Cytotoxic Alkaloids and Antibiotic Nordammarane Triterpenoids from the Marine-Derived Fungus *Aspergillus sydowi*

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Three new diketopiperazine alkaloids, 6-methoxyspirotryprostatin B (**1**), 18-oxotryprostatin A (**2**), and 14-hydroxyterezine D (**3**), with an oxaspiro[4.4]lactam moiety, 14-norpseurotin A (**4**), and the 29-nordammarane triterpenoid 6β,16β-diacetoxy-25-hydroxy-3,7-dioxy-29-nordammara-1,17(20)-dien-21-oic acid (**5**), as well as 12 known compounds (**6**−**17**), were isolated from the ethyl acetate extract of a marine-derived fungal strain, *Aspergillus sydowi* PFW1-13. The structures of compounds **1**−**5** were elucidated by comprehensive spectroscopic analysis. Compounds **1**−**3** exhibit weak cytotoxicity against A-549 cells, with IC_{50} values of 8.29, 1.28, and 7.31 μM, respectively. Compound **1** also shows slight cytotoxicity against HL-60 cells, with an IC_{50} value of 9.71 μM. Compounds **4** and **5** display significant antimicrobial activities against *Escherichia coli*, *Bacillus subtilis*, and *Micrococcus lysoleikticus* with MICs of 3.74, 14.97, and 7.49 μM and 10.65, 5.33, and 10.65 μM, respectively.

It has been demonstrated that marine fungal species have evolved unique metabolic mechanisms where the metabolites have potential applications in drug discovery.[1] In the course of screening "talented strains"[2] for the identification of structurally novel and bioactive metabolites from marine fungi, an EtOAc extract was obtained from the marine-derived fungal strain PFW1-13, named *Aspergillus sydowi*. The metabolites showed cytotoxicity against K562 cells. Bioassay-guided fractionation afforded three new diketopiperazine alkaloids (**1**−**3**), a new oxaspiro[4.4]lactam (**4**), and a new 29-nordammarane triterpenoid (**5**), together with 12 known compounds, terezine D (**6**),[3] pseustin A (**7**),[4,5] helvolic acid (**8**),[6–8] spirotryprostatin A (**9**),[9] 12,13-dihydroxyfumitremorgin C (**10**),[10] fumitremorgin C (**11**),[11] didehydrobisdethiobis(methylthio)gliotoxin (**12**),[12–14] verruculogen (**13**),[12,13] fumigaclavine C (**14**),[12–16] fumigaclavine B (**15**),[15] pyripyropene A (**16**),[17–20] and pyripyropene E (**17**).[21] The structures of the new compounds were elucidated using NMR and CD spectroscopy. Compounds **1**−**3** exhibited cytotoxicities against A-549 and HL-60 cells, and compounds **4** and **5** showed antimicrobial activities against *Escherichia coli*, *Bacillus subtilis*, and *Micrococcus lysoleikticus*.

Results and Discussion

The bioactive EtOAc extract of *A. sydowi* was subjected to column chromatography (CC) on Si gel and further purified by semipreparative HPLC, which gave 17 pure compounds, **1**−**17**.

6-Methoxyspirotryprostatin B (**1**) was isolated as a pale yellow, amorphous powder. Its molecular formula was determined as $C_{22}H_{23}N_3O_4$ according to the HRESIMS at *m/z* 392.1592 [M − H]$^-$ (calcd 392.1610). The ^{13}C NMR spectrum of **1** displayed 22 carbon signals, including three carbonyls, six quaternary carbons, seven methines, three methylenes, and three methyl groups (Tables 1). 1,2,4-Trisubstituted phenyl peaks in the ^{1}H NMR spectrum (δ 6.95, d, J = 8.2 Hz; δ 6.51, dd, J = 2.3, 8.3 Hz; δ 6.47, d, J = 2.3 Hz) and a methoxy singlet (δ 3.79, 3H, s) substituted the corresponding 1,2-disubstituted phenyl peaks in spirotryprostatin B at δ 7.06, 6.99, 7.23, and 6.89. Accordingly, a methoxy carbon (δ 55.5) and a quaternary carbon (δ 160.6) in the ^{13}C NMR spectrum substituted an aromatic methine carbon. Additionally, upfield shifts for H-4, H-5, H-7, C-3a, C-5, and C-7 were also observed in the 1D NMR spectra of **1**, due to the electron-donating effects of the methoxy group to its *ortho*- and *para*-positions. Thus, the structure of **1** was identified as 6-methoxyspirotryprostatin B, and it was further confirmed by the key HMBC correlations from methoxy protons and H-4 (δ 6.95) to C-6 (δ 160.6) and from H-5 (δ 6.51) and H-7 (δ 6.47) to C-3a (δ 118.8) (Figure 2). The configuration of **1** was determined as 3*S*,12*S*,18*S* by NOE difference effects between H-4 and H-19 (δ 5.20) and between H-12 (δ 4.35) and 13-H$_\alpha$ (δ 2.49, m), as well as by comparison of the specific rotation with that of spirotryprostatin B[9] and by comparison of the CD spectrum with that of **9**.

