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Rhizovarins A–F, Indole-Diterpenes from the Mangrove-Derived Endophytic Fungus *Mucor irregularis* QEN-189

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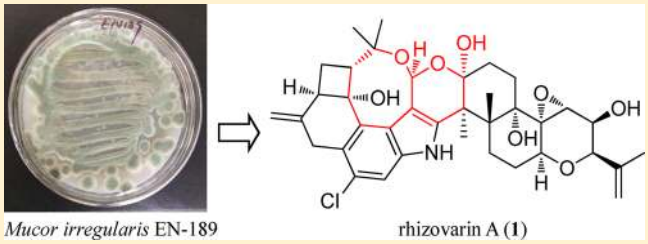
Supporting Information

ABSTRACT: Genome mining of the fungus *Mucor irregularis* (formerly known as *Rhizomucor variabilis*) revealed the presence of various gene clusters for secondary metabolite biosynthesis, including several terpene-based clusters. Investigation into the chemical diversity of *M. irregularis* QEN-189, an endophytic fungus isolated from the fresh inner tissue of the marine mangrove plant *Rhizophora stylosa*, resulted in the discovery of 20 structurally diverse indole-diterpenes including six new compounds, namely, rhizovarins A–F (**1**–**6**). Among them, compounds **1**–**3** represent the most complex members of the reported indole-diterpenes. The presence of an unusual acetal linked to a hemiketal (**1**) or a ketal (**2** and **3**) in an unprecedented 4,6,6,8,5,6,6,6,6-fused indole-diterpene ring system makes them chemically unique. Their structures and absolute configurations were elucidated by spectroscopic analysis, modified Mosher's method, and chemical calculations. Each of the isolated compounds was evaluated for antitumor activity against HL-60 and A-549 cell lines.



Cancer is considered one of the deadliest diseases in the medical field, and chemotherapy is still one of the main treatments used to combat it. Over the past few decades, there have been major advances in this field, and many antitumor compounds are available on the market, a great number of which are natural products or their derivatives, mainly produced by microorganisms.[1] Recently, marine-derived fungi have proven to be a prolific source of structurally unique and biologically active natural products,[2−4] and the majority of them are identified from the fungal genera *Aspergillus*, *Penicillium*, and *Talaromyces*.[5] However, a problem inherent in screening these well-investigated organisms is the high rediscovery rate of known compounds and scaffolds.[6] In addition to those well-studied fungi, neglected fungal species, the secondary metabolic potential of which has been poorly studied, might be an alternative source for the discovery of new bioactive compounds.

During our ongoing search for structurally unique and biologically active compounds from marine-derived fungi,[7−9] a mangrove-derived endophytic fungus, *Mucor irregularis* (formerly known as *Rhizomucor variabilis*) QEN-189, was obtained from the fresh inner tissue of the marine mangrove plant *Rhizophora stylosa*. It is likely that only one paper describing the chemical constituents of this fungus has been published so far, and two cyclic heptapeptides, unguisins E and F, were characterized from this fungal species.[10] Genome analysis of the strain *R. variabilis* B7584 has shown various unidentified biosynthesis gene clusters, including several terpene-based clusters, suggesting that this fungal strain has the potential to produce various secondary metabolites. Chemical investigation of *M. irregularis* QEN-189 resulted in the isolation of 20 indole-diterpenes (Scheme S1, Supporting Information), including six new compounds, namely, rhizovarins A–F (**1**–**6**), with compounds **1**–**3** possessing unprecedented scaffolds. Indole-diterpenes are a group of structurally interesting mycotoxins that have mainly been characterized from *Aspergillus* and *Penicillium* species,[11−15] have been reported to possess significant anti-insectan activity,[13,14] and have attracted great interest from synthetic chemists as well.[16] Recent research shows they are novel inhibitors of the Wnt/β-catenin pathway in breast cancer cells.[17]

This paper describes the details of fungal isolation, genome mining of the biosynthetic clusters, and isolation and structure

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elucidation of the novel indole-diterpenes, as well as their inhibitory activity against the HL-60 and A549 tumor cell lines. The possible biosynthetic pathway of **1** is also discussed.

RESULTS AND DISCUSSION

The marine mangrove plant *R. stylosa* was collected from Hainan Island, China. Following surface sterilization with 70% EtOH, the stems were rinsed with sterile water. To distinguish the remaining epiphytic fungi from endophytic fungi, an imprint from the surface of the stem on potato dextrose agar (PDA) was performed. Small tissue samples from the inside stems were cut aseptically and pressed onto PDA agar plates containing an antibiotic to suppress the growth of bacteria. After incubation at room temperature for 2 to 3 days, the fungal strain under investigation was found to grow exclusively out of the stem tissue, but not on the agar plates taken from the imprint of the stem surface. The pure strain was then obtained by repeated reinoculation onto PDA agar plates and identified as *M. irregularis* by DNA amplification and sequencing of the ITS region, as described in our previous report.[18]

The genome sequence of the strain *R. variabilis* B7584 (NCBI BioProject ID 211914) became publicly available in 2014, and we noticed that its genome harbors various secondary metabolite biosynthesis clusters, including at least four terpene-related biosynthesis gene clusters (Table S1 in the Supporting Information), implying the potential of this fungus to produce terpene-related compounds.[19] Given the presence of secondary metabolite clusters in the strain *R. variabilis* B7584, we argued that other strains of this fungus are likely to possess these genes. HPLC analysis of the culture extracts from the fungal strain *M. irregularis* QEN-189 under various fermentation conditions revealed its remarkable capacity to produce secondary metabolites. To fully understand the chemical diversity of natural products from this rarely studied species, a scale-up fermentation (30 L) in a modified Czapek medium was performed. The mycelia and culture broth were separated by filtration and exhaustively extracted with MeOH and EtOAc, respectively. Because the HPLC and TLC profiles of the two extracts were nearly identical, they were combined. The combined extracts were further purified by a combination of column chromatography on silica gel, Sephadex LH-20, and Lobar LiChroprep RP-18 as well as by semipreparative HPLC, and, as a result, 20 indole-diterpenes were isolated and identified (Chart 1). The structures and absolute configurations of the six new indole-diterpenes, rhizovarins A–F (**1**–**6**), were established by spectroscopic analysis, Mosher's method, and electronic circular dichroism (ECD) calculations.

