Two New Cytotoxic Tetracyclic Tetraterpenoids from the Soft Coral *Sarcophyton tortuosum*

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Two new cytotoxic tetracyclic tetraterpenoids, methyl tortuoate A (**1**) and methyl tortuoate B (**2**), along with the known methyl sartortuoate (**3**) were isolated from the soft coral *Sarcophyton tortuosum*. The structures of **1** and **2** were established by spectroscopic methods, mainly on the basis of 2D NMR techniques, and were confirmed by single-crystal X-ray diffraction analysis. The cytotoxic activities of these compounds were carried out in vitro on human nasophyringeal carcinoma (CNE-2) and murine lymphocytic leukemia (P-388) tumor cell lines.

In the course of our study on the chemical constituents of soft corals, we have isolated many chemically and biologically interesting secondary metabolites.[1−4] Among them, two tetraterpenoids, named methyl sartortuoate (**3**)[5] and methyl isosartortuoate (**4**),[6] from the soft coral *Sarcophyton tortuosum* were structurally the most interesting compounds. After inspection of the structures of **3** and **4**, we proposed that this unique framework might be derived from Diels−Alder addition of two cembranoid units as precursors. Later, two other biscembranoids, methyl sarcophytoate and methyl chlorosarcophytoate, together with the diterpenoid methyl sarcoate, one of the proposed biosynthetic precursors, from *S. glaucum* were reported.[7,8] The structure of methyl sarcoate was remarkably similar to the biosynthetic precursor that we had proposed previously. In addition, Bowden et al. isolated a biscembranoid, methyl neosartortuate acetate, and methyl sarcoate along with another precursor from *S. tortuo*sum.[9] These findings supported the biogenetic pathway of this type of biscembranoid.

Recently,we isolated two more new biscembranoids, name methyl tortuoate A (**1**) and methyl tortuoate B (**2**), along with the known methyl sartortuoate (**3**), from the soft coral *S. tortuosum* Tix.-Dur. (Alcyoniidae) collected from Sanya Bay Hainan Island of China, in 2002. Herein, we report the isolation and structural determination of these two new biscembranoids.

Compound **1** was obtained as colorless crystals, mp 242−244 °C, $[\alpha]^{25}_D$ +348° (*c* 0.025, $CHCl_3$). HRFABMS [*m/z* 705.4577 $(M + Na)^+$] revealed a molecular formula of $C_{41}H_{62}O_8$. The IR spectrum (KBr) indicated the presence of absorptions characteristic of hydroxyl groups (3381, 1104 cm^{-1}), an ester carbonyl group (1742, 1194 cm^{-1}), and ketone carbonyl groups (1707 cm^{-1}). Its NMR spectra (Table 1) showed the following functionalities: one methyl ester [δ_C 174.8, C-20; 51.4, C-41; δ_H 3.60 (3H, s, H-41)]; three ketone carbonyl groups (δ_C 213.6, C-10; 212.6, C-13; 212.0 C-3), two tri- and one tetrasubstituted double bond [δ_C 141.0, C-23; 134.4, C-27; 132.8, C-34; 129.1, C-35; 122.9, C-22; 120.2, C-28; δ_H 5.12 (1H, d, J = 10.0 Hz, H-22) and 5.55 (1H, dd, J = 4.5, 1.5 Hz, H-28)]; three allylic methyl groups [δ_H 1.64 (3H, s, H-39), 1.86 (3H, s, H-38), and 1.91 (3H, s, H-37)]; and an isopropyl group [δ_H 0.80 (3H, d, J = 7.0 Hz, H-15), 0.94 (3H, d, J = 7.0 Hz, H-17)]. In addition, four carbons were attached to oxygen (δ_C 79.7, C-26; 70.1, C-33; 68.3, C-30; 74.2, C-31), but only three oxygen atoms remained, plausibly as one ether linkage and one tertiary and one secondary hydroxyl. The presence of nine methyl signals and spectroscopic features that included the ketone and methyl ester groups led us to recognize a tetracyclic biscembranoid similar to those we had reported previously.[5,6]

