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NOTE

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Pestaloquinols A and B, Isoprenylated Epoxyquinols from *Pestalotiopsis* sp.

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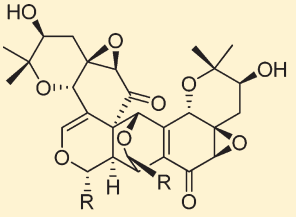
Supporting Information

ABSTRACT: Two new isoprenylated epoxyquinol derivatives, pestaloquinols A (**2**) and B (**3**), and their putative biosynthetic precursor, cytosporin D (**1**), were isolated from the crude extract of the plant endophytic fungus *Pestalotiopsis* sp. The structures of these compounds were elucidated primarily by NMR experiments. Pestaloquinols A (**2**) and B (**3**) possess a previously undescribed nonacyclic ring system and showed cytotoxicity against HeLa cells.



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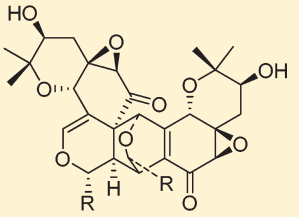
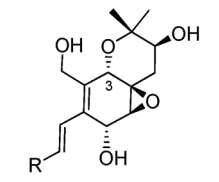


3 R = pentyl

Epoxyquinols A−C (epoxyquinol A; **4**) are potent angiogenesis inhibitors featuring a unique pentaketide skeleton derived from Diels−Alder dimerization of diastereomeric 3-methyl-7,8-dihydro-3*H*-isochromen-5(6*H*)-one derivatives.[1,2] Although the epoxyquinols represent a large family of natural products with a broad range of biological activities,[3] dimeric epoxyquinols are very rare. Examples include panepophenanthrin isolated from the mushroom *Panus rudis* Fr. IFO 8994,[4] epoxyquinols A−C[1,2] and epoxytwinol A[5] from an unidentified fungus, torreyanic acid (**5**) from the plant endophytic *Pestalotiopsis microspora*,[6] and its C-3 ketone reduced analogue from an endolichenic *Pestalotiopsis* sp.[7] Because of their structural novelty, potential as small-molecule probes to elucidate the mechanisms leading to angiogenesis, and the need to synthesize various derivatives for structure−activity relationship studies, the epoxyquinols have attracted much attention from the synthetic organic chemistry community. To date, total syntheses of epoxyquinols A−C have been accomplished by different research groups.[8]

Chemical studies of the fungal genus *Pestalotiopsis* have afforded a variety of bioactive natural products.[9,10] During an ongoing search for new cytotoxic agents from the plant endophytic fungi, a strain of *Pestalotiopsis* sp. isolated from the branches of *Podocarpus macrophyllus* (Thunb.) D. Don was subjected to chemical investigation. An EtOAc extract of its solid-substrate fermentation culture showed cytotoxicity against HeLa cells. Bioassay-guided fractionation of the extract led to the isolation of pestaloquinols A (**2**) and B (**3**), two isoprenylated heterodimers with a previously undescribed nonacyclic ring system that could be derived from Diels−Alder dimerization of diastereomeric 2*H*-pyran monomers. In addition, cytosporin D (**1**),[11] a putative biosynthetic precursor of **2** and **3**, was also isolated from the crude extract. We herein report the structure elucidation, cytotoxicity, and plausible biogeneses of **2** and **3**.



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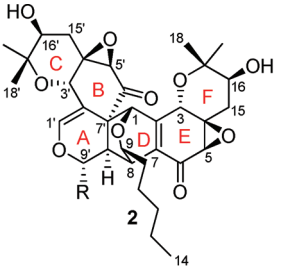

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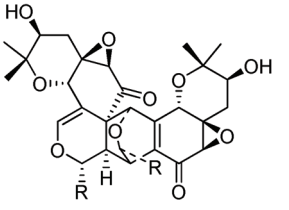

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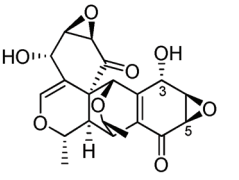

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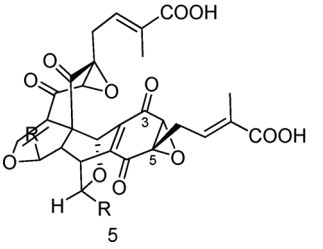