18-Oxotryprostatin A (**2**) was obtained as a yellow, amorphous powder. The molecular formula of **2** was determined as $C_{22}H_{25}N_3O_4$ by HRESIMS at *m/z* 394.1753 [M − H]$^-$ (calcd 394.1767). Except for the substitution of the 18-methylene group in tryprostatin A (δ_H/δ_C 3.40−3.46/25.1) for a carbonyl in **2** (δ 183.8), the 1D NMR spectra of **2** were similar to those of tryprostatin A (Table 1).[11,22,23] Downfield shifts for C-3, C-4, C-5, C-6, C-20, C-21, and C-22 and the upfield shift for C-2 were observed. These data showed that the 18-CH_2 in tryprostatin A was substituted by an 18-C═O in **2**. This deduction was supported by HMBC correlations between H-19 (δ 6.54, s) and C-18, C- 21 (δ 28.1) and C-22 (δ 21.4) and between H-22 (δ 2.17, s) and C-20 (δ 158.2) (Figure 2). The configuration of **2** was determined as 9*S*,12*S* by difference NOE effects between H-9 and H-12 and by comparing its specific rotation with that of tryprostatin A.[11,22,23]

14-Hydroxyterezine D (**3**) was isolated as a pale, amorphous powder. Its molecular formula was established as $C_{19}H_{23}N_3O_3$ by HRESIMS at *m/z* 340.1650 [M − H]$^-$ (calcd 340.1661), suggesting 16 amu more than the mass of terezine D (**6**).[3] Except for the substitution of 14-NH (δ 7.92) for 14-*N*-OH (δ 10.15), the 1D NMR spectra of **3** were very similar to those of **6**. In addition, the downfield shifts of H-9 and C-9 and the upfield shifts of C-8 and C-13 were also observed. These spectroscopic data showed that compound **3** was the 14-*N*-hydroxy derivative of **6**, and this was further supported by HMBC correlations from 11-NH (δ 7.96, d) to C-9 and C-13 and from H-12 (δ 3.61, dq) to C-10 (δ 165.1) (Figure 2). The configuration of **3** was determined as 9*S*,12*S* by comparison of the specific rotation and NMR data with those of **6**.

14-Norpseurotin A (**4**) was obtained as pale yellow power. Its HRESIMS at *m/z* 440.1324 [M + Na]$^+$ (calcd 440.1321) indicated that its molecular weight was 14 amu less than that of pseurotin A (**7**), with a molecular formula of $C_{21}H_{23}NO_8$. The ^{1}H and ^{13}C NMR

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Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1**−**5**[a]