Rhizovarin A (**1**) was found to have the molecular formula $C_{37}H_{44}ClNO_8$ on the basis of positive HRESIMS data. Its IR spectrum showed absorption bands for OH (3440 cm^{-1}), C=C (1633 cm^{-1}), and aromatic (1454, 1319, 934, and 842 cm^{-1}) functionalities. The ^1H NMR spectrum (Table 1) along with the HSQC data revealed resonances for five singlet methyl groups (H-34–H-36, H-39, and H-40), five aromatic or olefinic protons (H-7, H-33a, H-33b, H-38a, and H-38b), and five oxymethine protons (H-18, H-24–H-26, and H-28), as well as four exchangable protons (15-OH, 19-OH, 22-OH, and 25-OH). The ^{13}C NMR data along with the DEPT spectrum revealed the presence of 37 carbon atoms including 16 nonprotonated carbons (with five oxygenated, seven aromatic, and two olefinic), eight methines (with one aromatic and five oxygenated), eight methylenes (with two olefinic), and five methyls. Detailed analysis of the 1D and 2D NMR data of **1** revealed the presence of two fragments including a monoterpene unit featuring a bicyclo[4.2.0]octane skeleton (rings A and B) and an indole unit (rings C and E) as shown in Figure 1. The rings B and C are determined to be *ortho*-fused at C-4 and C-5, as supported by the observed HMBC correlations from H-10 to C-4, C-5, and C-6 (Figure 2). Thus, an indole nucleus connected to a monoterpene unit was established (rings A–C and E), with 19 carbon atoms remaining unassigned. A template-based search for similar natural products featuring a 4,6,6,5-fused ring system (rings A, B, C, and E) resulted in a large number of hits, most of which are indole-diterpenes including penitrems A–F, which were coisolated with **1**–**6**.[14,15] Further comparison with literature reports suggested that compound **1** was an indole-diterpene derivative related to penitrem A (**9**), which was isolated from the culture of *Penicillium crustosum*.[14,15] However, the eight-membered cyclic ether motif in **1** was evidenced to couple with a 3,6-dihydro-2*H*-pyran unit, which differed from that of penitrem A (**9**). This was supported by the fact that the two methine carbons C-18 (δ_C 72.4, CH) and C-19 (δ_C 58.8, CH) in the cyclopentene unit of penitrem A[13] were replaced by the deshielded carbons at δ_C 92.2 (CH, C-18) and 100.8 (C, C-19), respectively, in the ^{13}C NMR spectrum of **1** (Table 1). The above evidence, together with the observation of HMBC correlations from H-18 to C-2 and C-16 and from 19-OH proton to C-19, C-20, and C-32 (Figure 1) as well as with the consideration of the number of oxygen atoms presented in the molecular formula, resulted in the conclusion that C-18 was linked to C-19 via an oxygen atom and an additional hydroxy group substituted at C-19, which resulted in the formation of an acetal directly linked to a hemiketal unit in **1**. Compound **1** was thus identified as a new indole-diterpene with a novel scaffold.

The NOE correlations from the proton of 15-OH to H-12 and H-13α, from H_3-34 to H-13α and H-18, from the 19-OH proton to H-18 and H_3-40, from H-30α to H-28 and H_3-40, and from H-28 to the 22-OH proton, H-25, and H-26 (Figure 1b, blue arrows) indicated the cofacial orientation of these hydrogens. On the other hand, NOE correlations from H-35 to H-13β and H-14, from H-39 to H-21β, H-24, and H-30β, and from H-24 to H-21β (Figure 1b, red arrows) placed these protons on the opposite face. The energy-minimized conformer (Figure 2a) of **1** was generated by the Dreiding force field in MarvinSketch and further optimized using density functional theory (DFT) at the gas-phase B3LYP/6-31G(d) level via Gaussian 09 software,[20] which matched well with the above NOE data. In order to confirm the complicated structure and relative configuration of **1**, the energy-minimized conformer (Figure 2a) was subjected to ^{13}C NMR calculations using the gauge-independent atomic orbital (GIAO) method at the gas-phase B3LYP/6-31+G(d,p) level with tetramethylsilane as a reference.[21] The calculated ^{13}C NMR data, with deviations ranging from −5.4 to 8.0 ppm (mean absolute deviation 2.8 ppm), were in good agreement with the experimental data (Figure 2b), which further supported the structure and relative configuration of **1**. To determine the absolute configuration of **1**, the ECD spectrum was experimentally recorded, and it showed a strong negative Cotton effect at 237 nm (Figure 2c). The theoretical ECD was then calculated,[22,23] and the calculated curve matched well with the experimental one (Figure 2c), which indicated the absolute configurations of **1** were 12*R*, 14*S*, 15*R*, 18*S*, 19*S*, 22*S*, 23*S*, 24*R*, 25*S*, 26*R*, 28*S*, 31*R*, and 32*S*. This result was further confirmed by the modified Mosher's method.[24] Acylation of **1** with *R*-(−) and *S*-(+)-α-methoxy-α-(trifluoromethyl)phenyl acetyl chloride (MTPA-Cl) furnished 25-MTPA esters **1s** and **1r**, respectively.

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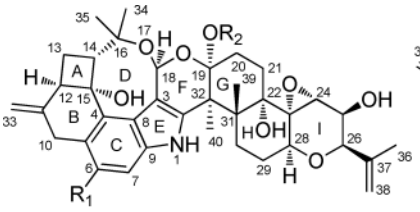
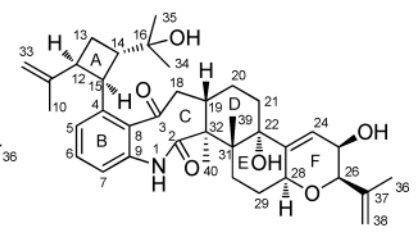
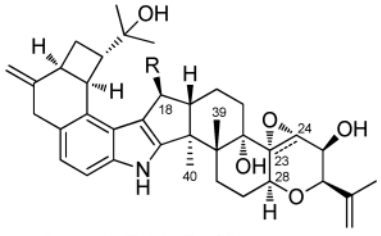
Chart 1



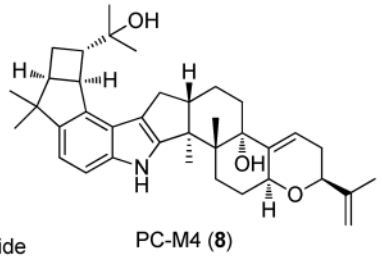
Rhizovarin A (**1**): R_1=Cl, R_2=H
Rhizovarin B (**2**): R_1=Cl, R_2=CH_3
Rhizovarin C (**3**): R_1=H, R_2=CH_3



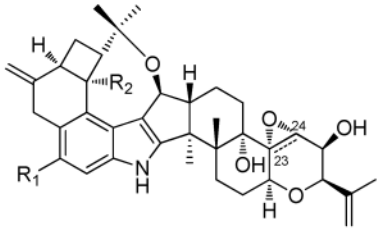
Rhizovarin D (**4**)

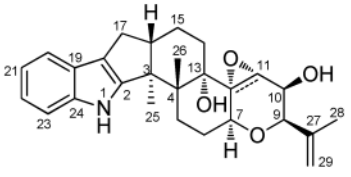


Rhizovarin E (**5**): R=OMe, 23α,24α-epoxide
Secopentrem D (**7**), R=H, 23,24-alkene



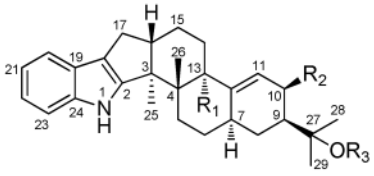
PC-M4 (**8**)





Rhizovarin F (**6**): 11α,12α-epoxide
Penijanthine A (**15**): 11,12-alkene

Penitrem A (**9**): R_1=Cl, R_2=OH, 23α,24α-epoxide
Penitrem B (**10**): R_1=R_2=H, 23α,24α-epoxide
Penitrem C (**11**): R_1=Cl, R_2=H, 23,24-alkene
Penitrem D (**12**): R_1=R_2=H, 23,24-alkene
Penitrem E (**13**): R_1=H, R_2=OH, 23α,24α-epoxide
Penitrem F (**14**): R_1=Cl, R_2=H, 23α,24α-epoxide



Paxilline (**16**): R_1=OH, R_2=ketone, R_3=H
27-*O*-acetylpaxilline (**17**): R_1=OH, R_2=ketone, R_3=Ac
13-Deoxy-27-*O*-acetylpaxilline (**18**): R_1=H, R_2=ketone, R_3=Ac
10-Deoxo-13-deoxypaxilline (**19**): R_1=H, R_2=H, R_3=H
10β-Hydroxy-13-desoxypaxilline (**20**): R_1=H, R_2=OH, R_3=H

The ^{1}H NMR signals of the two MTPA esters were assigned on the basis of their COSY spectra, and the $\Delta\delta_H(S-R)$ values were then calculated (Figure 2d). The results indicated that the absolute configuration of C-25 was S. Therefore, the absolute configurations of **1** were the same as those deduced from the ECD experiment and calculation.