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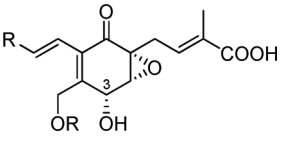

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Table 1. NMR Data of Pestaloquinol A (2) in $CDCl_3$

position	δ_C[a], mult.	δ_H[b] (J in Hz)	HMBC
1	71.7, CH	5.26, s	2, 3, 7, 9, 2′, 6′, 7′, 8′
2	151.3, qC		
3	65.2, CH	4.51, d (0.6)	1, 5, 7, 17
4	61.8, qC		
5	60.1, CH	3.31, d (1.2)	3, 7, 15
6	189.6, qC		
7	134.6, qC		
8	37.4, CH	3.16, dd (1.8, 1.2)	2, 6, 7, 10, 7′, 8′, 9′
9	71.3, CH	4.17, dd (6.6, 6.0)	1, 7, 8′
10	34.9, CH_2	0.92, m; 1.10, m	8, 11, 12
11	25.0, CH_2	1.22, m; 1.16, m	9, 10, 12, 13
12	31.7, CH_2	1.20, m; 1.16, m	10, 11, 13, 14
13	22.4, CH_2	1.24, m	11, 12, 14
14	13.9, CH_3	0.83, t (7.2)	12, 13
15	34.5, CH	2.30, dd (13, 11); 1.74, dd (13, 4.8)	3, 4, 5, 16, 17
16	72.4, CH	3.70, dd (11, 4.8)	4, 15, 18, 19
17	76.6, qC		
18	15.9, CH_3	1.35, s	16, 17, 19
19	26.7, CH_3	1.26, s	16, 17, 18
1′	143.9, CH	6.71, d (1.8)	2′, 3′, 7′, 9′
2′	111.7, qC		
3′	64.5, CH	4.85, br s	1′, 2′, 5′, 7′, 17′
4′	68.3, qC		
5′	61.1, CH	3.22, br s	3′, 6′, 7′, 15′
6′	200.1, qC		
7′	50.8, qC		
8′	36.0, qC	2.51, br s	1, 7, 2′, 6′, 7′, 10′
9′	78.7, CH	4.07, dd (9.6, 4.8)	8, 1′, 7′, 11′
10′	33.0, CH_2	1.42, m; 1.17, m	8′, 12′
11′	25.7, CH_2	1.24, m; 1.31, m	9′, 10′, 12′, 13′
12′	31.4, CH_2	1.21, m	10′, 11′, 13′, 14′
13′	22.5, CH_2	1.24, m	11′, 12′, 14′
14′	14.0, CH_3	0.86, t (6.0)	12′, 13′
15′	34.7, CH_2	2.27, dd (14, 3.0); 1.83, dd (14, 7.2)	3′, 4′, 5′, 17′
16′	72.1, CH	3.87, dd (7.2, 3.0)	4′, 17′, 18′, 19′
17′	75.6, qC		15′, 16′, 18′, 19′
18′	18.5, CH_3	1.35, s	16′, 17′, 19′
19′	27.9, CH_3	1.26, s	16′, 17′, 18′

[a] Recorded at 150 MHz. [b] Recorded at 600 MHz.

The structure of the known compound cytosporin D (**1**) was identified by comparison of its NMR and MS data with those reported.[11] It was previously reported as a secondary metabolite of the marine-derived fungus *Eutypella scoparia* isolated from the marine pulmonate mollusc *Onchidium* sp.