	1		**2**		**3**		**4**		**5**[b]	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	8.49 (s)		8.92 (s)		10.89 (br s)				7.30 (brd, 10.1)	157.2CH
2		179.0qC		133.6qC	7.07 (d, 2.3)	125.0CH		185.7qC	5.87 (brd, 10.1)	127.8CH
3		61.4qC		118.5qC		107.8qC		113.7qC		201.4qC
3a		118.8qC		122.6qC		127.7qC				
4	6.95 (d, 8.2)	128.4CH	7.66 (d, 9.1)	122.1CH	6.79 (d, 6.9)	119.9CH		196.4qC	2.78 (qd, 6.9, 8.7)	40.4CH
5	6.51 (dd, 8.3, 2.3)	107.1CH	6.83 (dd, 9.1, 2.3)	112.7CH	6.85 (dd, 7.8, 6.9)	118.7CH		92.6qC	2.24 (brd, 8.7)	47.2CH
6		160.6qC		159.8qC	7.35 (d, 7.8)	116.6CH		166.5qC	5.23 (brs)	73.8CH
7	6.47 (d, 2.3)	97.1CH	6.80 (d, 2.3	93.6CH		124.0qC	7.79 (brs)			208.8qC
7a		141.7qC		137.3qC		134.5qC				
8	5.76 (s)	116.9CH	3.47 (dd, 14.5, 8.7), 3.91 (dd, 14.6, 4.1)	25.6CH_2	3.30 (dd, 14.7, 3.7)	25.0CH_2		90.2qC		52.7qC
9		137.9qC	4.37 (dd, 8.7, 4.1)	56.8CH	4.31 (t, 3.7)	63.9CH	4.70 (brs)	73.2CH	2.61 (brd, 10.4)	41.6CH
10			7.18 (s)			165.1qC	4.62 (d, 5.0)	70.3CH		38.2qC
11		162.5qC		169.5qC			4.78 (dd, 8.7, 5.0)	70.4CH	1.60 (m), 1.96 (m)	23.9CH_2
12	4.35 (dd, 10.6, 5.9)	61.5CH	4.02 (dd, 7.8, 7.3)	59.1CH	3.61 (qd, 7.3, 1.8)	49.2CH	5.39 (dt, 1.4, 8.7)	128.0CH	1.83 (m), 2.43 (m)	25.9CH_2
13	2.49 (m,α-H), 1.99 (m,β-H)	29.2CH_2	1.99 (m), 2.31 (m)	28.2CH_2	7.96 (d, 1.8)	162.4qC	5.74 (qd, 8.7, 6.9)	129.8CH	2.59 (brd, 9.6)	49.1CH
14	1.99 (m), 2.13 (m)	22.1CH_2	1.97 (m), 1.85 (m)	22.6CH_2						46.6qC
15	3.58 (m), 3.84 (m)	44.8CH_2	3.58 (m), 3.61 (m)	45.4CH_2	3.47 (2H, br d, 7.4)	29. 0CH_2	1.74 (3H, dd, 6.9, 1.4)	13.7CH_3	1.91 (brd, 14.6), 2.27 (m)	40.6CH_2
16					5.35 (br t, 7.4)	122.4CH	1.69 (3H, s)	6.0CH_3	5.86 (brd,6.4)	73.4CH
17		155.1qC		165.6qC		131.7qC		194.8qC		147.5qC
18	5.39 (d, 9.2)	64.0CH		183.8qC	1.68 (3H, s)	25.5CH_3		132.3qC	1.18 (3H, s)	18.3CH_3
19	5.20 (d, 9.2)	120.4CH	6.54 (br s)	121.4CH	1.68 (3H, s)	17.7CH_3	8.32 (d, 7.3)	130.7CH	1.45 (3H, s)	27.5CH_3
20		138.3qC		158.2qC	0.13 (3H, d, 7.3)	19.6CH_3	7.50 (dd, 8.2,7.3)	128.8CH		130.8qC
21	1.59 (3H, s)	25.4CH_3	1.98 (3H, s)	28.1CH_3			7.66 (t, 8.2)	134.8CH		173.2qC
22	1.29 (3H, s)	18.3CH_3	2.17 (3H, s)	21.4CH_3			7.50 (dd, 8.2, 7.3)	128.8CH	2.53 (2H, m)	28.6CH_2
23							8.32 (d, 7.3)	130.7CH	1.49 (m), 2.41 (m)	24.2CH_2
24									1.49 (2H, m)	43.0CH_2
25										71.1qC
6-OCH_3	3.79 (3H, s)	55.5CH_3	3.85 (3H, s)	55.5CH_3						
8-OCH_3							3.42 (3H, s)	51.8CH_3		
9-OH										
10-OH					10.15 (br s)					

[a] Spectra were recorded in $CDCl_3$ at 600 and 150 MHz for ^{1}H and ^{13}C, respectively (TMS as internal standard). [b] NMR data for the rest of the moiety of **5**: 1.22 (6H, s, H-26/27), 1.28 (3H, d, $J = 6.9$ Hz, H-28), 0.92 (3H, s, H-30), 2.11 (3H, s, 6-COC$\underline{H}_3$), 1.96 (3H, s, 16-COC$\underline{H}_3$); 29.1(CH_3, C-26), 29.3 (CH_3, C-27), 13.1 (CH_3, C-28), 17.9 (CH_3, C-30), 20.7 (CH_3, 6-CO$\underline{C}H_3$), 168.9 (qC, 6-$\underline{C}$OCH_3), 20.6 (CH_3, 16-CO$\underline{C}H_3$), 170.4 (qC, 16-$\underline{C}$OCH_3).