Rhizovarin B (**2**) was assigned the molecular formula $C_{38}H_{46}ClNO_8$, having one CH_2 unit more than that of **1**, on the basis of HREIMS data. The ^{1}H and ^{13}C NMR data of **2** matched well with those for **1** and revealed the same structural features present in **1** except for the presence of the C-19 methoxy group, which is consistent with the difference in the molecular formula. Accordingly, the signal for the exchangeable proton of 19-OH at δ_H 4.74 in **1** was missing in the ^{1}H NMR spectrum of **2**. Instead, signals for an additional methoxy group at δ_H 3.26 (19-OCH_3) and δ_C 48.4 (19-OCH_3) were observed in the NMR spectra of **2**. These observations coupled with the MS data indicated that the hydroxy group at C-19 in **1** was replaced by a methoxy group in **2**. The location of the methoxy group at C-19 was further confirmed by the observed ^3J-HMBC correlation from the methoxy protons to C-19. The relative configuration and the absolute configuration for the stereogenic centers of **2** were determined to be the same as those of **1** by NOESY experiment and by the modified Mosher's method (Figure 2).

The molecular formula of rhizovarin C (**3**) was determined to be $C_{38}H_{47}NO_8$ by HREIMS data, indicating that the Cl atom in **2** was replaced by a H atom in **3**. The ^{1}H and ^{13}C NMR data of **3** were virtually identical to those observed for **2**, with the main differences being the proton and carbon signals at C-6.

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Table 1. NMR Data for Compounds 1 and 3−5 (500 MHz for ^{1}H, 125 MHz for ^{13}C)[a]

	1[b]		3[b]		4[c]		5[b]	
no.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1		10.07, s		9.83, s				9.83, s
2	140.1, C		138.7, C		174.7, C		153.1, C	
3	111.5, C		111.1, C		208.1, C		117.3, C	
4	125.5, C		128.2, C		138.0, C		133.3, C	
5	125.6, C		131.4, C		127.3, CH	7.27, brs	130.3, C	
6	133.6, C		121.2, CH	7.15, d (7.6)	129.1, CH	7.27, brs	122.5, CH	6.85, d (7.5)
7	111.6, CH	7.32, s	111.2, CH	6.74, d (7.6)	123.3, CH	6.83, dd (7.5, 2.2)	110.1, CH	7.07, d (7.5)
8	122.4, C		123.2, C		137.0, C		124.6, C	
9	136.6, C		136.7, C		133.7, C		141.4, C	
10	35.5, CH_2	3.70, d (15.8)	38.7, CH_2	3.55, d (15.9)	23.2, CH_3	1.48, s	38.7, CH_2	3.98, d (15.7)
		3.29, d (15.8)		3.29, d (15.9)				3.41, d (15.7)
11	148.6, C		150.0, C		144.9, C		151.4, C	
12	47.8, CH	2.94, m	48.4, CH	2.97, m	43.0, CH	2.93, m	38.0, CH	3.11, brt (9.0)
13	24.1, CH_2	α 2.39, m	24.1, CH_2	α 2.42, m	22.1, CH_2	2.08, m	26.7, CH_2	2.36, m
		β 2.18, m		β 2.18, m		1.94, m		1.73, m
14	54.5, CH	2.45, m	54.4, CH	2.42, m	48.3, CH	2.65, m	54.9, CH	2.44, m
15	81.5, C		81.7, C		40.1, CH	3.41, m	39.1, CH	4.12, t (8.5)
16	75.2, C		75.2, C		70.9, C		70.1, C	
18	92.2, CH	6.68, s	92.4, CH	6.61, s	49.8, CH_2	2.98, m	85.0, CH	4.60, d (8.4)
						2.41, m		
19	100.8, C		103.7, C		35.7, CH	2.80, m	57.9, CH	2.78, m
20	31.9, CH_2	2.29, m	26.1, CH_2	2.05, m	26.4, CH_2	1.93, m	21.0, CH_2	2.14, m
		1.79, m		2.01, m		1.72, m		1.64, m
21	24.9, CH_2	β 1.82, m	24.8, CH_2	β 1.79, m	32.0, CH_2	1.73, m	28.9, CH_2	2.05, m
		α 1.39, m		α 1.47, m		1.48, m		1.74, m
22	77.8, C		77.6, C		77.0, C		77.9, C	
23	66.3, C		66.3, C		148.1, C		66.3, C	
24	62.1, CH	3.54, d (1.8)	62.2, CH	3.56, brs	119.9, CH	5.72, d (4.6)	61.9, CH	3.57, brs
25	66.3, CH	4.02, brs	66.4, CH	4.05, brs	62.8, CH	3.92, m	66.0, CH	4.05, brs
26	74.7, CH	4.01, brs	74.7, CH	4.04, brs	78.8, CH	3.82, m	74.7, CH	4.03, brs
28	71.8, CH	4.21, t (9.3)	71.8, CH	4.22, t (9.2)	73.6, CH	4.46, t (9.3)	71.9, CH	4.28, t (9.1)
29	28.4, CH_2	2.10, m	28.4, CH_2	2.13, m	28.0, CH_2	2.10, m	27.0, CH_2	2.21, m
		1.84, m		1.80, m		1.57, m		2.04, m
30	27.4, CH_2	α 2.62, dt (13.8, 5.3)	27.3, CH_2	α 2.65, dt (14.0, 5.4)	25.6, CH_2	2.32, m	28.9, CH_2	2.59, dt (14.4, 5.6)
		β 1.45, m		β 1.46, m		1.77, m		1.59, m
31	44.3, C		44.5, C		44.1, C		43.4, C	
32	47.7, C		47.9, C		56.4, C		49.1, C	
33	107.1, CH_2	5.04, brs	105.4, CH_2	5.02, s	109.9, CH_2	4.92, brs	107.9, CH_2	4.80, brs
		4.91, s		4.83, s		4.89, brs		4.69, brs
34	20.4, CH_3	1.76, s	20.1, CH_3	1.82, s	27.3, CH_3	1.16, s	25.7, CH_3	1.06, s
35	31.3, CH_3	1.07, s	31.2, CH_3	1.10, s	27.8, CH_3	0.91, s	28.8, CH_3	0.85, s
36	19.7, CH_3	1.68, s	19.7, CH_3	1.68, s	19.7, CH_3	1.75, s	19.7, CH_3	1.70, s
37	143.2, C		143.2, C		141.4, C		143.3, C	
38	111.6, CH_2	5.01, brs	111.6, CH_2	4.95, s	111.8, CH_2	5.16, brs	111.6, CH_2	5.06, s
		4.83, s		4.83, s		5.02, brs		4.87, s
39	21.4, CH_3	1.06, s	21.5, CH_3	1.08, s	20.2, CH_3	0.93, s	18.9, CH_3	1.24, s
40	19.3, CH_3	1.66, s	19.1, CH_3	1.60, s	16.5, CH_3	1.48, s	18.7, CH_3	1.27, s
15-OH		4.43, s		4.30, s				
19-OH		4.74, s						
18-OCH_3							58.9, CH_3	3.73, s
19-OCH_3			48.4, CH_3	3.26, s				
22-OH		3.28, s		3.29, s				
25-OH		3.40, d (7.5)		3.33, d (8.5)				

[a]See Experimental Section for ^{1}H and ^{13}C NMR Data of compounds **2** and **6**. [b]Measured in acetone-d_6. [c]Measured in $CDCl_3$.