The molecular formula of pestaloquinol A (**2**) was determiend to be $C_{38}H_{52}O_{10}$ (13 degrees of unsaturation) by HRESIMS (m/z 691.3413 $[M + Na]^+$; Δ −4.0 mmu). Anaysis of its ^1H and ^{13}C NMR (Table 1) and HMQC data revealed the presence of six methyls, 10 methylenes, 11 methines (nine of which are oxygenated), five sp^3 quaternary carbons (four oxygenated), four olefinic carbons (one of which is protonated), and two ketone carbons including an α,β-conjugated one. These data accounted for all the ^1H and ^{13}C NMR resonances except for two exchangeable protons, suggesting that **2** was a nonacycle. The $^1H-^1H$ COSY NMR data showed three isolated spin systems, which were C-15−C-16, C-15′−C-16′, and C-14−C-14′ via C-8 and C-8′. HMBC correlations from H-1′ to C-2′, C-7′, and C-9′, from H-8′ to C-2′, C-7′, and C-10′, and from H-9′ to C-1′ and C-7′ established a 3,4-dihydro-2*H*-pyran moiety (ring A) with a pentyl group attached to C-9′. Cross-peaks from H-3′ to C-1′, C-2′, C-5′, and C-7′, from H-5′ to C-3′, C-6′, and C-7′, from H-8′ to C-6′, and from H_2-15′ and H-16′ to C-4′ completed a cyclohexanone unit (ring B) fused to ring A at C-2′/C-7′ with C-15′ attached to C-4′. In turn, correlations from H_2-15′, H-16′, H_3-18′, and H_3-19′ to C-17′ indicated that C-16′, C-18′, and C-19′ are all connected to C-17′. The correlation from H-3′ to C-17′ linked C-3′ and C-17′ via an ether bond, completing the substructure of ring C. Similarly, ring F was assigned the same planar structure as C, whereas ring E was deduced as the C-2/C-7

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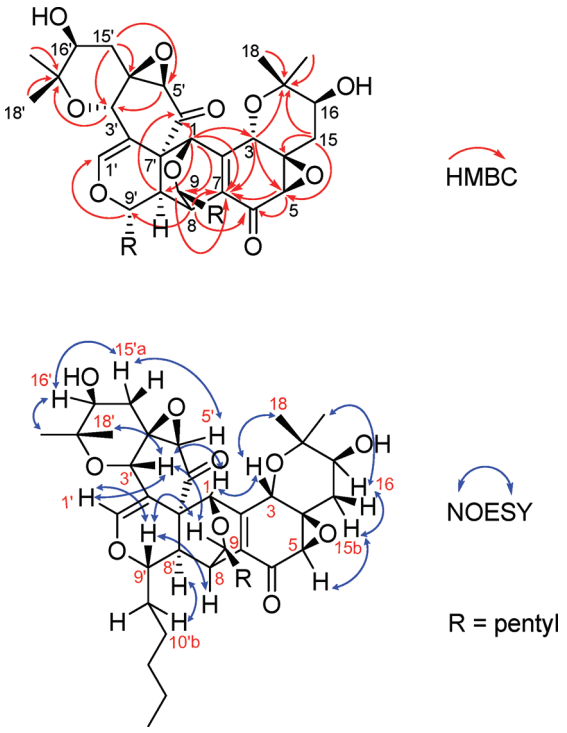

Figure 1. Selected HMBC and NOESY correlations of **2**.

olefinic counterpart of B by relevant HMBC data (Figure 1). Further correlations from H-1 to C-6, C-7, C-7′, C-8′, and C-9′ established a cyclohexene moiety (ring D) linking the left and right moieties. HMBC cross-peaks from H-1 to C-9 and from H-9 to C-1 revealed an ether bond between C-1 and C-9. Considering the relatively high-field ^{13}C chemical shifts of C-4, C-4′, C-5, and C-5′ (δ_C 61.8, 68.3, 60.1, and 61.1, respectively) and the unsaturation requirement, each of the C-4/C-5 and C-4′/C-5′ pairs in **2** was connected to an oxygen atom to form two epoxide moieties.[1,2] The remaining two exchangeable protons were assigned by default as C-16- and C-16′-OH, respectively, to complete the planar structure of **2** as depicted.

The relative configuration of **2** was deduced by NOESY data (Figure 1). NOESY correlations of H-15′a with H-5′ and H-16′ and of H-16′ with H_3-19′ indicated that these protons are on the same face of ring C, whereas those of H-3′ with H-1, H-9, and H_3-18′ placed these protons on the opposite face of the ring system. NOESY cross-peaks of H-9′ with H-8 and H-9 and of H-3 with H-1 and H_3-18 suggested that these protons adopt the same orientation as H-3′. Those of H-15b with H-5 and H-16, H-16 with H_3-19, and H-8′ with H-10′b revealed their spatial proximity. On the basis of these data, the relative configuration of **2** was determined as shown.