From detailed analysis of the ^1H and ^{13}C NMR data associated with C−H one-bond interactions and from cross-peaks observed in the $^1H-^{13}C$ 2D NMR shift-correlated spectrum, all the signals of H and C could be assigned (Table 1). The correlations of $^1H-^1H$ COSY revealed seven proton−proton networks, as shown in Figure 1.The carbon connectivity showed that compound **1** had the same carbon skeleton as that of methyl sartortuate (**3**). The structure of **1** was similar to that of **3**, except for the absence of the conjugated diene and one tertiary hydroxyl group. Instead, a new secondary hydroxyl was present. The HMBC connectivity, H-33/C-34, C-21, and C-31, allowed a decision for the placement of this secondary hydroxyl. Its HMBC spectrum also showed many informative $^1H-^{13}C$ long-range correlations, such as C-1/H-2, H-14, H-21; C-3/H-2, H-4, H-36; C-10/H-9, H-11, H-12, Me-18; C-13/H-12, H-14; C-23/H-22, H-24, Me-38; etc. (Figure 1). By combination of the $^1H-^1H$ COSY and HMBC correlations (Table 1 and Figure 1), the structure of **1** could be deduced. The relative configurations of the stereogenic carbons of **1**, depicted in Figure 2, were determined from 2D NOESY correlation peaks. The isopropyl group at C-12 had the same orientation as the methyl ester group at C-1. Thus the relative configuration of C-12 is *R**, as referred to the lowest carbon (C-1) having *R*-chirality. The 22*E*, 27*E*, and 34*Z* configurations of the olefinic bonds were deduced by the ^{13}C NMR chemical shifts of the olefinic methyl groups. The absolute configuration of **1** remains undetermined.

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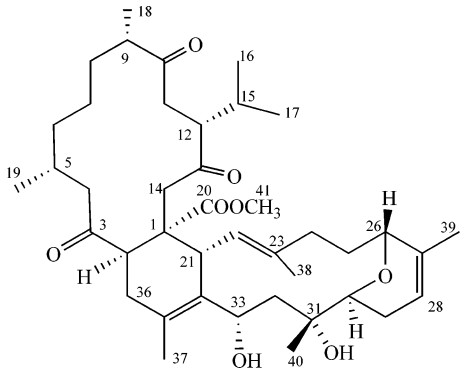

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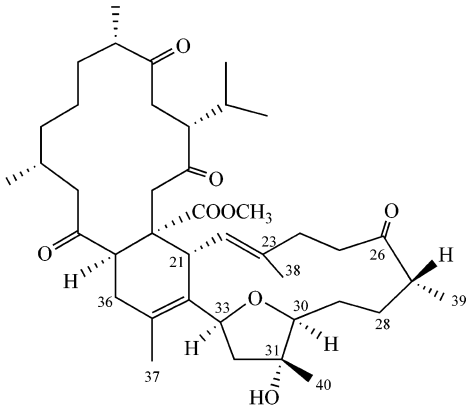

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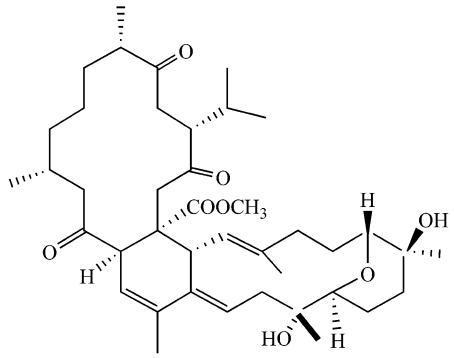

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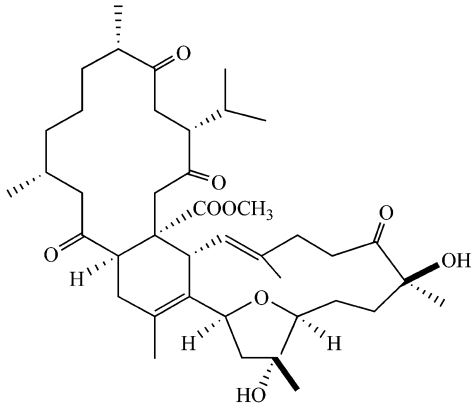

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A suitable single crystal of **1** was obtained from EtOH, and X-ray diffraction analysis was used to confirm the structure and determine the relative configuration. The

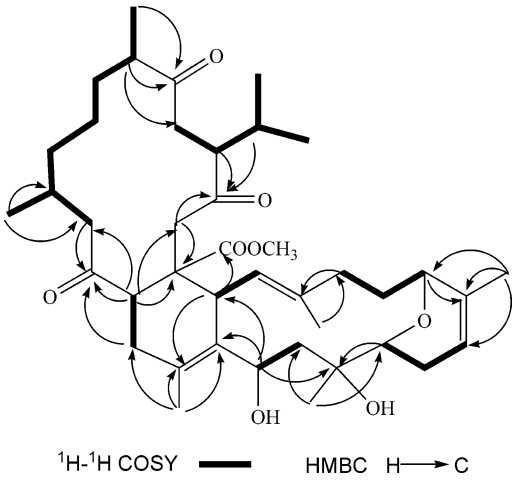

Figure 1. ^{1}H−^{1}H COSY and key HMBC correlations of **1**.

