*, *S. albus*, *Bacillus subtilis*, *B. cereus*, *Micrococcus tetragenus*, and *Kocuria rhizophila*, and two marine pathogenic bacteria, *Vibrio parahemolyticus* and *V. anguillarum* (Table 4). Compound **10** showed moderate activity against *B. cereus* and *V. parahemolyticus*, with MIC values of 6.25 μM for each. Compound **11** exhibited moderate inhibitory activity against *S. albus*, with an MIC value of 3.13 μM, and weak activity against *B. cereus*. All of the isolated compounds were also evaluated for their cytotoxic activity against human promyelocytic leukemia HL-60, human lung carcinoma A-549, HeLa cell human cervical carcinoma, and chronic leukemia K562 cell lines, but none of them exhibited cytotoxic activity.

Table 4. Antibacterial Activities of Compounds 4, 5, 8, and 10−14[a]

	MIC (μM)			
compound	*S. aureus*	*S. albus*	*B. cereus*	*V. parahemolyticus*
4	>25.0	12.5	>25.0	>25.0
5	>25.0	12.5	>25.0	>25.0
8	12.5	>25.0	>25.0	>25.0
10	12.5	>25.0	6.25	6.25
11	25.0	3.13	12.5	25.0
12	>25.0	12.5	>25.0	>25.0
13	12.5	25.0	12.5	>25.0
14	>25.0	25.0	25.0	25.0
ciprofloxacin[b]	0.160	0.312	0.625	0.160

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

In this paper, 10 new metabolites including three hydroisocoumarins, penicimarins A−C (**1**−**3**), three isocoumarins, penicimarins D−F (**6**−**8**), and four benzofurans, penicifurans A−D (**11**−**14**), were obtained from the sponge-derived fungus *Penicillium* sp. MWZ14-4, collected from the South China Sea. The absolute configurations of **1**−**4** were assigned by the modified Mosher's method and TDDFT ECD calculations together with comparison of their CD spectra. Among the naturally occurring isocoumarin derivatives, a 4-substituted isocoumarin with no substituent at the 3-position such as **1** is very rare.[21−27] During the past decade, only three compounds with such structural features have been reported.[26,27] Interestingly, the co-occurrence of isocoumarins and benzofurans in the same fungal species has also rarely been reported, only from lichen mycobionts and a Canadian thistle endophytic fungus.[28,29] In this paper, the isocoumarin derivatives with six-membered lactones and the benzofurans were simultaneously isolated from a marine-derived *Penicillium* fungus for the first time. All of the isolated isocoumarin derivatives and benzofurans may share a similar biosynthetic pathway from a common open-chain precursor.

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 spectrophotometer. CD spectra were recorded on a Jasco J-810 circular dichroism spectrometer. IR spectra were recorded on a Nicolet-Nexus-470 spectrometer using KBr pellets. ^{1}H NMR, ^{13}C NMR, and DEPT spectra of compounds **1**−**14** and 2D NMR spectra of compounds **1**−**3**, **8**, and **12**−**14** were recorded on a JEOL JNM-ECP NMR spectrometer (600 MHz for ^{1}H and 150 MHz for ^{13}C), using TMS as an internal standard. 2D NMR spectra of compounds **6**, **7**, and **11** were recorded on an Ultrashied 400 MHz Plus spectrometer. The ^{1}H NMR spectra of compounds **1a**, **1s**, and **1r** were recorded on an Agilent DD2 500 MHz NMR spectrometer. ESIMS and HRESIMS spectra were obtained from a Micromass Q-TOF spectrometer and Thermo Scientific LTQ Orbitrap XL spectrometer. EIMS spectra were measured on a Thermo DSQ EI-mass spectrometer, and HREIMS spectra on a Thermo MAT95XP high-resolution mass spectrometer. HPLC separations were performed using a Hitachi L-2000 prep-HPLC system coupled with a Hitachi L-2455 photodiode array detector. A Kromasil C_{18} preparative HPLC column (250 × 10 mm, 5 μm) was used. Silica gel (Qing Dao Hai Yang Chemical Group Co.; 200−300 mesh), Sephadex LH-20 (Amersham Biosciences), and octadecylsilyl silica gel (Unicorn; 45−60 μm) were used for column chromatography. Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co.; G60, F-254) were used for thin-layer chromatography.