The absolute configuration of **2** was assigned using the modified Mosher method.[12] Treatment of **2** with (*S*)- and (*R*)-MTPA Cl afforded the (*R*)- (**2a**) and (*S*)-MTPA (**2b**) diesters, respectively. The difference in chemical shifts ($\Delta\delta = \delta_S - \delta_R$) for **2b** and **2a** was calculated to assign the 16*S* and 16′*S* configuration (Figure 2). Considering the relative configuration deduced from NOESY data, the 1*S*, 3*S*, 3′*S*, 4*R*, 4′*R*, 5*R*, 5′*R*, 7′*R*, 8*S*, 8′*R*, 9*R*, and 9′*S* absolute onfiguration was assigned for **2**.

Pestaloquinol B (**3**) was given the same molecular formula, $C_{38}H_{52}O_{10}$, as **2** by HRESIMS (*m*/*z* 691.3437 $[M + Na]^+$; Δ −1.6 mmu). Interpretation of ^{1}H and ^{13}C NMR spectroscopic

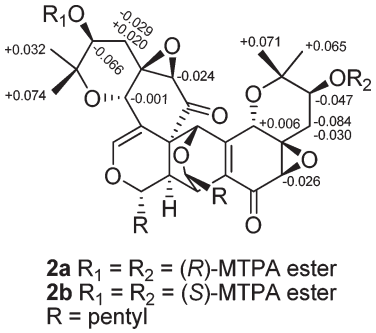

Figure 2. $\Delta\delta$ values (in ppm) = $\delta_S - \delta_R$ for (*R*)- and (*S*)-MPTA diesters **2a** and **2b**.

data (Table 2) revealed the same planar structure as **2**, which was confirmed by 2D NMR data, suggesting that **3** is a stereoisomer of **2**. The relative configuration of **3** was deduced by comparison of its NMR data with those of **2** and by analysis of its NOESY data (Figure 3). A NOESY correlation of H-8′ with H-9 revealed their proximity in space, which is opposite that observed in **2**. In addition, the correlations of H-9 with H-3′ and H-9′ found in **2** were not observed in the NOESY spectrum of **3**, whereas all other cross-peaks including the key ones of H-8′ with H-10′ and of H-8 with H-9′ remained unchanged, implying a change in orientation of the ether bridge between C-1 and C-9 with respect to ring D. Therefore, compound **3** was deduced to have the 1*R*, 8*R*, and 9*S* absolute configuration, while all other stereogenic centers have the same configuration as **2**.

Compounds **2** and **3** were tested for cytotoxicity against HeLa (cervical epithelium) cells, both showing an IC_{50} value of 8.8 μM (the positive controls VP-16 and D-24851 showed IC_{50} values of 1.63 and 0.88 μM, respectively).

Pestaloquinols A (**2**) and B (**3**) are unique isoprenylated epoxyquinol derivatives featuring a previously undescribed nonacyclic skeleton. Although compounds **2** and **3** are structurally related to the above-mentioned dimeric epoxyquinols, they possess two additional tetrahydropyran rings (C and F) *cis*-fused to the 7-oxabicyclo[4.1.0]hept-3-en-2-one (B and E). Biogenetically, compound **5** could be generated by Diels−Alder dimerization of diastereomeric ambuic acid (**6**) derivatives,[6,13,14] whereas **2** and **3** were presumably derived from the co-isolated precursor **1** via oxidation, electrocyclization, and Diels−Alder reaction cascade (Scheme 1).[6,8] In this scheme, the tetahydropyrans in **2** and **3** are derived from cyclization of the C-4/4′ isoprenyl group and C-3/3′-OH. The isoprenyl group in **5** is meta to the C-3-OH and does not favor such a cyclization as that which occurs in **2** and **3**. Structurally, pestaloquinols A (**2**) and B (**3**) are "hybrid"-type metabolites related to both the epoxyquinols and torreyanic acid. The discovery of these metabolites increases the structural diversity of the known epoxyquinols and demonstrates that the genus *Pestalotiopsis* deserves increased attention from both mycologists and natural products chemists.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV data were recorded on a Hitachi U-2800 spectrophotometer. IR data were recorded using a Shimadzu FTIR-8400S Bruker Vertex 70 spectrophotometer. ^{1}H and ^{13}C NMR data were acquired with Bruker Avance-500 and -600

