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Cytotoxic Norsesquiterpene Peroxides from the Endophytic Fungus *Talaromyces flavus* Isolated from the Mangrove Plant *Sonneratia apetala*

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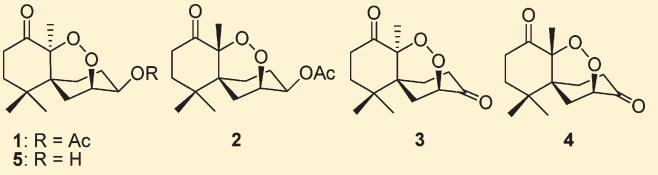
Supporting Information

ABSTRACT: Four new norsesquiterpene peroxides, named talaperoxides A−D (**1**−**4**), as well as one known analogue, steperoxide B (**5**, or merulin A), have been isolated from a mangrove endophytic fungus, *Talaromyces flavus*. Their structures were elucidated mainly by 1D and 2D NMR. Structures of **1**, **2**, and **5** were further confirmed by single-crystal X-ray diffraction, and their absolute configurations were also determined using copper radiation. Cytotoxic activities of compounds **1**−**5** were evaluated in vitro against human cancer cell lines MCF-7, MDA-MB-435, HepG2, HeLa, and PC-3. Compounds **2** and **4** showed cytotoxicity against the five human cancer cell lines with IC_{50} values between 0.70 and 2.78 μg/mL.



Terpene peroxides possess a wide range of biological activities, including antimalarial, antitumor, antimicrobial, and antiviral activities.[1−3] A typical example of sesquiterpene peroxide is artemisinin, which has already been clinically applied as an antimalarial drug.[4] Mangrove endophytes have proven to be a rich source of novel biologically active compounds.[5−8] Recently, in our ongoing search for new and potent antitumor natural products from mangrove endophytic fungi, four new norsesquiterpene peroxides, talaperoxides A−D (**1**−**4**), along with one known analogue, steperoxide B[9] (**5**, also named merulin A[10]), were isolated from the fungus *Talaromyces flavus*, which was isolated from the leaves of a mangrove plant, *Sonneratia apetala*, collected on the coastal saltmarsh of the South China Sea. In the in vitro cytotoxic assays, compounds **1**−**5** displayed cytotoxicity against human cancer cell lines MCF-7, MDA-MB-435, HepG2, HeLa, and PC-3. Herein, we report the isolation, structure elucidation, and in vitro cytotoxic activity of these sesquiterpene peroxides. A possible biosynthetic pathway for **1**−**5** was also proposed in this paper.

RESULTS AND DISSCUSION

Compound **1** was isolated as colorless crystals. The molecular formula of $C_{16}H_{24}O_5$ (five degrees of unsaturation) was determined by HRESIMS. The ^1H, ^{13}C, and DEPT NMR spectra (Table 1) supported the presence of four singlet methyls (Me-12, Me-13, Me-14, and Me-16), five methylenes (C-1, C-4, C-5, C-9, and C-10), two oxymethines (C-2 and C-3), three quaternary carbons (C-6, C-7, and C-11), one ketone carbonyl (C-8), and one ester carbonyl (3-OAc). With five degrees of unsaturation accounted for by the molecular formula, the structure of **1** was suggested to contain three rings, in association with two carbonyls. Analysis of the $^1H-^1H$ COSY spectrum revealed two spin systems (C-1 to C-5 and C-9−C-10) (Figure 1). The HMBC correlations of H-1, 2, 4, 5/C-6 established a six-membered ring (ring A, C-1−C-6). An acetoxy group was attached to ring A at

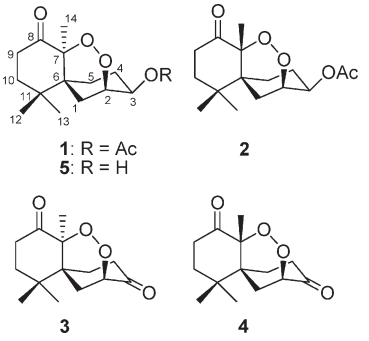


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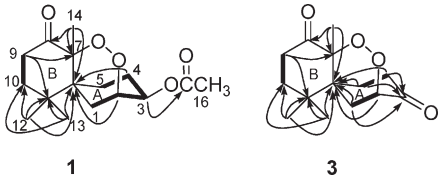
Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Spectroscopic Data for Compounds 1 and 2 ($CDCl_3$)