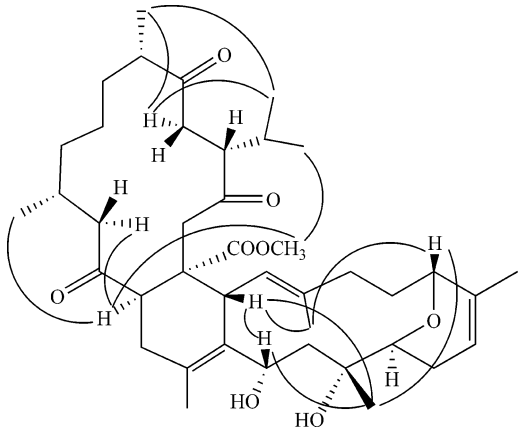

Figure 2. NOEs observed in the NOESY of **1**.

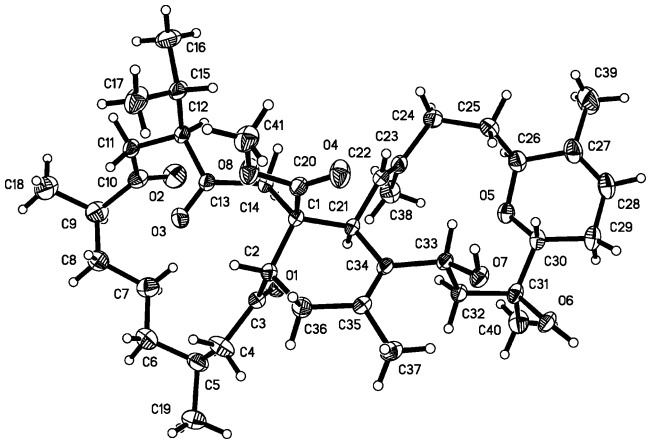

Figure 3. Perspective drawing of the X-ray structure of methyl tortuoate A (**1**).

results are shown in Figure 3 and in the Experimental Section. Similar to the structure of methyl sartotuate, X-ray showed that H-2 and the methyl ester have the same orientation; that is, they are cis to each other. The relative configuration of the 10 chiral carbons was determined as 1*R**, 2*R**, 5*R**, 9*S**, 12*R**, 21*R**, 26*R**, 30*S**, 31*R**, 33*R**.

Compound **2** was obtained as colorless crystals, mp 229−231 °C, $[\alpha]^{25}_D$ +84° (*c* 0.031, CH_3OH). HRFABMS [*m/z* 705.4577 $(M + Na)^+$] revealed a molecular formula of $C_{41}H_{62}O_8$. Its UV spectrum did not show any conjugated system. The IR spectrum (KBr) indicated the presence of hydroxyl (3506, 1071 cm^{-1}), ester (1740, 1210 cm^{-1}), and carbonyl groups (1705 cm^{-1}). The NMR spectra of **2** (Table

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds **1** and **2** ($CDCl_3$)