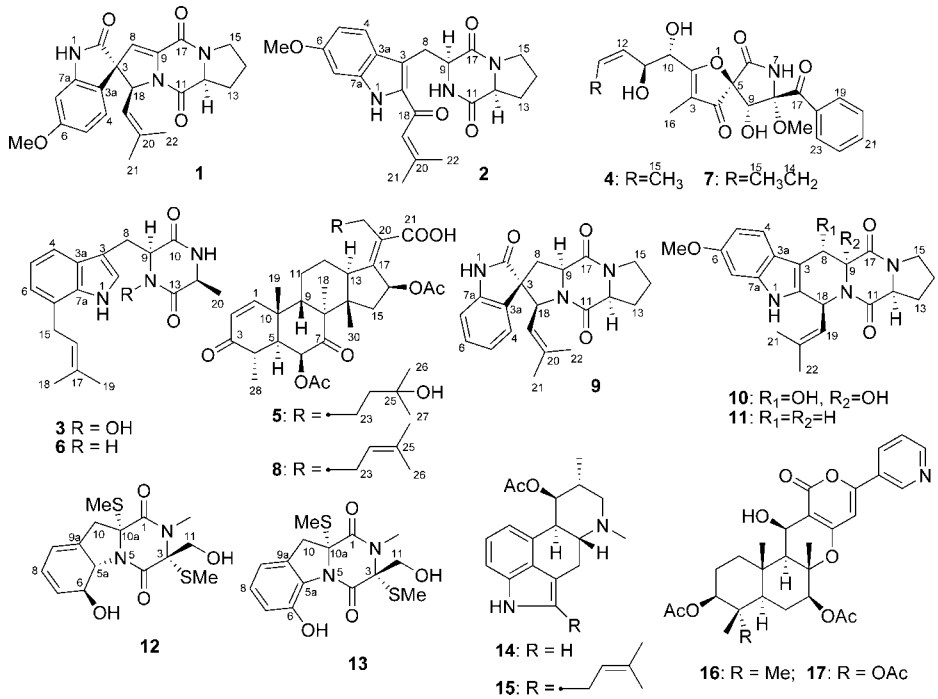


Figure 1. Structures of alkaloids **1**−**17**.

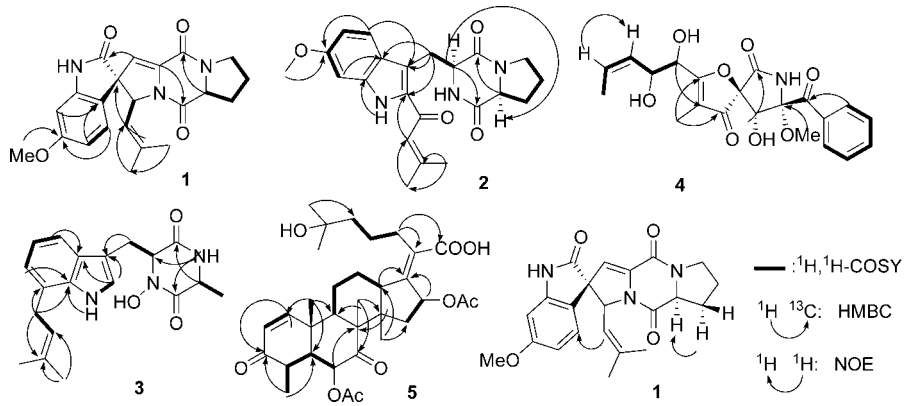


Figure 2. Key HMBC, ^{1}H−^{1}H COSY, and NOE correlations of **1**−**5**.