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Table 2. NMR Data of Pestaloquinol B (3) in $CDCl_3$

position	δ_C[a], mult.	δ_H[b] (J in Hz)	HMBC
1	70.2, CH	5.50, s	2, 3, 7, 9, 2′, 6′, 7′, 8′
2	150.7, qC		
3	65.5, CH	4.31, br s	1, 5, 7, 17
4	62.6, qC		
5	60.3, CH	3.34, br s	3, 7, 15
6	190.8, qC		
7	132.7, qC		
8	35.5, CH	3.16, br s	2, 6, 7, 10, 7′, 8′, 9′
9	76.0, CH	3.82, t (6.5)	1, 7, 8′
10	34.1, CH_2	1.04, m; 0.94, m	8, 11, 12
11	24.6, CH_2	1.17, m	9, 10, 12, 13
12	31.3, CH_2	1.18, m	10, 11, 13, 14
13	22.4, CH_2	1.21, m	11, 12, 14
14	13.9, CH_3	0.85, t (7.0)	12, 13
15	34.5, CH_2	2.30, dd (14, 12); 1.84, dd (14, 4.5)	3, 4, 5, 16, 17
16	72.5, CH	3.79, dd (12, 4.5)	4, 15, 18, 19
17	76.5, qC		
18	15.8, CH_3	1.39, s	16, 17, 19
19	27.2, CH_3	1.33, s	16, 17, 18
1′	145.4, CH	6.51, s	2′, 3′, 7′, 9′
2′	110.7, qC		
3′	64.9, CH	4.69, br s	1′, 2′, 5′, 7′,17′
4′	68.0, qC		
5′	60.0, CH	3.35, br s	3′, 6′, 7′, 15′
6′	200.8, qC		
7′	53.6, qC		
8′	40.0, qC	3.14, dd (5.5, 2.0)	1, 7, 2′, 6′, 7′, 10′
9′	77.4, CH	3.36, m	8, 1′, 7′, 11′
10′	32.4, CH_2	1.43, m; 1.28, m	8′, 12′
11′	25.0, CH_2	1.20, m	9′, 12′, 13′
12′	31.7, CH_2	1.15, m	10′, 11′, 13′, 14′
13′	22.5, CH_2	1.21, m	11′, 12′, 14′
14′	13.9, CH_3	0.85, t (7.5)	12′, 13′
15′	34.3, CH_2	2.35, dd (15, 2.5); 1.79, dd (15, 7.0)	3′, 4′, 5′, 17′
16′	71.7, CH	3.89, dd (6.5, 2.5)	4′, 17′, 18′, 19′
17′	75.8, qC		15′, 16′, 18′, 19′
18′	18.3, CH_3	1.33, s	16′, 17′, 19′
19′	27.7, CH_3	1.23, s	16′, 17′, 18′

[a] Recorded at 150 MHz. [b] Recorded at 600 MHz.

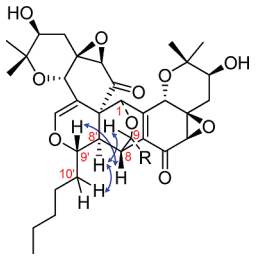

Figure 3. Selected NOESY correlations of **3**.

spectrometers using solvent signals ($CDCl_3$; δ_H 7.26/δ_C 77.6) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. HRESIMS data were acquired using a LTQ Orbitrap XL mass spectrometer.

Fungal Material. The culture of *Pestalotiopsis* sp. was isolated from the branches of *Podocarpus macrophyllus* (Thunb.) D. Don at Kunming World Horticultural Exposition Garden, Kunming, People's Republic of China, in November 2002. The isolate was identified by one of the authors (L.G.) based on morphology and sequence analysis of the ITS region of the rDNA and assigned the accession number 789 at L.G.'s culture collection of Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. Agar plugs were cut into small pieces (about 0.5×0.5×0.5 cm^3) under aseptic conditions, and 15 of these pieces were used to inoculate in three Erlenmeyer flasks (250 mL), each containing 50 mL of media (0.4% glucose, 1% malt extract, and 0.4% yeast extract; final pH adjusted to 6.5). The three inoculated flasks were incubated at 25 °C on a rotary shaker at 170 rpm for five days to prepare the seed culture. Spore inoculum was prepared by suspending the seed culture in sterile, distilled H_2O to give a final spore/cell