	1			**2**		
C/H	^{13}C δ m	^{1}H δ (m, *J* in Hz)	^{1}H−^{1}H COSY	^{13}C δ m	^{1}H δ (m, *J* in Hz)	^{1}H−^{1}H COSY
1	49.2 s			50.5 s		
2	46.6 d	3.82 (1H, dd, 5.0, 6.6)	H-36	43.4 d	3.51 (1H, m)	H-36
3	212.0 s			208.6 s		
4	50.4 t	2.60 (1H, dd, 19.0, 8.0)	H-5	53.8 7	2.38 (1H,dd, 20.0, 2.0)	H-5
		2.29 (1H, dd, 19.0, 4.0)	H-5		3.16 (1H,dd, 20.0, 10.0)	H-5
5	26.4 d	2.10 (1H, m)	H-4, H-6, H-19	27.4 d	1.74 ((1H, m)	H-4, H-6, H-19
6	36.4 t	1.16 (2H, m)	H-5, H-7	37.2 t	1.15 (2H, m)	H-5, H-7
7	32.5 t	1.80 (2H, m)	H-6, H-8	32.5 t	1.36 (1H, m)	H-6, H-8
					0.55 (1H, t, 12.0)	H-7, H-9
8	32.9 t	1.43 (1H, m)	H-7, H-9	33.8 t	1.51 (2H, m)	
		1.60 (1H, m)				
9	47.3 d	2.39 (1H, m)	H-8, H-18	47.5 d	2.48 (1H, m)	H-8, H-18
10	213.6 s			213.8 s		
11	35.5 t	2.16 (1H, d, 16.5)	H-12	31.4 s	1.88 (2H, dd, 17.5, 2.0)	H-12
		2.89 (1H, m)				
12	50.9 d	2.94 (1H, m)	H-11, H-15	51.6 d	3.03 (1H, m)	H-11, H-15
13	212.6 s			213.5 s		
14	47.4 t	2.29 (1H, dd, 19.0, 8.0)		45.8 t	2.48 (2H, d, 11.5)	
		2.60 (1H, dd, 19.0, 4.0)				
15	29.1 d	1.94 (1H, m)	H-12, H-16, H-17	28.9 d	2.22 (1H, m)	H-12, H-16, H-17
16	18.5 q	0.80 (3H, d, 7.0)	H-15	17.6 q	0.70 (3H, d, 7.0)	H-15
17	20.0 q	0.94 (3H, d, 7.0)	H-15	21.2 q	0.99 (3H, d, 7.0)	H-15
18	17.0 q	1.14 (3H, d, 7.0)	H-9	17.4 q	1.13 (3H, d, 7.0)	H-9
19	21.7 q	0.89 (3H, d, 6.5)	H-5	22.2 q	0.86 (3H, d, 7.0)	H-5
20	174.8 s			174.4 s		
21	42.1 d	3.62 (1H, d, 10.0)	H-22	45.7 d	2.50 (1H, d, 10.5)	H-22
22	122.9 d	5.12 (1H, d, 10.0)		127.4 d	4.89 (1H, d, 10.5)	
23	141.0 s			136.5 s		
24	38.3 t	1.86 (1H, m)	H-25	30.3 t	2.24 (2H, m)	H-25
		2.53 (1H, m)				
25	24.0 t	1.02 (2H, m)	H-24, H-26	37.7 t	2.24 (1H, m)	H-24
					2.76 (1H, m)	
26	79.7 d	4.01 (1H, br.d, 6.5)	H-25	215.9 s		
27	134.4 s			49.0 d	2.37 (1H, m)	H-28, H-39
28	120.2 d	5.55 (1H, dd, 4.5, 1.5)	H-29	31.5 t	1.36 (2H, m)	H-27, H-29
29	24.7 t	2.02 (1H, m)	H-28, H-30	25.4 t	1.10 (2H, m)	H-28, H-30
		2.14 (1H, m)	H-28, H-30			
30	68.3 d	3.67 (1H, dd, 6.6, 5.0)	H-29	87.0 d	3.47 (1H, m)	H-29
31	74.2 s			80.2 s		
32	42.2 t	1.87 (1H, m)	H-33	42.1 t	1.73 (2H, m)	H-33
		1.60 (1H, m)				
33	70.1 d	4.81 (1H, br d, 9.5)	H-32	75.3 d	5.05 (1H, dd, 10.5, 6.0)	H-32
34	132.8 s			135.2 s		
35	129.1 s			126.1 s		
36	34.6 t	2.01 (1H, m)	H-2	33.1 t	2.13 (2H, dd, 19.0, 9.0)	
		2.54 (1H, m)				
37	19.9 q	1.91 (3H, s)		19.0 q	1.83 (3H, s)	
38	19.8 q	1.86 (3H, s)		19.2 q	1.64 (3H, s)	
39	20.3 q	1.64 (3H, s)		17.4 q	1.08 (3H, d, 7.0)	H-27
40	23.1 q	1.09 (3H, s)		23.5 q	1.28 (3H, s)	
41	51.4 q	3.57 (3H, s)		51.1 q	3.48 (3H, s)	

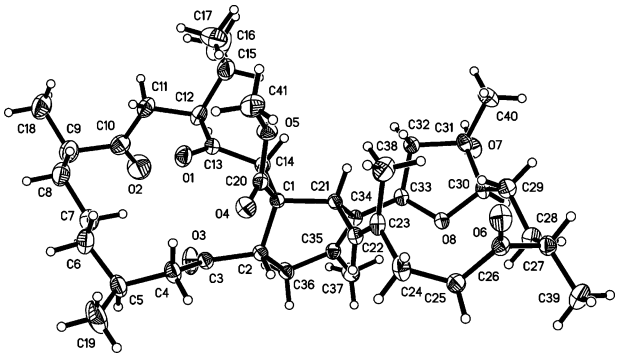

Figure 4. Perspective drawing of the X-ray structure of methyl tortuoate B (**2**).