Fungal Material. The fungal strain *Penicillium* sp. (MWZ14-4) was isolated from a piece of fresh tissue from the inner part of an unidentified sponge (GX-WZ-20080014), collected from the Weizhou coral reef in the South China Sea in September 2008. The fungus was identified as a *Penicillium* sp. according to morphological traits and a molecular biological protocol by amplification and sequencing of the DNA sequences of the ITS region of the rRNA gene. The 532 base pair ITS sequence had 100% sequence identity to that of *Penicillium* sp. 3TMS-2011 (HQ631007). The sequence data have been submitted

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to GenBank, accession number JN693500. 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.

Fermentation, Extraction, and Isolation. Thirty-five Erlenmeyer flasks of the fungal strain were cultivated in solid medium (Erlenmeyer flasks each containing rice 80 g, water 120 mL, sea salt 2.0 g) at 27 °C for four weeks. The fermented solid medium was extracted three times with EtOAc. The combined EtOAc layers were evaporated to dryness under reduced pressure to give an EtOAc extract. The EtOAc extract (9.0 g) was subjected to vacuum liquid chromatography (VLC) on silica gel using step gradient elution with EtOAc–petroleum ether (0–100%) and then with MeOH–EtOAc (0–100%) to afford eight fractions (Fr. 1–Fr. 8). Fr. 3 was isolated by column chromatography (CC) on silica gel eluted with petroleum ether–EtOAc (v/v, 7:3), then subjected to Sephadex LH-20 CC with petroleum ether–$CHCl_3$–MeOH (v/v/v, 2:1:1), and further purified by using semipreparative HPLC on an ODS column (Kromasil C_{18}, 250 × 10 mm, 5 μm, 2 mL/min) eluted with 70% MeOH–H_2O for **5** (10.0 mg) and 60% MeOH–H_2O for **7** (20.0 mg) and **12** (2.3 mg). Fr. 5 was first subjected to repeated silica gel CC ($CHCl_3$–MeOH, v/v, 15:1), then separated by Sephadex LH-20 CC ($CHCl_3$–MeOH, v/v, 1:1), and further purified on HPLC with 70% MeOH–H_2O for **4** (12.0 mg) and 20% MeOH–H_2O for **13** (3.0 mg). Fr. 6 was applied to CC on silica gel (petroleum ether–EtOAc, v/v, 3:2), then separated by Sephadex LH-20 CC ($CHCl_3$–MeOH, v/v, 1:1), and further purified on HPLC with 30% MeOH–H_2O to obtain **1** (4.0 mg) and **14** (2.0 mg), 65% MeOH–H_2O for **3** (8.0 mg), 40% MeOH–H_2O for **10** (45.0 mg), and 45% MeOH–H_2O for **11** (3.0 mg). Fr. 7 was fractionated on silica gel CC ($CHCl_3$–MeOH, v/v, 10:1), then purified by Sephadex LH-20 CC (MeOH), and further purified on HPLC with 40% MeOH–H_2O to afford **2** (6.0 mg) and **8** (8.0 mg) and 60% MeOH–H_2O for **6** (5.0 mg) and **9** (11.0 mg).

Penicimarin A (1): colorless, amorphous powder; $[\alpha]^{25}_D$ −36 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 219 (3.91), 233 (3.99), 270 (3.93), 303 (3.74) nm; CD (0.92 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 220 (+7.52), 234 (−3.65), 244 (+1.24), 270 (−11.61) nm; IR (KBr) ν_{max} 3204, 1650, 1402, 1243, 1166, 1118, 850, 713 cm^{-1}; ^1H and ^{13}C NMR see Table 1; EIMS *m/z* 224 $[M]^+$; HREIMS *m/z* 224.0680 $[M]^+$ (calcd for $C_{11}H_{12}O_5$, 224.0679).