The chlorine-substituted aromatic carbon signal at δ_C 133.4 (C-6) in the ^{13}C NMR spectrum of **2** was replaced by an aromatic methine signal at δ_C 121.2 (C-6) in **3** (Table 1). Accordingly, an additional aromatic methine proton signal at δ_H 7.15 (1H, d, J = 7.6 Hz, H-6) was observed in the ^{1}H NMR spectrum of **3**. In addition, the singlet aromatic methine proton signal of H-7 at δ_H 7.31 in **2** was replaced by a doublet signal at δ_H 6.74 (1H, d, J = 7.6 Hz, H-7) in **3**. The COSY correlation from H-7 to H-6

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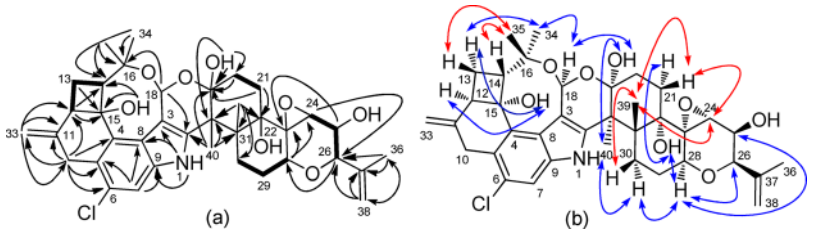

Figure 1. (a) Key COSY (bold lines) and HMBC (arrows) of **1**. (b) Key NOE correlations of **1**.

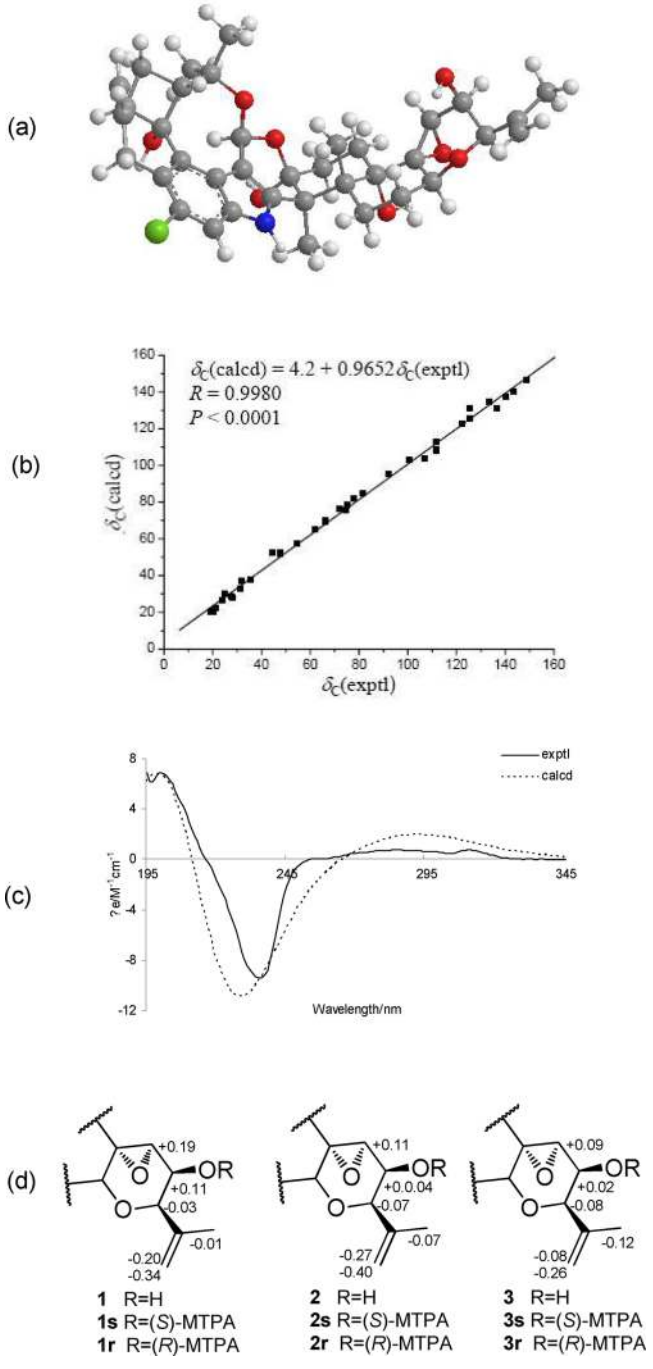

Figure 2. (a) Energy-minimized conformer of **1**. (b) Deviations of ^{13}C NMR data for **1** [δ_C(calcd) − δ_C(exptl)]. (c) Experimental and calculated ECD spectra of **1**. (d) $\Delta\delta_{H(S-R)}$ value (in acetone-d_6) of the MTPA esters of **1**−**3**.

as well as the HMBC correlations from H-6 to C-4, C-9, and C-10 supported the above deduction.

The relative configuration and the absolute configuration for the stereogenic centers of **3** were also determined to be the same as that of **1** and **2** by NOESY experiments and by the modified Mosher's method, respectively (Figure 2). The structure of compound **3** was thus assigned, and this compound was named as rhizovarin C.

Considering that **2** and **3** are possible artifacts due to the use of MeOH during the purification procedures, an experiment simulating chromatographic conditions of the purification was performed. A sample of **1** (0.5 mg) was dissolved in 2.5 mL of MeOH−$CHCl_3$ (1:1) and mixed with 0.4 g of silica. The mixture was stirred at room temperature for 24 h and then checked by HPLC, which showed that both compounds **1** and **2** were present in the HPLC profile (Figure S1), indicating that **2** can be formed from **1** under mild conditions. However, attempted identification of the corresponding peaks for **1**−**3** in the HPLC traces of the extracts is inconclusive because of the complexity of the HPLC traces.

Rhizovarin D (**4**) was assigned the molecular formula $C_{37}H_{49}NO_6$ based on HREIMS. The general features of its ^{1}H and ^{13}C NMR data (Table 1) suggested that **4** is also an indole-diterpene with a similar structure to those of **1**−**3**. However, two carbonyl carbon signals observed at δ_C 174.7 (C-2) and 208.1 (C-3) in the ^{13}C NMR spectrum of **4** suggested the presence of two additional carbonyl carbons in **4**, which were not present in the spectra of **1**−**3**. Comparing the ^{1}H and ^{13}C NMR data of **4** (Table 1) with those of known compounds shearinine C[25] and sulpinine C[26] indicated the presence of an eight-membered keto-amide central ring (ring C) in **4**, presumably formed via oxidation of the indole C-2−C-3 bond, which was confirmed by the HMBC correlations from H_3-40 and H-18 to the carbonyl carbons C-2 and C-3, respectively (Figure 3). The structure of rings D−F in

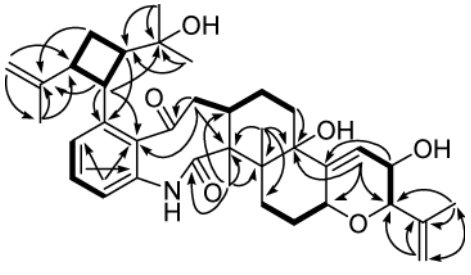

Figure 3. Key COSY (bold lines) and HMBC (arrows) correlations of **4**.