	1			2		
position	δ_C, type	δ_H, mult. (J in Hz)	HMBC	δ_C, type	δ_H, mult. (J in Hz)	HMBC
1eq	27.5, CH_2	2.41, m	C-3, 5, 6	26.3, CH_2	2.52, ddd (13.5, 5.2, 3.6)	C-3, 5, 6
1ax		2.02, m	C-2, 3, 5, 11		1.40, dd (13.5, 1.2)	C-3, 5, 11
2	76.0, CH	4.23, m	C-1, 3, 4, 6	77.3, CH_2	4.30, m	C-3, 4, 6
3	71.7, CH	4.87, ddd (11.3, 7.6, 3.8)	C-1, 2	72.8, C	4.88, ddd (11.1, 7.9, 3.1)	C-1, 2, 4
4eq	30.5, CH_2	1.99, m	C-2, 3, 6	28.3, CH_2	2.61, m	C-3
4ax		1.57, m	C-6		2.00, m	C-3, 6
5eq	25.4, CH_2	2.14, m	C-1, 4, 6, 11	28.1, CH_2	1.85, m	C-1, 4, 6, 11
5ax		1.61, m	C-7		1.77, m	C-1, 3, 4, 6, 7
6	41.4, C			43.3, C		
7	90.0, C			89.8, C		
8	207.9, C			208.1, C		
9eq	35.7, CH_2	2.65, ddd (15.1, 14.6, 6.6)	C-8, 10, 11	35.5, CH_2	2.81, ddd (15.4, 13.0, 7.5)	C-8, 10, 11
9ax		2.39, m	C-7, 8, 11		2.30, ddd (15.4, 5.7, 2.4)	C-7, 8, 10, 11
10eq	35.8, CH_2	1.97, m	C-6, 8, 9, 11	37.0, CH_2	2.03, m	C-6, 8, 11
10ax		1.55, m	C-6, 8, 9, 11		1.67, ddd (14.2, 7.5, 2.4)	C-6, 8
11	37.4, C			36.1, C		
12	26.3, CH_3	0.95, s	C-6, 10, 11, 13	25.2, CH_3	1.25, s	C-6, 10, 11, 12
13	24.7, CH_3	1.23, s	C-6, 10, 11, 12	27.5, CH_3	0.99, s	C-6, 10, 11, 13
14	21.5, CH_3	1.38, s	C-6, 7, 8	24.2, CH_3	1.90, s	C-6, 7, 8
15	171.0, C			171.3, C		
16	21.3, CH_3	2.06, s	C-15	21.4, CH_3	2.08, s	C-15



1 3

Figure 1. ^{1}H−^{1}H COSY (bold) and selected HMBC (arrow) correlations of **1** and **3**.

C-3 on the basis of the HMBC correlation of H-3/C-15. The HMBC correlations from *gem*-dimethyl groups (H_3-12 and H_3-13) to C-6, C-10, and C-11 and from H_3-14 to C-6, C-7, and C-8, as well as the correlations of H-9/C-7, C-11 and of H-10/C-8, determined a 2,4,4-trimethyl-3-bisubstituted-cyclohexanone (ring B). Rings A and B were fused at spiro carbon C-6. Considering the requirement of the unsaturations and the molecular formula for **1**, a peroxide bridge was assumed to connect C-2 and C-7 by the two remaining oxygen atoms. The ^{13}C NMR chemical shift values of C-2 at δ_C 76.0 and C-7 at δ_C 90.0 were consistent with this assumption. The structure of **1** was subsequently confirmed by a single-crystal X-ray diffraction experiment using Cu Kα radiation, and its absolute configuration was also established as 2*S*, 3*S*, 6*R*, and 7*S* (Figure 2). The new norsesquiterpene peroxide (**1**) was named talaperoxide A.