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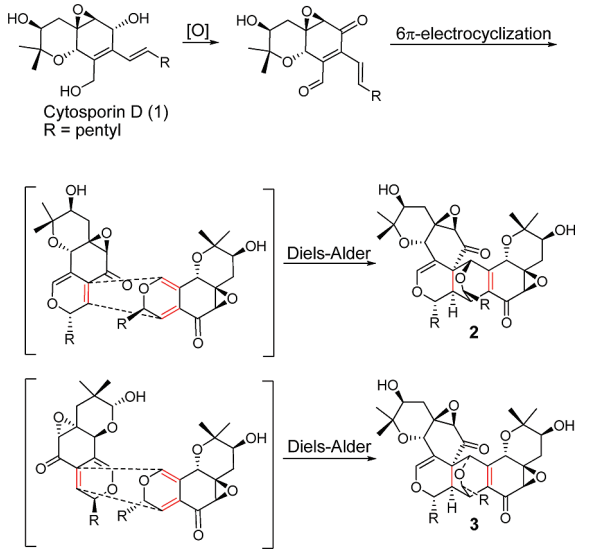
Scheme 1. Hypothetical Biosynthetic Pathways for Compounds 2 and 3



suspension of 1×10^6/mL determined by microscope and hemocytometer. Fermentation was carried out in 12 Fernbach flasks (500 mL) each containing 80 g of rice. Distilled H_2O (120 mL) was added to each flask, and the contents were soaked overnight before autoclaving at 15 psi for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 mL of the spore inoculum and incubated at 25 °C for 40 days.

Extraction and Isolation. The fermented material was extracted successively with petroleum ether (oil, 15 g) and EtOAc (3 × 500 mL). The organic solvent EtOAc was evaporated to dryness under vacuum to afford a crude extract (2.5 g), which was fractionated by silica gel column chromatography (CC) (5 × 25 cm) using CH_2Cl_2−MeOH gradient elution. The fraction (205 mg) eluted with 99:1 and 99:2 CH_2Cl_2−MeOH was further separated by silica gel CC using CH_2Cl_2−MeOH gradient elution to afford pestaloquinol A (**2**; 10 mg) and a 12 mg mixture. Further separation of the mixture by RP HPLC (Agilent Zorbax SB-C_{18} column; 5 μm; 9.4 × 250 mm; 85% MeOH in H_2O for 5 min, followed by 85−100% for 30 min) afforded pestaloquinol B (**3**; 2.0 mg, t_R 25.0 min). The fraction (234 mg) eluted with 96:4 CH_2Cl_2−MeOH was separated by silica gel CC using CH_2Cl_2−MeOH gradient elution to afford a subfraction (56 mg), and further purification of this subfraction by RP HPLC (35% CH_3CN in H_2O for 5 min, followed by 35−40% for 60 min) afforded cytosporin D (1; 3.6 mg, t_R 29.0 min).

*Pestaloquinol A (**2**):*. amorphous powder; $[\alpha]^{25}_D$ −59.0 (*c* 0.30, MeOH); UV (MeOH) λ_{max} (log ε) 209 (1.20), 250 (0.59) nm; IR (KBr) ν_{max} 3438, 2954, 2931, 1716, 1681, 1458, 1379, 1058 cm^{-1}; ^1H and ^{13}C NMR and HMBC data see Table 1; HRESIMS *m/z* 691.3413 (calcd for $C_{38}H_{52}O_{10}Na$, 691.3453).

*Preparation of (R)- (**2a**) and (S)-MTPA (**2b**) Esters.* A sample of **2** (1.0 mg, 0.0015 mmol), (*R*)-MPTA Cl (5.0 μL, 0.026 mmol), and pyridine-d_5 (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 20 h. The mixture was purified by RP HPLC (Cap cell pack column; 5 μm; 4.6 × 250 mm; 20% MeOH in H_2O for 2 min, followed by 20−100% for 16 min) to afford **2a** (1.0 mg, t_R 27.5 min): ^1H NMR ($CDCl_3$, 600 MHz) δ 6.67 (1H, d, *J* = 1.8 Hz, H-1′), 5.13 (1H, dd, *J* = 3.0, 6.0 Hz, H-16′), 5.09 (1H, s, H-1), 4.98 (1H, dd, *J* = 5.4, 11 Hz, H-16), 4.73 (1H, s, H-3′), 4.52 (1H, s, H-3), 4.14 (1H, br t, *J* = 6.0 Hz, H-9), 4.07 (1H, dd, *J* = 5.4 Hz, 9.0, H-9′), 3.39 (1H, br s, H-5), 3.18 (1H, br s, H-5′), 3.18 (1H, br s, H-8), 2.55 (1H, br d, *J* = 2.4 Hz, H-8′), 2.51 (1H, dd, *J* = 3.0, 15 Hz, H-15′a), 2.27 (1H, br t, *J* = 12 Hz, H-15a), 2.00 (1H, dd, *J* = 4.8, 13 Hz, H-15b), 1.90 (1H, dd, *J* = 6.0, 15 Hz, H-15′b), 1.30 (3H, s, H-18′), 1.26 (3H, s, H-18), 1.20 (3H, s, H-19′), 1.15 (3H, s, H-19), 0.87 (3H, *J* = 7.2 Hz, H-14′), 0.84 (3H, t, *J* = 7.2 Hz, H-14).