4 was readily established by comparison with those of known indole-diterpenes penitrems C (**11**) and D (**12**)[15] and further confirmed by extensive analysis of 2D NMR data (Figure 3). Thus, an indole nucleus connected to a cyclized diterpene unit was established for **4** (rings B−F), with 10 carbon atoms unassigned, which could constitute a monoterpene unit like that in **1**−**3**.

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However, detailed analysis of the ^{1}H and ^{13}C NMR data revealed that the proton and carbon signals for the C-10 methylene in **1**−**3** disappeared in the spectra of **4**. Instead, signals for an additional methyl group were observed at δ_C 23.2 and δ_H 1.48, indicating that the C-10 methylene in **1**−**3** was replaced by a methyl group in **4** and the linkage between C-10 and C-5 observed in **1**−**3** no longer existed in **4**. This deduction was confirmed by the HMBC correlations from H_3-10 to C-11 and C-12 (Figure 3). The structure of compound **4** was thus identified as a new indole-diterpene with a complex 6,8,6,6,6-fused ring system. The relative configuration for the stereogenic centers of **4** was determined to be the same as that of known diterpenes penitrems A−F by NOESY experiments.[15]

The molecular formula of compound **5** was determined as $C_{38}H_{49}NO_6$ by HRESIMS data. The structure elucidation of **5** was straightforward due to its close relationship to the reported indole-diterpene secopenitrem D (**7**),[27] which was isolated from a strain of *P. crustosum* Thom. The only differences between the two compounds were in regard to the H-18β and the 23,24-alkene in **7**, which were replaced by a β-oriented methoxy group and a 23α,24α-epoxide in **5**, respectively. The replacement of H-18β by a methoxy group was supported by the deshielded chemical shift of C-18 (δ_C 85.0, CH) in **5**, which is at δ_C 30.1 (CH_2) in **7**, the observed HMBC correlations from the methoxy protons (δ_H 3.73, 18-OCH_3) to C-18, and the NOE correlations from the oxygenated methine proton H-18 (δ_H 4.60) to the α-oriented H_3-40. In addition, the olefinic carbon signals at δ_C 148.4 (C-23) and 119.6 (C-24) for the 23,24-alkene in **7** disappeared in the ^{13}C NMR spectrum of **5**, whereas two additional oxygenated carbon signals resonating at δ_C 66.3 (C, C-23) and 61.9 (CH, C-24) were observed (Table 1), implying the presence of a 23α,24α-epoxide in **5**, which is a common structural feature shared with some of the known indole-diterpenes.[11−15] This deduction was confirmed by the COSY correlation from H-24 (δ_H 3.57) to H-25 and by the HMBC correlations from H-28 and H-29 to C-23. The NOE correlation from H-24 to the β-oriented H_3-39 allowed the assignment of the α-orientation of the epoxide unit. The structure of compound **5** was thus assigned and named rhizovarin E.

The molecular formula of **6** was determined as $C_{27}H_{33}NO_4$ on the basis of positive HREIMS data. The general features of its ^{1}H and ^{13}C NMR data closely resembled those of penijanthine A (**15**).[28,29] However, the olefinic proton and carbon signals for the 11,12-alkene at δ_H 5.85/δ_C 118.7 (CH-11) and δ_C 148.1 (C-12) in **15** disappeared in the spectra of **6**. Instead, oxygenated signals resonating at δ_H 3.55/δ_C 62.1 (CH, C-11) and δ_C 66.4 (C, C-12) were observed in the NMR spectra of **6**. These observations, along with the fact that there is one more oxygen atom present in the molecular formula compared to **15**, indicated that the 11,12-alkene in **15** was replaced by an epoxide unit in **6**. The observed COSY correlation from H-11 to H-10 and the HMBC correlations from H-11 to C-9 and C-12 confirmed this deduction. The relative configuration of **6** was determined to be similar to that of **15**, except that the additional methine proton H-11 is on the same side with H_3-26 and H-16, as confirmed by the NOE correlations from H_3-26 to H-16 and H-11. Thus, the structure of **6** was assigned and named rhizovarin F.

The absolute configurations of compounds **4**−**6** remain unassigned due to the shortage of samples available after the bioassays, but from a biosynthetic point of view, these compounds are assumed to have the same absolute configurations as those of **1**−**3**.

In addition to rhizovarins A−F (**1**−**6**), 14 known indole-diterpenes, including secopenitrem D (**7**),[27] PC-M4 (**8**),[30] penitrems A−F (**9**−**14**),[15] penijanthine A (**15**),[28,29] paxilline (**16**),[31] 1′-*O*-acetylpaxilline (**17**),[32] 4b-deoxy-1′-*O*-acetylpaxilline (**18**),[33] 3-deoxo-4b-deoxypaxilline (**19**),[34] and 3b-hydroxy-4b-desoxypaxilline (**20**),[35] were also isolated and identified. The structures of these compounds were determined by spectroscopic analysis.

Rhizovarin A (**1**) possesses a unique skeleton that incorporates an unprecedented linkage of an acetal to a hemiketal system among the indole-diterpenes, which features a six-membered pyran ring fused to the indole nucleus instead of a five-membered ring in the previously reported structures, while rhizovarins B and C (**2** and **3**) have an unusual acetal connected to a ketal system. All of these compounds possess an unprecedented eight-membered cyclic ether system coupled with five other rings including cyclobutane, methylenecyclohexane, indole, and 3,6-dihydro-2*H*-pyran motifs.

Rhizovarins A−C (**1**−**3**) represent the most complex members of the indole-diterpene derivatives.[11−15] Even though the main structural elements resemble those of other reported indole-diterpenes, the presence of an unusual acetal linked to a hemiketal (**1**) or a ketal (**2**) unit makes them chemically unique. These structural features are unprecedented among indole-diterpenes reported so far and are rarely reported in other kinds of natural products.

Biogenetically, indole-diterpenes, such as penitrem A (**9**), are regarded to be derived from tryptophan, geranylgeranyldiphosphate, and two isopentenyl-diphosphate units.[36] Recently, the biosynthetic pathway of penitrem A (**9**) has been elucidated by reconstitution of the biosynthetic genes in *Aspergillus oryzae*, which includes a prenylation-initiated cationic cyclization to install the bicyclo[3.2.0]heptane skeleton, a two-step P450-catalyzed oxidative process forming the tricyclic penitrem skeleton, and five sequential oxidative transformations to form the final product.[37] For rhizovarin A (**1**), the biosynthetic pathway may involve more oxidative steps than that of penitrem A (**9**), due to its unprecedented linkage of the acetal to the hemiketal system. A plausible biosynthetic pathway for rhizovarin A (**1**) is outlined in Scheme 1.

In this pathway, thomitrem E,[11] the known indole-diterpene derivative isolated from the fungus *P. crustosum*, is regarded as the biosynthetic precursor of compound **1**. Selective peroxidation of C-18 and C-19 in thomitrem E would produce the intermediate I, which, by cleavage of the C-18−C-19 bond, could form the key intermediate II. The intramolecular 16-OH group in II can react with the carbonyl group C-18 to give hemiacetal III. Similar intramolecular reactions in III from the 18-OH to carbonyl C-19 would yield the last intermediate, IV, which contains a linked acetal to hemiketal unit in the molecule. Compound **1** could then be formed from IV by chlorination.