Penicimarin B (2): colorless oil; $[\alpha]^{25}_D$ −69 (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 221 (3.32), 244 (2.35), 306 (3.27) nm; CD (0.63 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 239 (+2.01), 259 (−4.15), 290 (−1.42), 302 (−1.96) nm; IR (KBr) ν_{max} 3521, 1710, 1598, 1477, 1279, 1250, 1232, 1111, 1088, 1063, 803 cm^{-1}; ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 265.3 $[M + H]^+$, 287.3 $[M + Na]^+$, 303.3 $[M + K]^+$, 551.6 $[2\ M + Na]^+$; HRESIMS *m/z* 265.1433 $[M + H]^+$ (calcd for $C_{15}H_{21}O_4$, 265.1434).

Penicimarin C (3): colorless oil; $[\alpha]^{25}_D$ −84 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 220 (3.39), 250 (3.48), 319 (3.38) nm; CD (0.71 mM, MeOH) λ_{max} ($\Delta\varepsilon$) 235 (−0.85), 256 (−2.88), 283 (−0.73), 317 (−1.98) nm; IR (KBr) ν_{max} 3354, 1708, 1489, 1430, 1373, 1295, 1128, 1053, 894, 802 cm^{-1}; ^1H and ^{13}C NMR see Table 1; ESIMS *m/z* 281.3 $[M + H]^+$, 303.3 $[M + Na]^+$, 319.3 $[M + K]^+$, 583.6 $[2\ M + Na]^+$; HRESIMS *m/z* 281.1382 $[M + H]^+$ (calcd for $C_{15}H_{21}O_5$, 281.1384).

Penicimarin D (6): colorless, amorphous powder; $[\alpha]^{25}_D$ +69 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 248 (4.07), 279 (3.60), 327 (3.57) nm; IR (KBr) ν_{max} 3367, 1732, 1680, 1626, 1370, 1252, 1176, 1037, 846, 713 cm^{-1}; ^1H and ^{13}C NMR see Table 2; ESIMS *m/z* 427.2 $[M + H]^+$, 449.2 $[M + Na]^+$, 465.2 $[M + K]^+$, 853.5 $[2\ M + H]^+$, 875.5 $[2\ M + Na]^+$; HRESIMS *m/z* 427.1229 $[M + H]^+$ (calcd for $C_{19}H_{23}O_{11}$, 427.1235).

Penicimarin E (7): colorless, amorphous powder; UV (MeOH) λ_{max} (log ε) 246 (4.37), 325 (3.83) nm; IR (KBr) ν_{max} 3197, 1681, 1618, 1372, 1266, 1166, 1032, 847, 740 cm^{-1}; ^1H and ^{13}C NMR see Table 2; EIMS *m/z* 264 $[M]^+$; HREIMS *m/z* 264.0625 $[M]^+$ (calcd for $C_{13}H_{12}O_6$, 264.0628).

Penicimarin F (8): colorless, amorphous powder; UV (MeOH) λ_{max} (log ε) 249 (3.73), 329 (3.17) nm; IR (KBr) ν_{max} 3444, 3258, 1681, 1621, 1484, 1378, 1243, 1184, 1004, 853, 722 cm^{-1}; ^1H and ^{13}C NMR see Table 2; EIMS *m/z* 238 $[M]^+$; HREIMS *m/z* 238.0476 $[M]^+$ (calcd for $C_{11}H_{10}O_6$, 238.0472).

Penicifuran A (11): pale brown, semicrystalline solid; mp 118–119 °C; UV (MeOH) λ_{max} (log ε) 218 (3.74), 250 (3.63), 290 (3.31) nm; IR (KBr) ν_{max} 3398, 3182, 1632, 1601, 1438, 1386, 1276, 1216, 1152, 1000, 970, 812 cm^{-1}; ^1H and ^{13}C NMR see Table 3; EIMS *m/z* 208 $[M]^+$; HREIMS *m/z* 208.0726 $[M]^+$ (calcd for $C_{11}H_{12}O_4$, 208.0730).

Penicifuran B (12): pale brown, semicrystalline solid; mp 103–104 °C; UV (MeOH) λ_{max} (log ε) 223 (3.94), 250 (3.88), 294 (3.47) nm; IR (KBr) ν_{max} 3321, 1710, 1603, 1436, 1384, 1237, 1151, 1022, 972, 836 cm^{-1}; ^1H and ^{13}C NMR see Table 3; EIMS *m/z* 250 $[M]^+$; HREIMS *m/z* 250.0835 $[M]^+$ (calcd for $C_{13}H_{14}O_5$, 250.0836).