Compound **2** was obtained as colorless crystals. Its molecular formula was established as $C_{16}H_{24}O_5$ by HRESIMS data. The ^{1}H and ^{13}C spectroscopic data for **2** were similar to those of **1** except for the signals of H-1ax, 4eq, 4ax, and 14 (Table 1). Analysis of COSY and HMBC spectroscopic data for **2** suggested that it had the same skeleton structure as that of **1**. The different chemical shifts between **2** and **1** were presumably the result of the opposite configuration at C-7. This structural assumption was confirmed on the basis of a single-crystal X-ray diffraction analysis using Cu Kα radiation (Figure 2). The absolute configuration of **2** was consequently established to be 2*S*, 3*S*, 6*R*, and 7*R*. Thus, compound **2** was determined to be an epimer of **1** at C-7. It was named talaperoxide B.

The molecular formula of compound **3** was determined as $C_{14}H_{20}O_4$ (five degrees of unsaturation) by HRESIMS. The ^{1}H and ^{13}C NMR spectroscopic data for **3** (Table 2) showed three singlet methyls (Me-12, Me-13, and Me-14), five methylenes (C-1, C-4, C-5, C-9, and C-10), one oxymethine (C-3), three quaternary carbons (C-6, C-7, and C-11), and two ketone carbonyls (C-8 and C-3). Close comparison of the ^{1}H and ^{13}C NMR spectroscopic data with those of **1** revealed that the oxymethine (C-3) in **1** was oxidized to a ketone carbonyl (C-3) in **3**. The HMBC correlations from H-1 to C-3 and from H-4 and H-5 to C-3 gave further evidence for the existence of the ketone carbonyl. COSY and HMBC correlations for the rest of the molecule were identical to those observed for compound **1**. A NOESY experiment was carried out to determine the relative configuration of **3**. The correlations of H-5eq/H_3-14, H-13 established the cis configurations of Me-14/C-5 and of Me-13/C-5 (Figure 3). In addition, the correlations of H_3-12/H-1eq, H-1ax led to the trans configuration of Me-12/C-5. On the basis of these experimental data and the probable biosynthetic relationship (see below) to **1** and **2**, whose absolute configurations have been determined, the absolute configuration of **3** was assumed to be 2*S*, 6*R*, and 7*S*. Therefore, **3** was determined to be a new norsesquiterpene peroxide and was named talaperoxide C.

Compound **4** was obtained as a white solid. Its molecular formula of $C_{14}H_{20}O_4$ was deduced by HRESIMS, which was identical with that of **3**. The ^{1}H and ^{13}C NMR spectroscopic data