All of the indole-diterpenes isolated in this study were evaluated for their antitumor activity. Compounds **1**, **2**, **9**, **11**, **14**, and **20** showed activity against the human A-549 and HL-60 cancer cell lines, while compound **5** exhibited activity only against the A-549 cancer cell line (Table 2). The other indole-diterpenes showed weak or no activity (IC_{50} > 10 μM) against these two cell lines. In this screening, all of the chlorinated compounds (**1**, **2**, **9**, **11**, and **14**) showed activity against both A-549 and HL-60 cancer cell lines. On the other hand, the chlorinated derivatives including compounds **2**, **9**, **11**, and **14** showed stronger activity than their chlorine-free analogous **3**, **13**, **12**, and **10**, respectively. These results indicated that the chlorine substitution might be essential for the activity against these cell targets. It is worth noting that **20** is the only compound of the paxilline-type indole-diterpenes that displayed activities against the two cell lines. Compared to paxilline (**16**), the 13-hydroxy group is missing and the 10-keto in **16** is replaced by 10β-hydroxy

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Scheme 1. Possible Biosynthetic Pathway of Rhizovarin A (1)

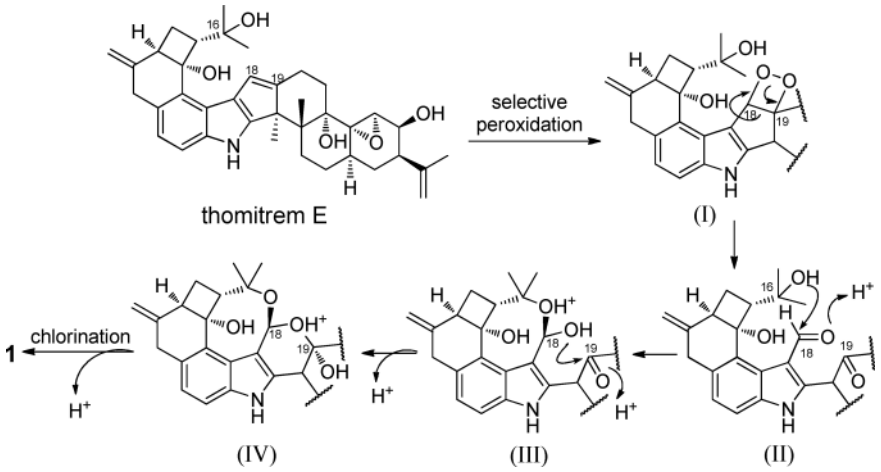

Table 2. Antitumor Activity of Isolated Indole-Diterpenes (IC_{50}, μM)

	1	2	5	9	11	14	20	adriamycin[a]
A-549	11.5	6.3	9.2	8.4	8.0	8.2	4.6	0.30
HL-60	9.6	5.0	–[b]	7.0	4.7	3.3	2.6	0.067

[a]Positive control. [b]No activity (IC_{50} > 10 μM).

in **20**, both of which may contribute to its activity. Compound **19** is inactive against the two cell lines, and this result also indicates that the 10β-hydroxy is essential for the activity of the paxilline-type indole-diterpenes (**20** vs **19**).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were obtained on a PuXi TU-1810 UV–visible spectrophotometer. ECD spectra were recorded on a Chirascan CD spectrometer. IR spectra were obtained on a Nicolet NEXUE 470 infrared spectrophotometer. 1D and 2D NMR were recorded on a Bruker Avance 500 or AVANCE DMX 600 NMR spectrometer. Mass spectra were obtained on a VG Autospec 3000 mass spectrometer. Column chromatography was performed with silica gel (200–300 mesh, Qingdao Haiyang Chemical Co.), Lobar LiChroprep RP-18 (40–63 μm; Merck), and Sephadex LH-20 (18–110 μm, Merck). Semipreparative HPLC was performed using an HPLC system equipped with a Dionex P680 pump, ASI-100 automated sample injector, and UVD340U multiple wavelength detector controlled using Chromeleon software, version 6.80.

Fungal Material. *Mucor irregularis* QEN-189 was isolated from the inner tissue of the stems of the mangrove plant *Rhizophora stylosa* collected in Hainan Island, China. Fungal identification was carried out by DNA amplification and sequencing of the ITS region as described in our previous report.[18] The sequence from the fungal strain has been deposited at GenBank with accession number HQ891659. A BLAST search result showed that the sequence was the same (100%) as that of *R. variabilis* (compared to EU196747.1 GI:159024333). The strain is preserved at the Key Laboratory of Experimental Marine Biology, Institute of Oceanology of the Chinese Academy of Sciences (IOCAS).

Fermentation, Extraction, and Isolation. Mass growth of the fungus for the isolation and identification of secondary metabolites was carried out in Erlenmeyer flasks (1 L each). The fungus was grown in a modified Czapek medium (dextrose 10 g, mannitol 20 g, maltose 20 g, yeast extract 3 g, corn syrup 1 g, monosodium glutamate 10 g, tryptophan 0.5 g, K_2HPO_4 0.5 g, $MgSO_4{\cdot}7H_2O$ 0.3 g, and 1000 mL of natural seawater that was obtained from Huiquan Bay near the campus of IOCAS, pH 7.2–7.8, adjusted with 10% NaOH or 36.5% HCl, 300 mL/flask, 100 flasks, 30 L total) at room temperature under static conditions for 30 days.

The mycelia and culture broth of *M. irregularis* QEN-189 were separated by filtration and were exhaustively extracted with MeOH and EtOAc, respectively. Because the TLC and HPLC profiles of the two extracts were nearly identical, they were combined before further separation. The combined extract (45 g) was subjected to vacuum liquid chromatography (VLC) over silica gel, eluting with different solvents of increasing polarity from petroleum ether (PE) to MeOH to yield eight fractions (Frs. 1–8) on the basis of TLC analysis. Fr. 3 (1.3 g) was further purified by VLC on silica gel eluting with a EtOAc–PE gradient (from 1:5 to 2:1), Sephadex LH-20 (MeOH), and semipreparative HPLC (Elite ODS-BP column, 10 μm; 10.0 × 30.0 mm; 85% MeOH–H_2O, 4 mL/min) to afford compounds **4** (1.3 mg, t_R 21.3 min), **5** (1.1 mg, t_R 18.6 min), **7** (13.9 mg, t_R 15.5 min), **8** (16.1 mg, t_R 26.7 min), and **12** (18.9 mg, t_R 24.3 min). Fr. 4 (2.3 g) was further purified by VLC on silica gel eluting with a EtOAc–PE gradient (from 1:5 to 2:1), Sephadex LH-20 (MeOH), and semipreparative HPLC (Elite ODS-BP column, 10 μm; 10.0 × 30.0 mm; 78% MeOH–H_2O, 4 mL/min) to afford **10** (11.1 mg, t_R 19.5 min), **11** (13.5 mg, t_R 21.3 min), **13** (16.5 mg, t_R 16.3 min), and **14** (15.3 mg, t_R 17.4 min). Fr. 5 (4.5 g) was further purified by VLC on silica gel eluting with a $CHCl_3$–MeOH gradient (from 50:1 to 1:1), Sephadex LH-20 (MeOH), and semipreparative HPLC (Elite ODS-BP column, 10 μm; 10.0 × 30.0 mm; 67% MeOH–H_2O, 4 mL/min) to yield compounds **1** (10.1 mg, t_R 15.4 min), **2** (8.3 mg, t_R 20.7 min), **3** (7.8 mg, t_R 22.8 min), **9** (10.1 mg, t_R 20.4 min), and **19** (7.3 mg, t_R 16.5 min). Fr. 6 (1.9 g) was further purified by VLC on silica gel eluting with a $CHCl_3$–MeOH gradient (from 20:1 to 1:2), Sephadex LH-20 (MeOH), and semipreparative HPLC (Elite ODS-BP column, 10 μm; 10.0 × 30.0 mm; 51% MeOH–H_2O, 4 mL/min) to obtain compounds **6** (1.2 mg, t_R 18.9 min), **15** (6.7 mg, t_R 16.1 min), **16** (7.5 mg, t_R 20.2 min), **17** (6.3 mg, t_R 22.3 min), **18** (4.1 mg, t_R 24.6 min), and **20** (12.1 mg, t_R 21.5 min).