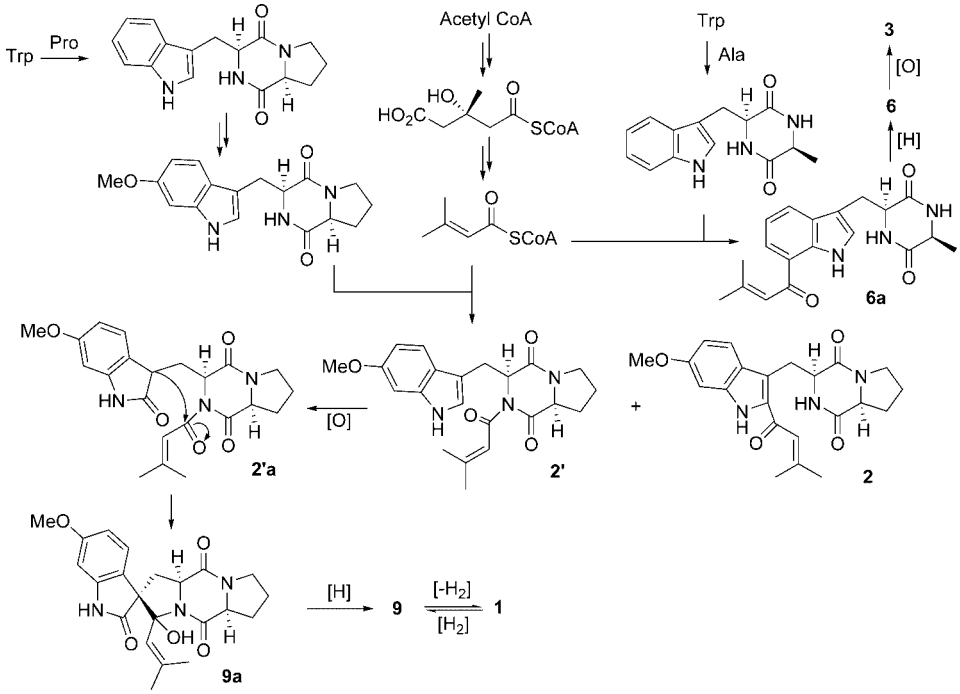
spectra of **4** were similar to those of **7**.[4,5] The differences include the absence of methylene signals at δ_H 2.08, 2.14 (m, H-14) and δ_C 21.3 (CH_2) and the downfield shifts for H-12, H-13, H-15, and C-12 and the upfield shifts for C-13 and C-15 observed in **4**. These data supported **4** as the 14-nor-derivative of **7**, and this was confirmed by the ^{1}H−^{1}H COSY correlations between H-15 (δ 1.74, 3H) and the olefinic proton H-13 (δ 5.74), between H-13 and H-12 (δ 5.39), between H-12 and H-11 (δ 4.78), and between H-11 and H-10 (δ 4.62) (Figure 2). The configuration of **4** was established as 5*S*,8*S*,9*R*,10*S*,11*S*,12*Z* by NOE correlation between H-12 and H-13 and by comparing the specific rotation ($[\alpha]_D$ −6.5) with that of pseurotin A ($[\alpha]_D$ −3.0).[4,5]

Compound **5** was obtained as white needles from MeOH−$CHCl_3$ (v/v 1:1). Its molecular formula was determined as $C_{33}H_{46}O_9$ by HRESIMS at *m*/*z* 609.3034 $[M + Na]^+$ (calcd 609.3040). Except for the side chain, its ^{1}H and ^{13}C NMR spectra were similar to those of helvolic acid (**8**) (Table 1),[6−8] indicating that **5** was a 29-nordammarane triterpenoid. The differences include the absence of the trisubstituted ethenyl moiety signals at δ_H 5.10 (t, J = 7.0 Hz, H-24), δ_C 122.7 (CH, C-24), and δ_C 132.9 (qC, C-25), while 1,1,2-trisubstituted ethanol moiety signals at δ_H 1.49 (m, 2H, H-24), δ_C 43.0 (CH, C-24), and δ_C 71.1 (qC, C-25) were present. Additionally, downfield shifts for C-26 and C-27 and an upfield shift for C-23 were observed. These data, combined with a mass increase of 18 amu (H_2O) compared to **8**, supported **5** as the hydrated derivative of the C_{24}=C_{25} ethylenic bond of **8**, which was further confirmed by ^{1}H−^{1}H COSY correlations from H-22 to H-24 and the key HMBC correlation between H-26 (δ 1.22, s) and C-24. The configuration of **5** was determined to be the same as **8** by comparison of the specific rotation ($[\alpha]_D$ −118.9 vs −105.1) and their NMR data. Thus, the structure of **5** was established as (4*S*,5*S*,6*S*,8*S*,9*S*,10*R*,13*R*,14*S*,16*S*,17*Z*)-6,16-diacetoxy-25-hydroxy-3,7-dioxy-29-nordammara-1,17(20)-dien-21-oic acid.