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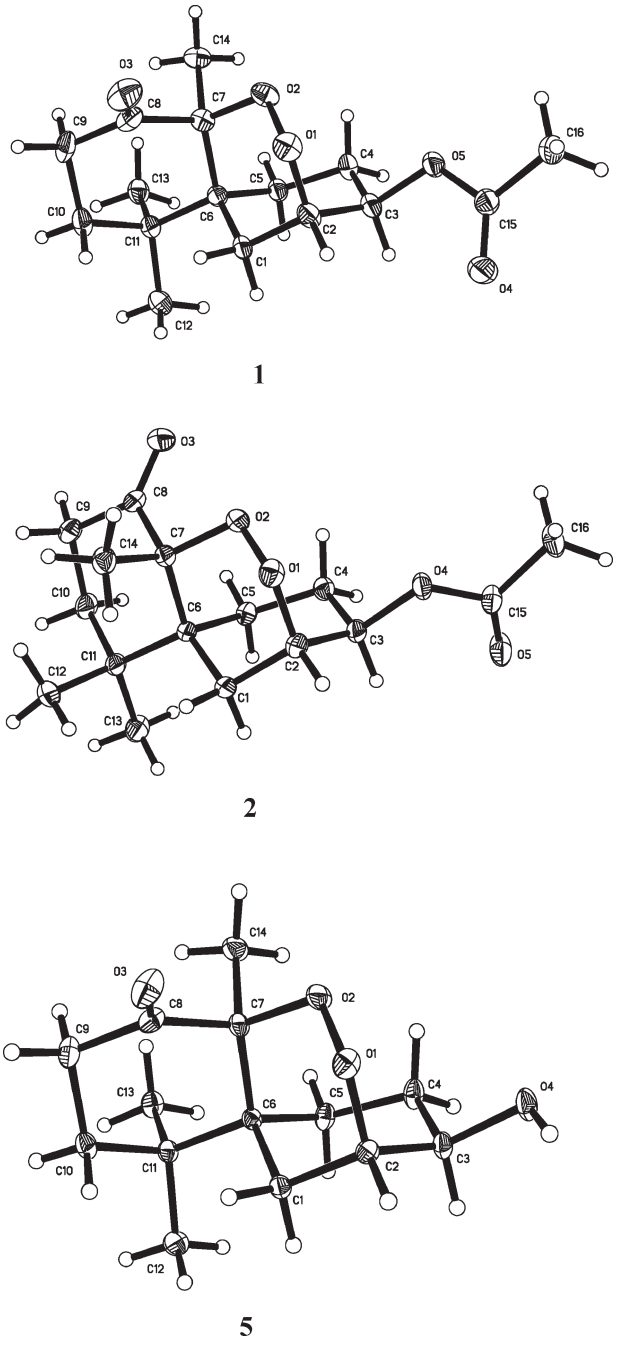

Figure 2. Perspective ORTEP drawings for **1**, **2**, and **5**.

for **4** were quite similar to those of **3**, except that the chemical shifts of H_3-14 and H-1eq were changed from δ_H 1.50 to δ_H 1.94 and from δ_H 2.27 to δ_H 2.69, respectively. The NOESY correlations of H-1eq/H-14, H-12 and of H-12/H-14 assigned cis configurations of Me-14/C-1 and Me-12/C-1 (Figure 3). On the basis of chemical shift comparison with that of **3** and biosynthetic considerations (see below), the absolute configuration of **4** was presumed to be 2*S*, 6*R*, and 7*R*. Consequently, this new compound (**4**) was assigned to be the C-7 epimer of **3**, namely, talaperoxide D.

In the case of **5**, the HRESIMS showed a pseudomolecular ion peak at *m*/*z* 277.1410 $[M + Na]^+$ (calcd 277.1416 for $C_{14}H_{22}O_4Na$). The ^1H and ^{13}C NMR spectra (Table 3) displayed three singlet methyls, five methylenes, two oxymethines, three quaternary carbons, and one ketone carbonyl. This compound was identified as steperoxide B (also named merulin A) by comparing the ^1H and ^{13}C NMR and MS data with those reported.[9,10] However, the absolute configuration of **5** was not determined in the previous reports. On the basis of Cu Kα X-ray diffraction data, it was here established to be 2*S*, 3*S*, 6*R*, and 7*S* (Figure 2).

The norsesquiterpene peroxides **1**−**5**, possessing a 6,6,6-tricyclic framework and a peroxide bridge, are structurally unique. A possible biosynthetic pathway for these compounds is proposed (Figure 4). The two pairs of epimers (**1**/**2**, **3**/**4**) and **5** are thought to be biogenetically related to the chamigrane skeleton.[11] The chamigrenyl cation generates **6** through a proton elimination reaction. The double bond of **6** is oxidized from the *re* or *si* face to form two hydroperoxides (**6a** and **6b**),[12] and then **6a** is transformed to **1**, **3**, and **5**, while **6b** is transformed to **2** and **4** by a series of oxidation, dehydration and rearrangement, reduction, and acetylation reactions (Figure S21, Supporting Information).[13]