Penicifuran C (13): pale brown, amorphous powder; UV (MeOH) λ_{max} (log ε) 218 (3.41), 240 (3.28), 272 (2.97) nm; IR (KBr) ν_{max} 3357, 1668, 1504, 1384, 1314, 1180, 1141, 835 cm^{-1}; ^1H and ^{13}C NMR see Table 3; EIMS *m/z* 192 $[M]^+$; HREIMS *m/z* 192.0415 $[M]^+$ (calcd for $C_{10}H_8O_4$, 192.0417).

Penicifuran D (14): pale brown, semicrystalline solid; mp 190–192 °C; UV (MeOH) λ_{max} (log ε) 217 (3.23), 236 (3.12), 264 (3.31), 305 (2.89) nm; IR (KBr) ν_{max} 3299, 1650, 1506, 1358, 1279, 1153, 841 cm^{-1}; ^1H and ^{13}C NMR see Table 3; EIMS *m/z* 192 $[M]^+$; HREIMS *m/z* 192.0414 $[M]^+$ (calcd for $C_{10}H_8O_4$, 192.0417).

Preparation of the (*R*)- and (*S*)-MTPA Esters of 1–4. Compound **1** (1.0 mg) was dissolved in 500 μL of pyridine, and dimethylaminopyridine (2.0 mg) and (*R*)-MTPACl (8 μL) were then added in sequence. The reaction mixture was stirred for 14 h at room temperature (rt), and 1 mL of H_2O was then added. The solution was extracted with 5 mL of CH_2Cl_2, and the organic phase was concentrated under reduced pressure. Then the residue was purified by semipreparative HPLC (90% MeOH–H_2O) to yield the (*S*)-MTPA ester **1s** (0.9 mg). By the same procedure, the (*R*)-MTPA ester **1r** (0.8 mg) was obtained from the reaction of **1** (1 mg) with (*S*)-MTPACl (8 μL).

By the same procedure as for the preparation of the (*S*)- and (*R*)-MTPA esters of **1**, (*S*)-MTPA esters **2s**, **3s**, **4s** and (*R*)-MTPA esters **2r**, **3r**, **4r** were obtained.

(S)-MTPA ester (1s): ^1H NMR (CD_3OD, 500 MHz) δ 7.71–6.88 (17H, m, aromatic protons), 5.51 (1H, m, H-9), 4.63 (1H, dd, *J* = 12.0, 3.5 Hz, H-3a), 4.59 (1H, dd, *J* = 12.0, 2.5 Hz, H-3b), 3.74 (3H, s, OCH_3-MTPA), 3.68 (3H, s, OCH_3-MTPA), 3.44 (1H, m, H-4), 3.37 (3H, s, OCH_3-MTPA), 1.35 (3H, d, *J* = 6.5 Hz, H-10); ESIMS *m/z* 895.2 $[M + Na]^+$, 911.2 $[M + K]^+$. (*R*)-MTPA ester (**1r**): ^1H NMR (CD_3OD, 500 MHz) δ 7.73–6.84 (17H, m, aromatic protons), 5.44 (1H, m, H-9), 4.51 (1H, dd, *J* = 12.0, 3.5 Hz, H-3a), 4.41 (1H, dd, *J* = 12.0, 2.5 Hz, H-3b), 3.71 (3H, s, OCH_3-MTPA), 3.70 (3H, s, OCH_3-MTPA), 3.55 (3H, s, OCH_3-MTPA), 3.35 (1H, m, H-4), 1.48 (3H, d, *J* = 6.0 Hz, H-10); ESIMS *m/z* 895.2 $[M + Na]^+$, 911.2 $[M + K]^+$.