The new diketopiperazines **1**−**3** were probably biosynthesized via a mixed amino acid−mevalonic acid pathway. Compound **3** could be formed from the oxidation of **6** that may result from tryptophan and alanine and mevalonic acid. Cyclo(Trp-Pro) apparently derives from tryptophan and proline, which was further subjected to oxidation and methylation to form methoxylated cyclo(Trp-Pro). The latter may then react with mevalonic acid to form **2** and **2′**. The oxidized product **2′a** may be subjected to intramolecular aldol reaction to form **9a**, which may be further deoxygenated to yield **9**. Compound **1** could be produced from the dehydrogenation of **9** (Scheme 1).

In the MTT bioassay,[24] compounds **1**−**3** exhibited weak cytotoxicity against A-549 cells with IC_{50} values of 8.29, 1.28, and

Scheme 1. Postulated Biosynthesis Pathway of New Compounds **1**−**3**



7.31 μM, respectively. Compound **1**was also slightly active against HL-60 cells with an IC_{50} value of 9.71 μM. Compounds **4** and **5** were assayed for their antimicrobial activities using the disk diffusion method.[25] Both compounds **4** and **5** displayed significant growth inhibition against *Escherichia coli*, *Bacillus subtilis*, and *Micrococcus lysoleikticus* with MICs of 3.74, 14.97, and 7.49 μM and 10.65, 5.33, and 10.65 μM, respectively. Compounds **7** and **8** also showed antibiotic activities against *Escherichia coli*, *Bacillus subtilis*, and *Micrococcus lysoleikticus* with MICs of 14.49, 14.49, and 7.24 μM and 87.92, 21.98, and 10.99 μM, respectively. These observations indicated that compounds **4** and **5** were more potent than the corresponding compounds **7** and **8** in antimicrobial activity, respectively.

Experimental Section

General Experimental Procedures. Melting points were measured using a Yanaco MP-500D micromelting point apparatus and were uncorrected. Specific rotations were obtained on a JASCO P-1020 digital polarimeter. CD spectra were obtained on a JASCO J-810 spectropolarimeter. UV spectra were recorded on a Beckman DU 640 spectrophotometer. ^{1}H, ^{13}C NMR and DEPT spectra and 2D NMR were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard, and chemical shifts were recorded as δ values. ESIMS was measured on a Q-TOF Ultima Global GAA076 LC mass spectrometer. Semipreparative HPLC was performed using an ODS column [YMC-pack ODS-A, 10 × 250 mm, 5 μm, 4 mL/min].

Fungal Material. The fungus *Aspergillus sydowi* was isolated from a driftwood sample (PFW1) collected from the beach of Baishamen, Hainan, China, and identified by Professor Li Tian from the First Institute of Oceanography, State Oceanic Administration of China, Qingdao, China. The voucher specimen PFW1-13 is deposited in our laboratory at −80 °C. The producing strain was prepared on potato dextrose agar slants and stored at 4 °C.

Fermentation and Extraction. *A. sydowi* was grown under static conditions at 20 °C for 40 days in 1 L conical flasks (150) containing the liquid medium (300 mL/flask) composed of mannitol (20 g/L), maltose (20 g/L), glucose (10 g/L), monosodium glutamate (10 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.3 g/L), yeast extract (3 g/L), corn steep liquor (1 g/L), and seawater (45 L) at pH 6.5. The fermented whole broth (45 L) was filtered through cheesecloth into supernatants and mycelia. The supernatants were concentrated under reduced pressure to about a quarter of the original volume under reduced pressure and then extracted three times with an equal volume of EtOAc. The mycelia were extracted three times with acetone. The acetone solution was evaporated *in vacuo* to remove the acetone, and the residue was extracted three times with equal volumes of EtOAc to give an EtOAc solution. Both EtOAc solutions were combined and concentrated under reduced pressure to give a crude extract (86.7 g).