All norsesquiterpene peroxides **1**−**5** exhibited lethal activity (LD_{50} < 10 ppm) in a preliminary test of the brine shrimp, *Artemia salina*. Compounds **1**−**5** were further evaluated for cytotoxic activity against human breast cancer cell lines MCF-7 and MDA-MB-435, human hepatoma cell line HepG2, human cervical cancer cell line HeLa, and human prostatic cancer cell line PC-3 by the MTT method as described previously.[14] Compounds **2** and **4** showed cytotoxicity toward these five cancer cell lines with IC_{50} values between 0.70 and 2.78 μg/mL. In particular, compound **4** showed promising growth-inhibitory effects on MDA-MB-435, HepG2, and PC-3 with IC_{50} values of 0.91, 0.90, and 0.70 μg/mL, respectively. It should be noted that compounds **2** and **4**, possessing the *R* configuration at C-7, showed more potent cytotoxicity toward the five cancer cell lines than compounds **1** and **3**, with the *S* configuration at C-7. These results indicated that the *R* configuration at C-7 might contribute to the cytotoxic activity. In addition, compound **5**, with a hydroxyl group at C-3, displayed better antiproliferative activities than **1**, with an acetoxy group at C-3, and **3**, with a 3-carbonyl, implying that the hydroxy group probably has an effect on the cytotoxic activity. The results of the bioassays are listed in Table 4.

The antimicrobial activities of metabolites **1**−**5** were also tested; none of the compounds showed inhibitory effects against *Staphylococcus aureus* (ATCC 27154), *Escherichia coli* (ATCC 25922), *Sarcina ventriculi* (ATCC 29068), *Pseudomonas aeruginosa* (ATCC 25668), *Candida albicans* (ATCC 10231), or *Aspergillus niger* (ATCC 13496) at a concentration of 50 μg/mL.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on an X-4 micromelting point apparatus and are uncorrected. Optical rotations were measured on a Polartronic HHW5 digital polarimeter. IR spectra were measured on a Bruker Vector 22 spectrophotometer using KBr pellets. The NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 MHz for ^1H and 100 MHz for ^{13}C in $CDCl_3$. All chemical shifts (δ) are given in ppm with reference to the solvent signal ($CDCl_3$, δ_H 7.26 for ^1H, δ_C 77.23 for ^{13}C), and coupling constants (*J*) are given in Hz. LRESIMS spectra were recorded on a Finnigan LCQ-DECA mass spectrometer. HRESIMS spectra were recorded on a Shimadzu LCMS-IT-TOF mass spectrometer. Single-crystal data were measured on an Oxford Gemini S Ultra diffractometer. Column chromatography (CC) was performed on silica gel (200−300 mesh,

Table 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Spectroscopic Data for Compounds 3 and 4 ($CDCl_3$)

	3			4		
position	δ_C, type	δ_H, mult. (*J* in Hz)	HMBC	δ_C, type	δ_H, mult. (*J* in Hz)	HMBC
1eq	32.3, CH_2	2.27, m	C-2, 3, 5, 6, 7, 11	25.7, CH_2	2.69, ddd (14.3, 4.0, 2.1)	C-3
1ax		1.90, m	C-5, 6, 7, 11		1.81, m	C-3, 6, 7, 11
2	81.7, C	4.16, m	C-1, 6	80.7, C	4.21, dd (4.0, 1.8)	C-3, 6
3	206.7, C			207.7, C		
4eq	39.4, CH_2	2.93, ddd (17.3, 12.0, 8.5)	C-3, 5	38.6, CH_2	2.88, m	C-3
4ax		2.59, m	C-3, 5, 6		2.47, m	C-3, 5
5eq	26.3, CH_2	2.33, m	C-1, 3, 4, 6, 7	27.2, CH_2	2.11, m	C-1, 3, 4, 6, 7, 11
5ax		1.93, m	C-1, 4, 6, 7, 11		1.84, m	C-1, 3, 4, 6, 7
6	42.1, C			43.7, C		
7	91.1, C			90.6, C		
8	207.0, C			207.4, C		
9eq	35.8, CH_2	2.73, ddd (15.2, 14.4, 6.6)	C-7, 8, 9, 11	35.4, CH_2	2.85, m	C-8, 10, 11
9ax		2.48, ddd (15.2, 4.8, 2.5)	C-8, 9, 11		2.33, ddd (15.5, 5.5, 2.1)	C-7, 8, 10, 11
10eq	35.9, CH_2	2.04, td (14.4, 4.8)	C-8, 9, 11, 12, 13	36.5, CH_2	2.06, m	C-6, 8, 11, 13
10ax		1.64, ddd (14.4, 6.6, 2.5)	C-6, 8, 9, 11, 12		1.67, ddd (14.2, 7.4, 2.1)	C-6, 8
11	37.8, C			36.4, C		
12	26.5, CH_3	1.02, s	C-6, 10, 11, 13	24.7, CH_3	1.30, s	C-6, 10, 11, 12
13	24.9, CH_3	1.30, s	C-6, 10, 11, 12	26.6, CH_3	1.05, s	C-6, 10, 11, 13
14	22.1, CH_3	1.50, s	C-6, 7, 8	23.0, CH_3	1.94, s	C-6, 7, 8