(S)-MTPA ester (2s): ^1H NMR ($CDCl_3$, 600 MHz) δ 6.92 (1H, d, *J* = 8.4 Hz, H-7), 6.78 (1H, d, *J* = 7.8 Hz, H-5), 5.15 (1H, m, H-4′), 4.34 (1H, m, H-3), 3.95 (3H, s, 8-OCH_3), 3.54 (3H, s, OCH_3-MTPA), 2.89 (1H, dd, *J* = 16.2, 12.0 Hz, H-4a), 2.78 (1H, dd, *J* = 16.2, 2.4 Hz, H-4b), 1.84 (1H, m, H-1′a) and 1.75 (1H, m, H-1′b), 1.63 (2H, m, H-2′), 1.52 (2H, m, H-3′), 1.28 (3H, d, *J* = 6.0 Hz, H-5′); ESIMS *m/z* 481.0 $[M + H]^+$, 502.9 $[M + Na]^+$, 519.0 $[M + K]^+$, 982.9 $[2\ M + Na]^+$. (*R*)-MTPA ester (**2r**): ^1H NMR ($CDCl_3$, 600 MHz) δ 6.92 (1H, d, *J* = 9.0 Hz, H-7), 6.78 (1H, d, *J* = 7.2 Hz, H-7), 5.15 (1H, m, H-4′), 4.25 (1H, m, H-3), 3.95 (3H, s, 8-OCH_3), 3.57 (3H, s, OCH_3-MTPA), 2.87 (1H, dd, *J* = 15.6, 11.4 Hz, H-4a), 2.81 (1H, dd, *J* = 15.6, 3.9 Hz, H-4b), 1.79 (1H, m, H-1′a) and 1.69 (1H, m, H-1′b), 1.57 (2H, m, H-2′), 1.47 (2H, m, H-3′), 1.35 (3H, d, *J* = 6.0 Hz, H-5′); ESIMS *m/z* 480.9 $[M + H]^+$, 502.9 $[M + Na]^+$, 518.9 $[M + K]^+$, 982.9 $[2\ M + Na]^+$.

(S)-MTPA ester (3s): ^1H NMR ($CDCl_3$, 600 MHz) δ 7.20 (1H, d, *J* = 7.8 Hz, H-6), 6.99 (1H, d, *J* = 7.8 Hz, H-5), 5.16 (1H, m, H-4′), 4.43 (1H, m, H-3), 3.81 (3H, s, 8-OCH_3), 3.73 (3H, s, OCH_3-MTPA), 3.54 (3H, s, OCH_3-MTPA), 2.92 (1H, dd, *J* = 16.2, 12.0 Hz, H-4a), 2.83 (1H, dd, *J* = 16.2, 3.0 Hz, H-4b), 1.87 (1H, m, H-1′a) and 1.76 (1H, m, H-1′b), 1.69 (2H, m, H-2′), 1.52 (2H, m, H-3′), 1.29 (3H, d, *J* = 6.0 Hz, H-5′); ESIMS *m/z* 712.9 $[M + H]^+$, 734.8 $[M + Na]^+$, 750.9

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$[M + K]^+$. (*R*)-MTPA ester (**3r**): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.24 (1H, d, J = 7.8 Hz, H-6), 7.00 (1H, d, J = 7.8 Hz, H-5), 5.16 (1H, m, H-4′), 4.31 (1H, m, H-3), 3.75 (3H, s, 8-OCH_3), 3.72 (3H, s, OCH_3-MTPA), 3.57 (3H, s, OCH_3-MTPA), 2.84 (1H, dd, J = 16.2, 11.1 Hz, H-4a), 2.77 (1H, dd, J = 16.2, 3.0 Hz, H-4b), 1.80 (1H, m, H-1′a) and 1.69 (1H, m, H-1′b), 1.61 (2H, m, H-2′), 1.48 (2H, m, H-3′), 1.36 (3H, d, J = 6.0 Hz, H-5′); ESIMS m/z 712.9 $[M + H]^+$, 734.9 $[M + Na]^+$.