Purification. The crude extract (86.7 g) showing significant cytotoxicity against K562 cells at 100 μg/mL was separated into 18 fractions on Si gel CC using a step gradient elution with $CHCl_3$−petroleum ether (0−100%) and then with MeOH−$CHCl_3$ (0−50%). Fraction 10 (5.5 g) was separated further into 16 subfractions by Sephadex LH-20, eluted with MeOH−$CHCl_3$ (1:1). Compound **5** (15 mg) was obtained from fraction 10-2 after recrystallization from MeOH−$CHCl_3$. Fraction 10-3 (78 mg) was further purified by semipreparative HPLC (85% MeOH−H_2O, 4.0 mL/min) to yield **16** (6.4 mg) and **17** (9.1 mg). Fraction 10-12 (62 mg) was further purified by semipreparative HPLC (70% MeOH−H_2O, 4.0 mL/min) to yield **14** (6.4 mg) and **15** (9.1 mg). Fraction 11 (4.87 g) was further separated into 12 subfractions by Si gel CC using MeOH−$CHCl_3$ (99:1) as the eluent. Fraction 11-11 (1.82 g) was further separated into six subfractions by Sephadex LH-20. Then subfraction 11-11-2 (0.89 mg) was further separated into five subfractions by Si gel CC, and the obtained subfraction 11-11-2-1 (0.64 g) was recrystallized from MeOH−$CHCl_3$ to yield **8** (11 mg). Subfraction 11-11-2-2 (52 mg) was purified by semipreparative HPLC (50% MeOH−H_2O, 4.0 mL/min) to give **1** (7.6 mg) and **9** (11.2 mg). Subfraction 11-11-2-5 (24 mg) was further purified by semipreparative HPLC (65% MeOH−H_2O, 4.0 mL/min) to yield **2** (7.0 mg). Fraction 13 (4 g) was separated into four subfractions by Sephadex LH-20 (MeOH−$CHCl_3$, 1:1). Subfraction 13-1 (1.68 g) was further separated into five subfractions by Si gel CC using a step gradient elution of petroleum ether−acetone and then MeOH−$CHCl_3$. The obtained subfraction 13-1-4 (0.21 g) was purified by semipreparative HPLC to give **4** (8.6 mg) and **7** (150 mg). Fraction 15 (2.16 g) was separated into five subfractions by Sephadex LH-20 (MeOH−$CHCl_3$ 1:1). Fraction 15-3 (292 mg) was further separated into four subfractions by Si gel CC using a step gradient elution of petroleum ether−acetone (100−0%), and subfraction 15-3-2 (67 mg) was then purified by semipreparative HPLC (60% MeOH−H_2O, 4.0 mL/min) to give **3** (7.0 mg). Fraction 15-2 (732 mg) was separated into nine subfractions by Si gel CC using a step gradient elution of petroleum ether−acetone (100−0%), and subfraction 15-2-6 (141 mg) was further purified by semipreparative HPLC (70% MeOH−H_2O, 4.0 mL/min) to give **10** (8.2 mg), **6** (23.1 mg), and **11** (3.0 mg). Fraction 11-10 (521 mg) was separated into four subfractions by Sephadex LH-20 (MeOH−$CHCl_3$,

1:1). Subfraction 11-10-4 (32 mg) was further purified by semipreparative HPLC (55% MeOH−H_2O, 4.0 mL/min) to yield **12** (2.2 mg) and **13** (5.1 mg).

Biological Assays. Cytotoxicity was evaluated by the MTT method[23] using A549 and HL-60 cell lines. The cell lines were grown in RPMI-1640 supplemented with 10% FBS under a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Then 200 μL of these cell suspensions at a density of 5×10^4 cell mL^{-1} was plated in 96-well microtiter plates and incubated for 24 h under the above condition. Then 2 μL of the test compound solutions (in DMSO, concentration: 10 mM, 1 mM, 100 μM, 10 μM, 1 μM) at different concentrations was added to each well and further incubated for 72 h under the same conditions. Then 20 μL of the MTT solution (5 mg/mL in IPMI-1640 medium) was added to each well and incubated for 4 h. The old medium (150 μL) containing MTT was then gently replaced by DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined on a Spectra Max Plus plate reader at 520 nm.