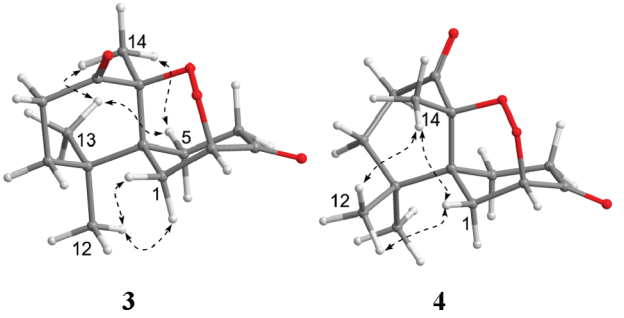

Figure 3. Selected NOESY correlations of **3** and **4**.

Qingdao Marine Chemical Factory) and Sephadex LH-20 (Amersham Pharmacia).

Fungal Material. The fungus used in this study was isolated from fresh, healthy leaves of *S. apetala*, which were collected in April 2009 from Dongzhaigang Mangrove National Nature Reserve in Hainan Island, China. The fungus was identified as *Talaromyces flavus* by the ITS region (deposited in GenBank, accession no. HQ700311).[15] A voucher strain was deposited in the China Center for Type Culture Collection under patent depository number CCTCC M 2010266.

Extraction and Isolation. The fungus *T. flavus* was fermented on autoclaved rice solid-substrate medium (twelve 500 mL Erlenmeyer flasks, each containing 50 g of rice and 50 mL of distilled water) for 40 days at 25 °C. Following incubation, the mycelia and solid rice medium were extracted with MeOH. The organic solvent was filtered and concentrated under reduced pressure to yield 3.6 g of organic extract. The residue was then divided into 15 fractions (Fr.1−F.15) by column chromatography on silica gel eluted by a gradient of $CHCl_3$/MeOH from 1:0 to 0:1. Fr.4 (215 mg) was applied to Sephadex LH-20 CC, eluted with petroleum ether/$CHCl_3$/MeOH (2:1:1), to obtain compounds **1** (3.9 mg) and **2** (1.2 mg). Fr.5 (832 mg) was rechromatographed on silica gel (gradient of $CHCl_3$/MeOH from 1:0 to 1:1) to give five fractions (Fr.5-1−Fr.5-5). Fr.5-2 (74 mg), Fr.5-3 (154 mg), and Fr.5-5 (31 mg) were applied to Sephadex LH-20 CC eluted with $CHCl_3$/MeOH (1:1) to yield compounds **3** (3.2 mg), **4** (1.7 mg), and **5** (2.6 mg), respectively.