Similarly, a sample of **2** (1.0 mg, 0.0015 mmol), (*R*)-MPTA Cl (5.0 μL, 0.026 mmol), and pyridine-d_5 (0.5 mL) were allowed to react in an NMR tube at ambient temperature for 20 h. The mixture was purified by analytic RP HPLC (Cap cell pack column; 5 μm; 4.6×250 mm; 20% MeOH in H_2O for 2 min, followed by 20−100% for 16 min) to afford **2b** (0.9 mg, t_R 27.8 min): ^1H NMR ($CDCl_3$, 600 MHz) δ 6.67 (1H, d, *J* = 1.8 Hz, H-1′), 5.20 (1H, dd, *J* = 3.0, 6.0 Hz, H-16′), 5.07 (1H, s, H-1), 5.02 (1H, dd, *J* = 4.8, 11 Hz, H-16), 4.73 (1H, s, H-3′), 4.51 (1H, s, H-3), 4.14 (1H, br t, *J* = 6.0 Hz, H-9), 4.07 (1H, dd, *J* = 4.8, 9.0 Hz, H-9′), 3.39 (1H, br s, H-5), 3.21 (1H, br s, H-5′), 3.18 (1H, br s, H-8), 2.55 (1H, br s, H-8′), 2.54 (1H, dd, *J* = 3.0, 15 Hz, H-15′a), 2.35 (1H, br t, *J* = 12 Hz, H-15a), 2.03 (1H, dd, *J* = 4.8, 12 Hz, H-15b), 1.88 (1H, dd, *J* = 6.0, 15 Hz, H-15′b), 1.27 (3H, s, H-18′), 1.20 (3H, s, H-18), 1.13 (3H, s, H-19′), 1.09 (3H, s, H-19), 0.87 (3H, *J* = 7.2 Hz, H-14′), 0.84 (3H, t, *J* = 7.2 Hz, H-14).

*Pestaloquinol B (**3**):*. colorless oil; $[\alpha]^{25}_D$ +11.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 206 (1.22), 247 (0.60) nm; IR (KBr) ν_{max} 3446, 2955, 2932, 1717, 1682, 1458, 1379, 1059 cm^{-1}; ^1H and ^{13}C NMR, and HMBC data see Table 2; HRESIMS *m/z* 691.3437 (calcd for $C_{38}H_{52}O_{10}Na$, 691.3453).

*Cytosporin D (**1**):*. ^1H and ^{13}C NMR and ESIMS data were consistent with literature.[11]

MTT Assay (ref 15). The assay was performed in triplicate. In a 96-well plate, each well was plated with 10^4 cells. After cell attachment overnight, the medium was removed, and each well was treated with 50 μL of medium containing 0.2% DMSO, or appropriate concentrations of the test compounds, or the positive control cisplatin (10 mg/mL as stock solution of a compound in DMSO and serial dilutions; the test compounds showed good solubility in DMSO and did not precipitate when added to the cells). Cells were treated at 37 °C for 4 h in a humidified incubator and an atmosphere containing 5% CO_2 and then allowed to grow for another 48 h after the medium was changed to fresh Dulbecco's modified Eagle medium (DMEM). The medium was removed from the wells, and in the dark, 50 μL of a solution containing 0.5 mg/mL MTT (Sigma) dissolved in serum-free medium or phosphate-buffered saline (PBS) was added and then incubated at 37 °C for 3 h. Upon removal of MTT/medium, 100 μL of DMSO was added to each well and agitated at 60 rpm for 5 min to dissolve the precipitate. The assay plate was measured at 540 nm using a microplate reader.

■ ASSOCIATED CONTENT

Supporting Information. ^1H and ^{13}C NMR spectra of **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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