Antimicrobial activity was assayed by the disk diffusion method (DDM).[25] Briefly, 5 μL of each of the compounds at 1000, 100, 10, and 1 μg/mL in MeOH was dropped on a standard disk ($\Psi = 6$ mm), which was subsequently placed on an agar plate preinoculated with a 0.2 mL suspension in LB (yeast extract 5 g/L, peptone 10 g/L, NaCl 5 g/L, and agar 20 g/L, pH 7.0). All test plates were then incubated for 24 h at 37 °C, followed by measurement of diameters of the inhibition zone around each of the extract-carrying disks. According to the above results, final concentrations of each compound in the medium were set at 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 μg/mL by the continuous dilution method. The procedure was repeated. The minimum inhibitory concentrations (MICs) were defined as the lowest concentration at which no microbial growth could be observed.

6-Methoxyspirotryprostatin B (1): pale yellow, amorphous powder; $[\alpha]_D^{21}$ −47.7 (*c* 0.26, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (4.1), 251(3.6), 336 (3.5) nm; CD (MeOH) λ_{max} ($\Delta\epsilon$) 268 (−46.3), 242 (+148.7), 209 (+7.0), 198 (−24.2); IR (KBr) ν_{max} 3450, 3234, 2966, 1722, 1677, 1636, 1454, 1300, 1156, 800 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 1; HRESIMS *m/z* 392.1592 $[M - H]^-$ (calcd for $C_{22}H_{22}N_3O_4$ 392.1610).

Spirotryprostatin A (9): pale yellow, amorphous powder; $[\alpha]_D^{21}$ −94.1 (*c* 0.16, $CHCl_3$); CD (MeOH) λ_{max} ($\Delta\epsilon$) 265 (−4.6), 237 (+9.8), 213 (+20.6), 193 (−24.1).

18-Oxotryprostatin A (2): yellow, amorphous powder; $[\alpha]_D^{21}$ −31.5 (*c* 0.14, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (4.0), 255 (3.6), 350 (3.4) nm; IR (KBr) ν_{max} 3446, 1650, 1541, 1518, 671 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 1; HRESIMS *m/z* 394.1753 $[M - H]^-$ (calcd for $C_{22}H_{24}N_3O_4$ 394.1767).

14-Hydroxyterezine D (3): pale yellow, amorphous powder; $[\alpha]_D^{22}$ +17.8 (*c* 0.13, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (4.0), 265 (3.4) nm; IR (KBr) ν_{max} 3393, 3262, 2970, 2928, 1670, 1655, 1456, 1316, 1024, 1001, 994, 819, 667 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 1; HRESIMS *m/z* 340.1650 $[M - H]^-$ (calcd for $C_{19}H_{22}N_3O_3$ 340.1661).

14-Norpseurotin A (4): pale yellow power; $[\alpha]_D^{25}$ −6.5 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.0), 256 (3.6), 284 (3.7) nm; IR (KBr) ν_{max} 3390, 3277, 2963, 2846, 1724, 1700, 1634, 1440, 1282, 1168, 1102, 667 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 1; HRESIMS *m/z* 440.1324 $[M + Na]^+$ (calcd for $C_{21}H_{23}NO_8Na$ 440.1321).

(4*S*,5*S*,6*S*,8*S*,9*S*,10*R*,13*R*,14*S*,16*S*,17*Z*)-6,16-Diacetoxy-25-hydroxy-3,7-dioxy-29-nordammara-1,17(20)-dien-21-oic acid (5): white needles; mp 206−208 °C; $[\alpha]_D^{26}$ −118.9 (*c* 0.07, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 223 (3.4) nm; IR (KBr) ν_{max} 3445, 1747, 1716, 1673, 1375, 1258, 1219, 1145, 1031 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table 1; HRESIMS *m/z* 609.3034 $[M + Na]^+$ (calcd for $C_{33}H_{46}O_9Na$ 609.3040).

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