Table 3. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Spectroscopic Data for Compound 5 ($CDCl_3$)

position	δ_C, type	δ_H, mult. (*J* in Hz)
1eq	30.7, CH_2	2.03, m
1ax		1.54, m
2	79.3, CH	4.11, m
3	69.6, CH	3.75, m
4	32.3, CH_2	2.14, m
5eq	25.7, CH_2	2.09, m
5ax		1.57, m
6	41.4, C	
7	90.3, C	
8	208.2, C	
9eq	35.8, CH_2	2.67, td (15.4, 6.7)
9ax		2.42, ddd (15.4, 4.6, 2.3)
10eq	36.0, CH_2	1.99, m
10ax		1.56, m
11	37.5, C	
12	26.4, CH_3	0.96, s
13	24.9, CH_3	1.24, s
14	21.6, CH_3	1.39, s

Talaperoxide A (***1***): colorless crystals (MeOH); mp 125−127 °C; $[\alpha]^{25}_D$ +191 (*c* 0.11 MeOH); IR (KBr) ν_{max} 2961, 2881, 1729, 1370, 1255, 1044, and 965 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; ESIMS *m/z* 319.2 $[M + Na]^+$; HRESIMS *m/z* 319.1518 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_5Na$, 319.1521).

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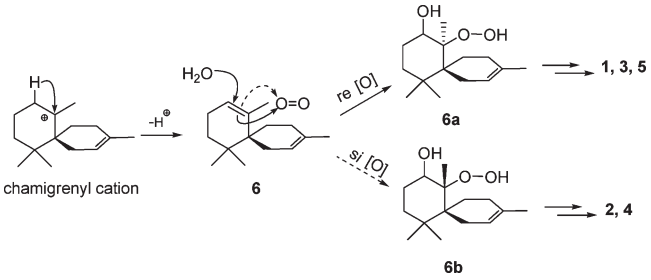

Figure 4. Possible biosynthetic pathway of compounds **1**−**5**.

Table 4. Cytotoxic Activities of Compounds 1−5[a]

	cell lines				
compound	MCF-7	MDA-MB-435	HepG2	HeLa	PC-3
1	19.77	11.78	12.93	13.7	5.70
2	1.33	2.78	1.29	1.73	0.89
3	6.63	2.64	15.11	12.71	4.34
4	1.92	0.91	0.90	1.31	0.70
5	4.17	1.90	6.79	7.97	1.82
EPI[b]	0.56	0.33	0.56	0.51	0.16

[a] Data are expressed in IC_{50} values (μg/mL). MCF-7 and MDA-MB-435: human breast cancer cell lines; HepG2: human hepatoma cell line; HeLa: human cervical cancer cell line; PC-3: human prostatic cancer cell line. [b] EPI (epirubicin) used as positive control.

Talaperoxide B (**2**): colorless crystals (MeOH); mp 91−93 °C; $[\alpha]^{25}_{D}$ + 261 (*c* 0.07 MeOH); IR (KBr) ν_{max} 2969, 2878, 1720, 1382, 1247, 1102, and 931 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; ESIMS *m*/*z* 319.1 $[M + Na]^+$; HRESIMS *m*/*z* 319.1525 $[M + Na]^+$ (calcd for $C_{16}H_{24}O_5Na$, 319.1521).

Talaperoxide C (**3**): white solid; mp 148−150 °C; $[\alpha]^{25}_{D}$ +225 (*c* 0.12 MeOH); IR (KBr) ν_{max} 2952, 2888, 1739, 1723, 1384, 1114, and 1019 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; ESIMS *m*/*z* 275.1 $[M + Na]^+$; HRESIMS *m*/*z* 275.1256 $[M + Na]^+$ (calcd for $C_{14}H_{20}O_4Na$, 275.1259).

Talaperoxide D (**4**): white solid; mp 120−122 °C; $[\alpha]^{25}_{D}$ +126 (*c* 0.08 MeOH); IR (KBr) ν_{max} 2961, 2922, 1736, 1716, 1469, 1378, 1066, and 1044 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; ESIMS *m*/*z* 275.1 $[M + Na]^+$; HRESIMS *m*/*z* 275.1261 $[M + Na]^+$ (calcd for $C_{14}H_{20}O_4Na$, 275.1259).