*Rhizovarin A (**1**):* white, amorphous powder; $[\alpha]^{25}_D$ –25.7 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ε) 233 (4.2), 281 (3.7) nm; ECD (*c* 0.9 mM, MeOH), λ_{max} ($\Delta\varepsilon$) 285 (+0.75), 235 (–9.38), 200 (+6.90) nm; IR (KBr) ν_{max} 3440, 2927, 1633, 1454, 1385, 1319, 1128, 1067, 1011, 934, 842, 782, 584 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; ESIMS *m/z* 666 $[M + H]^+$; HRESIMS *m/z* 666.2835 $[M + H]^+$ (calcd for $C_{37}H_{45}ClNO_8$, 666.2833).

*Rhizovarin B (**2**):* white, amorphous powder; $[\alpha]^{25}_D$ –15.1 (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ε) 229 (4.3), 282 (3.6) nm; IR (KBr) ν_{max} 3439, 2924, 1630, 1452, 1385, 1208, 1067, 1012, 933, 897, 841, 817, 597 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6), δ_H 10.05 (1H, s, NH-1), 7.31 (1H, s, H-7), 6.58 (1H, S, H-18), 5.05 (1H, s, H-33a), 5.01 (1H, s, H-38a), 4.92 (1H, s, H-33b), 4.83 (1H, s, H-38b), 4.50 (1H, s, 15-OH), 4.21 (1H, t, 9.3, H-28), 4.03 (1H, brs, H-25), 4.02 (1H, brs, H-26), 3.70 (1H, d, 16.0, H-10a), 3.56 (1H, d, 1.8, H-24), 3.41 (1H, d, 7.4, 25-OH), 3.30 (1H, s, 22-OH), 3.29 (1H, d, 16.0, H-10b), 3.26 (3H, s, 19-OCH_3), 2.96 (1H, m, H-12), 2.61 (1H, dt, 14.0 and 5.1, H-30a), 2.47 (1H, m, H-14), 2.43 (1H, m, H-13a), 2.21 (1H, m, H-13b), 2.08 (1H, m, H-29a),

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2.07 (1H, m, H-20a), 2.01 (1H, m, H-20b), 1.81 (1H, s, H-34), 1.79 (1H, m, H-29b), 1.77 (1H, m, H-21a), 1.67 (1H, s, H-36), 1.60 (3H, m, H-40), 1.48 (2H, m, H-21b and 30b), 1.09 (1H, s, H-35), 1.07 (3H, m, H-39); ^{13}C NMR (125 MHz, acetone-d_6), δ_C 148.5 (C, C-11), 143.1 (C, C-37), 139.7 (C, C-2), 136.3 (C, C-9), 133.4 (C, C-6), 125.7 (C, C-5), 125.5 (C, C-4), 122.3 (C, C-8), 111.7 (CH_2, C-38), 111.5 (CH, C-7), 111.3 (C, C-3), 107.1 (CH_2, C-33), 103.6 (CH, C-19), 92.2 (CH, C-18), 81.5 (C, C-15), 77.6 (C, C-22), 75.2 (C, C-16), 74.6 (CH, C-26), 71.7 (CH, C-28), 66.3 (CH, C-25), 66.2 (C, C-23), 62.1 (CH, C-24), 54.3 (CH, C-14), 48.4 (CH_3, 19-OCH_3); 47.9 (CH, C-12), 47.8 (C, C-44.5 (C, C-31), 35.5 (CH_2, C-10), 31.2 (CH_3, C-35), 28.3 (CH_2, C-29), 27.1 (CH_2, C-30), 26.0 (CH_2, C-20), 24.5 (CH_2, C-21), 24.1 (CH_2, C-13), 21.4 (CH_3, C-39), 20.2 (CH_3, C-34), 19.7 (CH_3, C-36), 19.0 (CH_3, C-40); EIMS m/z (%) 679 (35) 647 (35), 593 (36), 579 (39) 561 (40), 408 (83) 395 (35), 385 (35), 340 (51), 326 (43), 298 (36), 284 (40), 258 (37), 69 (100); HREIMS m/z 679.2911 $[M]^+$ (calcd for $C_{38}H_{46}ClNO_8$, 679.2912).

Rhizovarin C (**3**): white, amorphous powder; $[\alpha]^{25}_D$ −21.3 (*c* 0.51, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.0), 283 (3.5) nm; IR (KBr) ν_{max} 3440, 2928, 2853, 1637, 1610, 1449, 1384, 1310, 1214, 1130, 1080, 947, 701, 540 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; EIMS m/z (%) 645 (39), 613 (48), 596 (52), 559 (57), 545 (52), 527 (50), 517 (32), 374 (100), 361 (50), 306 (51), 292 (41), 264 (39), 250 (42), 224 (35), 69 (81); HREIMS m/z 645.3311 $[M]^+$ (calcd for $C_{38}H_{47}NO_8$, 645.3302).

Rhizovarin D (**4**): white, amorphous powder; $[\alpha]^{25}_D$ −73.6 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.5), 264 (4.1) nm; IR (KBr) ν_{max} 3413, 2935, 2861, 1708, 1658, 1376, 1261, 1130, 1041, 983, 875 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; EIMS m/z (%) 603 ([M$^+$], 25), 585 (10), 544 (35), 476 (40), 458 (10), 430 (10), 406 (10), 360 (15), 268 (15), 252 (15), 227 (15), 212 (20), 201 (30), 159 (40), 146 (60), 59 (100); HREIMS m/z 603.3554 $[M]^+$ (calcd for $C_{37}H_{49}NO_6$, 603.3560);

Rhizovarin E (**5**): white, amorphous powder; $[\alpha]^{25}_D$ −43.5 (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ε) 230 (3.6), 281 (2.9) nm; IR (KBr) ν_{max} 3355, 2927, 2857, 1677, 1627, 1403, 1284, 1218, 1168, 1099, 1006, 937, 836 cm^{-1}; ^{1}H and ^{13}C NMR data, Table 1; ESIMS m/z 616 $[M + H]^+$; HRESIMS m/z 616.3644 $[M + H]^+$ (calcd for $C_{38}H_{50}NO_6$, 616.3638).