*(S)-MTPA ester (**4s**):* ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.19 (1H, d, J = 7.8 Hz, H-7), 6.97 (1H, d, J = 7.8 Hz, H-5), 5.16 (1H, m, H-4′), 4.48 (1H, m, H-3), 3.75 (3H, s, OCH_3-MTPA), 3.53 (3H, s, OCH_3-MTPA), 2.96 (1H, dd, J = 16.2, 12.0 Hz, H-4a), 2.88 (1H, dd, J = 16.2, 3.0 Hz, H-4b), 1.85 (1H, m, H-1′a) and 1.75 (1H, m, H-1′b), 1.66 (2H, m, H-2′), 1.49 (2H, m, H-3′), 1.28 (3H, d, J = 6.6 Hz, H-5′); ESIMS m/z 704.8 $[M + Na]^+$. (*R*)-MTPA ester (**4r**): ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.17 (1H, d, J = 8.1 Hz, H-7), 6.98 (1H, d, J = 8.1 Hz, H-5), 5.15 (1H, m, H-4′), 4.36 (1H, m, H-3), 3.77 (3H, s, OCH_3-MTPA), 3.57 (3H, s, OCH_3-MTPA), 2.90 (1H, dd, J = 16.2, 12.0 Hz, H-4a), 2.82 (1H, dd, J = 16.2, 3.0 Hz, H-4b), 1.77 (2H, m, H-1′), 1.58 (2H, m, H-2′), 1.45 (2H, m, H-3′), 1.35 (3H, d, J = 6.0 Hz, H-5′); ESIMS m/z 704.8 $[M + Na]^+$.

Acetylation of Compound 1. Compound **1** (1.0 mg) was dissolved in 1.0 mL of pyridine, and 3.0 mL of acetic anhydride was then added. The solution was allowed to react by stirring for 14 h at rt. The solvent and excess reagents were removed with a high-vacuum pump, and the crude mixture was subjected to semipreparative HPLC (45% MeOH−H_2O) to obtain the acetyl derivative **1a** (1.0 mg): ^{1}H NMR of **1a** (DMSO-d_6, 500 MHz) δ 7.27 (1H, d, J = 2.0 Hz, H-7), 7.13 (1H, d, J = 2.0 Hz, H-5), 5.03 (1H, m, H-9), 4.60 (1H, dd, J = 12.0, 0.5 Hz, H-3a), 4.49 (1H, dd, J = 12.0, 3.5 Hz, H-3b), 2.89 (1H, m, H-4), 2.31 (3H, s, 7-$OCOCH_3$), 2.26 (3H, s, 5-$OCOCH_3$), 1.90 (3H, s, 9-$OCOCH_3$), 1.21 (3H, d, J = 6.5 Hz, H-10); ESIMS m/z 351.2 $[M + H]^+$, 373.1 $[M + Na]^+$, 389.1 $[M + K]^+$.

Conversion of 4 to 5. Dess−Martin periodinane (6.5 mg) was added to a solution of **4** (2.0 mg) in dry CH_2Cl_2 (2 mL). The reaction mixture was stirred for 10 h at rt. Purification by preparative TLC (silica gel) using 30% EtOAc in petroleum ether as eluent gave **5** (1.2 mg) with $[\alpha]^{25}_D$ −90 (c 0.06, $CHCl_3$) [lit. $[\alpha]^{20}_D$ −10.3 (c 0.27, $CHCl_3$)].[11]

Biological Assays. Antibacterial activities were evaluated by the conventional broth dilution assay.[30] Nine bacterial strains, *E. coli* (ATCC 25922), *S. aureus* (ATCC 27154), *S. albus* (ATCC 8799), *B. subtilis* (ATCC 6633), *B. cereus* (ACCC 11077), *M. tetragenus* (ATCC 13623), *K. rhizophila* (ATCC 9341), *V. parahemolyticus* (ATCC 17802), and *V. anguillarum* (ATCC 19019), were used, and ciprofloxacin was used as a positive control. Cytotoxic activity was evaluated by the MTT method as described previously.[31] Four human cancer cell lines, human promyelocytic leukemia HL-60, human lung carcinoma A-549, human cervical carcinoma HeLa, and chronic leukemia K562, were used. Adriamycin was used as a positive control.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, HMQC, HMBC, and MS spectra of the new compounds **1**−**3**, **6**−**8**, and **11**−**14** and COSY spectra of **1**−**3** and **6**; ^{1}H, MS spectra of the (*R*)- and (*S*)-MTPA esters of **1**−**4**, ^{1}H, MS spectra of the triacetyl derivative **1a** of **1**, and computational calculation details and the original data for each conformer of **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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