X-ray Crystallographic Analysis of 1, 2, and 5. All single-crystal X-ray diffraction data were collected at 123 K on an Oxford Gemini S Ultra diffractometer with Cu Kα radiation (λ = 1.54178 Å). The structures were solved by direct methods (SHELXS-97) and refined using full-matrix least-squares difference Fourier techniques. Hydrogen atoms bonded to carbons were placed on the geometrically ideal positions by the "ride on" method. Hydrogen atoms bonded to oxygen were located by the difference Fourier method and were included in the calculation of structure factors with isotropic temperature factors. Crystallographic data for **1**, **2**, and **5** have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033, or e-mail: deposit@ccdc.cam.ac.uk).

Crystal data of **1**: $C_{16}H_{24}O_5$, M_r = 296.35, orthorhombic, *a* = 10.9434(2) Å, *b* = 12.4201(2) Å, *c* = 22.1019(4) Å, α = 90°, β = 90°, γ = 90°, *V* = 3004.05(9) $Å^3$, space group $P2_12_12_1$, *Z* = 8, D_x = 1.311 mg/m^3, μ(Cu Kα) = 0.792 mm^{-1}, and *F*(000) = 1280. Crystal dimensions: 0.48 × 0.35 × 0.28 mm^3. Independent reflections: 4524 (R_{int} = 0.0229). The final R_1 values were 0.0361, wR_2 = 0.0912 ($I > 2\sigma(I)$). Flack parameter = 0.002(16). CCDC number: 807999.

Crystal data of **2**: $C_{16}H_{24}O_5$, M_r = 296.35, monoclinic, *a* = 8.73800(10) Å, *b* = 12.27280(10) Å, *c* = 14.31590(10) Å, α = 90°, β = 97.1630(10)°, γ = 90°, *V* = 1523.25(2) $Å^3$, space group $P2_1$, *Z* = 4, D_x = 1.292 mg/m^3, μ(Cu Kα) = 0.781 mm^{-1}, and *F*(000) = 640. Crystal dimensions: 0.20 × 0.15 × 0.10 mm^3. Independent reflections: 5761 (R_{int} = 0.0400). The final R_1 values were 0.0293, wR_2 = 0.0736 ($I > 2\sigma(I)$). Flack parameter = 0.05(11). CCDC number: 808000.

Crystal data of **5**: $C_{14}H_{22}O_4$, M_r = 254.32, orthorhombic, *a* = 7.3125(2) Å, *b* = 12.4303(5) Å, *c* = 13.9596(7) Å, α = 90°, β = 90°, γ = 90°, *V* = 1268.88(9) $Å^3$, space group $P2_12_12_1$, *Z* = 4, D_x = 1.331 mg/m^3, μ(Cu Kα) = 0.785 mm^{-1}, and *F*(000) = 552. Crystal dimensions: 0.48 × 0.46 × 0.40 mm^3. Independent reflections: 1984 (R_{int} = 0.0210). The final R_1 values were 0.0258, wR_2 = 0.0661 ($I > 2\sigma(I)$). Flack parameter = 0.03(7). CCDC number: 807998.

Biological Assays. Antimicrobial activity,[16] brine shrimp lethality assay,[17] and cytotoxic activity[18] were determined according to established procedures. Four bacteria (*S. aureus* (ATCC 27154), *E. coli* (ATCC 25922), *S. ventriculi* (ATCC 29068), *P. aeruginosa* (ATCC 25668)) and two fungi (*C. albicans* (ATCC 10231) and *A. niger* (ATCC 13496)) were used in the antimicrobial test. Ampicillin and nystatin were used as antibacterial and antifungal positive controls, respectively. Five human cancer cell lines (human breast cancer cell lines MCF-7 and MDA-MB-435, human hepatoma cell line HepG2, human cervical cancer cell line HeLa, and human prostatic cancer cell line PC-3) were used in the cytotoxicity bioassay. Epirubicin (EPI) was used as positive control.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra of compounds **1**−**4** and CIF files for compounds **1**, **2**, and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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