Rhizovarin F (**6**): white, amorphous powder; $[\alpha]^{25}_D$ −41.3 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.1), 280 (3.5) nm; IR (KBr) ν_{max} 3382, 2931, 2861, 1700, 1454, 1407, 1272, 1191, 1091, 963, 937, 883, 755, 667 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6), δ_H 7.33 (1H, dd, 6.7 and 2.1, H-20), 7.28 (1H, dd, 6.5 and 2.5, H-23), 6.95 (1H, m, H-21), 6.93 (1H, m, H-22), 5.07 (1H, s, H-29a), 4.88 (1H, s, H-29b), 4.30 (1H, t, 8.9, H-7), 4.03 (1H, brs, H-9), 4.02 (1H, brs, H-10), 3.55 (1H, brs, H-11), 2.86 (1H, m, H-16), 2.67 (1H, dt, 13.8 and 5.2, H-5a), 2.66 (1H, m, H-17a), 2.24 (1H, m, H-6a), 2.37 (1H, m, H-17b), 2.06 (1H, m, H-6b), 1.99 (1H, m, H-14a), 1.98 (1H, m, H-15a), 1.71 (3H, s, H-28), 1.68 (1H, m, H-14a), 1.67 (1H, m, H-5b) 1.65 (1H, m, H-14b), 1.59 (1H, m, H-14b), 1.58 (1H, m, H-15b),1.33 (3H, s, H-25), 1.24 (3H, s, H-26); ^{13}C NMR (125 MHz, acetone-d_6), δ_C 153.6 (C, C-2), 143.1 (C, C-27), 141.4 (C, C-24), 126.3 (CH, C-19), 120.5 (CH, C-22), 119.6 (CH, C-21), 118.8 (CH, C-20), 117.1 (C, C-18), 112.6 (CH, C-23), 111.7 (CH_2, C-29), 78.4 (C, C-13), 74.7 (CH, C-9), 72.1 (CH, C-7), 66.4 (C, C-12), 66.2 (CH, C-10), 62.1 (CH, C-11), 51.7 (C, C-3), 51.1 (CH, C-16), 43.5 (C, C-4), 30.5 (CH_2, C-14), 28.9 (CH_2, C-6), 27.9 (CH_2, C-17), 27.3 (CH_2, C-5), 21.6 (CH_2, C-15), 19.7 (CH_3, C-28), 18.9 (CH_3, C-26), 16.6 (CH_3, C-25); EIMS m/z (%) 435 (100), 420 (90), 304 (33), 273 (25), 232 (50), 182 (70); HREIMS m/z 435.2403 $[M]^+$ (calcd for $C_{27}H_{33}NO_4$, 435.2410).

Preparation of the (*R*)- and (*S*)-MTPA Esters of Compounds 1−3.[24] (*S*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 μL) and 4-(dimethylamino)pyridine (6 mg) were added to rhizotrem A (1, 2.5 mg) that was dissolved in dried pyridine (400 μL). The mixture was kept at room temperature for 12 h, and the acylation product was then purified by preparative TLC on silica gel (eluent: petroleum ether−EtOAC, 1:1, v/v) to yield corresponding (*R*)-MTPA ester **1r**. Treatment of **1** (2.5 mg) with (*R*)-MTPA-Cl (10 μL) as described above yielded the corresponding (*S*)-MTPA ester **1s**. Compounds **2** and **3** were also reacted with (*S*)- and (*R*)-MTPA-Cl to afford the respective Mosher esters.

(S)-MTPA ester of 1 (1s): white, amorphous powder; ^{1}H NMR (500 MHz, acetone-d_6), δ_H 5.67 (1H, d, 2.8, H-25), 3.86 (1H, d, 2.8, H-24), 4.22 (1H, brs, H-26), 4.89 (1H, brs, H-38a), 4.73 (1H, H-38b), 1.65 (3H, s, H-36), 4.37 (1H, t, 9.1), 2.09 (1H, m, H-29a), 1.71 (3H, s, H-40), 1.59 (1H, m, H-29b), 2.65 (1H, m, H-30a), 1.26 (1H, m, H-30b), 1.19 (3H, s, H-39).

(R)-MTPA ester of 1 (1r): white, amorphous powder; ^{1}H NMR (500 MHz, acetone-d_6), δ_H 5.56 (1H, d, 2.8, H-25), 5.09 (1H, brs, H-38a), 5.07 (1H, H-38b), 4.25 (1H, brs, H-26), 3.67 (1H, d, 2.8, H-24), 2.60 (1H, m, H-30a), 2.29 (1H, t, 8.6, H-28), 2.08 (1H, m, H-29a), 1.72 (3H, s, H-36), 1.64 (1H, m, H-29b), 1.61 (3H, s, H-40) 1.43 (1H, m, H-30b), 1.13 (3H, s, H-39).

(S)-MTPA ester of 2 (2s): white, amorphous powder; ^{1}H NMR (500 MHz, acetone-d_6), δ_H 5.59 (1H, d, 2.8, H-25), 4.81 (1H, brs, H-38a), 4.66 (1H, H-38b), 4.27 (1H, t, 9.1, H-28), 4.18 (1H, brs, H-26), 3.75 (1H, d, 2.8, H-24), 2.61 (1H, m, H-30a), 2.06 (1H, m, H-29a), 1.67 (1H, m, H-29b), 1.61 (3H, s, H-40), 1.60 (3H, s, H-36), 1.44 (1H, m, H-30b), 1.05 (3H, s, H-39).

(R)-MTPA ester of 2 (2r): white, amorphous powder; ^{1}H NMR (500 MHz, acetone-d_6), δ_H 5.56 (1H, d, 2.8, H-25), 5.08 (1H, brs, H-38a), 5.06 (1H, H-38b), 4.25 (1H, brs, H-26), 4.28 (1H, t, 9.4, H-28), 3.64 (1H, d, 2.8, H-24), 2.60 (1H, m, H-30a), 2.08 (1H, m, H-29a), 1.67 (3H, s, H-36), 1.65 (1H, m, H-29b), 1.61 (3H, s, H-40), 1.45 (1H, m, H-30b), 1.08 (3H, s, H-39).

(S)-MTPA ester of 3 (3s): white, amorphous powder; ^{1}H NMR (500 MHz, acetone-d_6), δ_H 5.58 (1H, d, 2.8, H-25), 4.95 (1H, brs, H-38a), 4.66 (1H, H-38b), 4.27 (1H, t, 8.8), 4.18 (1H, brs, H-26), 3.75 (1H, d, 2.8, H-24), 2.61 (1H, t, 14.5, H-30a), 2.05 (1H, m, H-29a), 1.65 (1H, m, H-29b), 1.62 (3H, s, H-40), 1.59 (3H, s, H-36), 1.47 (1H, m, H-30b), 1,07 (3H, s, H-39).

(R)-MTPA ester of 3 (3r): white, amorphous powder; ^{1}H NMR (500 MHz, acetone-d_6), δ_H 5.56 (1H, d, 2.8, H-25), 5.03 (1H, brs, H-38a), 4.92 (1H, H-38b), 4.28 (1H, t, 9.5, H-28), 4.26 (1H, brs, H-26), 3.66 (1H, d, 2.8, H-24), 2.60 (1H, m, H-30a), 2.09 (1H, m, H-29a), 1.71 (3H, s, H-36), 1.65 (1H, m, H-29b), 1.60 (3H, s, H-40), 1.47 (1H, m, H-30b), 1.10 (3H, s, H-39).

Cytotoxicity Assay. Cytotoxicity of compounds **1**−**20** against HL-60 (human leukemia) and A549 (human lung adenocarcinoma) cell lines was evaluated using the MTT[38] and SRB[39] methods, respectively. Adriamycin was used as the positive control.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00403.

Selected 1D and 2D NMR spectra of compounds **1**−**6** and a table of potential secondary metabolite gene clusters from genome mining results (PDF)

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Notes

The authors declare no competing financial interest.

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