1) showed one methyl ester (δ_C 174.4, C-20; 51.1, C-41; δ_H 3.60, 3H, s), four ketone carbonyl groups (δ_C 215.9, C-26; 213.8, C-10; 213.5, C-13; and 208.6, C-3), one trisubstituted and one tetrasubstituted double bond (δ_C 126.1, C-35; 135.2, C-34; 136.5, C-23; 127.4, C-22; δ_H 4.89, d, J = 10.5 Hz, H-22). In addition, one tertiary hydroxyl (δ_C 80.2, C-31) and an ether linkage (δ_C 87.0, C-30; 75.3, C-33) were revealed. The ^{13}C NMR DEPT, HMQC, and ^{1}H−^{1}H COSY led to the assignment of all H and C signals. A single-crystal X-ray diffraction analysis (Figure 3 and Experimental Section) was performed, and the relative configuration of **2** was also determined. There are 10 chiral carbons in **2**; the relative configuration of the stereocenters (1*R**, 2*R**, 5*R**, 9*S**, 12*R**, 21*R**, 27*R**, 30*S**, 31*R**, 33*S**) was almost the same as those of **1**, except for 27*R** and 33*S** in **2** instead of 26*R** and 33*R** in **1**. Its structure was similar to that of compound **4**, except for the absence of one tertiary hydroxyl group at C-27. It is worthy to note that the orientation of the isopropyl group relative to the ring junction in **1** and **2** is the same as for methyl

sartortuate,[5] methyl isosartortuate,[6] and methyl neosartortuate acetate.[9]

Methyl tortuoate A (**1**) and methyl tortuoate B (**2**) exhibited in vitro cytotoxicity against the human nasophyringeal carcinoma CNE-2 cell line, with IC_{50} values of 22.7 and 24.7 μg/mL, and the murine P-388 tumor cell line, with IC_{50} values of 3.5 and 5.0 μg/mL, respectively.

The known compound **3** was identified by comparison with literature data.[5]

Experimental Section

General Experimental Procedures. The melting points were determined using an X-6 micromelting point apparatus and are uncorrected. ^{1}H and ^{13}CNMR spectra were recorded at 500 and 125 MHz, respectively, in $CDCl_3$ using TMS as internal standard. Optical rotations were measured on a Schmidt+Haensch Polaptronic hnqw5 polarimeter. The X-ray diffraction data were collected on a Bruker SMART 1000 CCD X-ray diffractometer. Si gel (200−300 mesh) was used for column chromatography.

Animal Material. The soft coral *S. tortuosum* Tix.-Dur. (Alcyoniidae) was collected from Sanya Bay, Hainan Island, China, in 2002 and was identified by Dr. Zhican Tang, Institute of Oceanology, Academia Sinica. A voucher specimen is deposited in the Research Center of Organic Natural Products, Sun Yat-sen University, Guangzhou, China.

Extraction and Isolation. The soft coral *S. tortuosum* (300 g, dried wt) was extracted with CH_2Cl_2−MeOH (1:1) to give a brown extract, which was extracted with petroleum ether and then with EtOAc. The residue in EtOAc was chromatographed on silica gel. Fraction A, eluted with petroleum ether−EtOAc (3:1), was further purified by chromatography on silica gel eluting with a gradient elution of EtOAc−MeOH to afford methyl tortuoate A (**1**) (13 mg). Fraction B, eluted with petroleum ether−EtOAc (2:1), after further purification, gave methyl tortuoate B (**2**) (18 mg). Fraction C, eluted with petroleum ether−EtOAc (1:1), after chromatography twice on silica gel, gave methyl sartortuoate (**3**) (5 mg).

Methyl tortuoate A (1): colorless prisms, mp 242−244 °C (EtOH); $[\alpha]^{25}_D$ +345° (*c* 0.025, $CHCl_3$); IR (KBr) ν_{max} 3381, 1742, 1707, 1194, 1104 cm^{-1}; ^{1}H NMR and ^{13}C NMR data in Table 1; HRFABMS *m/z* 705.4359 (calcd for $C_{41}H_{62}O_8Na$,-705.4342 $[M + Na]^+$).

X-ray Crystal Data for 1. Crystal data were as follows: colorless crystal, $C_{41}H_{62}O_8$, fw 682.91; crystal dimensions 0.50 × 0.28 × 0.26 mm; crystal system monoclinic; space group $P2_1$; unit cell dimensions a = 11.6213(15) Å, α = 90°, b = 9.9065-(12) Å, β = 92.201(2)°, c = 16.638(2) Å, γ = 90°; Z = 2; V = 1914.1(4) Å^3; calculated density 1.185 g/cm^3; F_{000} = 744. The R (R_w) value of **1** was 0.0402 (0.1106). The X-ray measurements were made on a Bruker SMART 1000 CCD X-ray diffractometer with graphite-monochromated Mo Kα (λ 0.71073Å) radiation at 293(2) K. A total of 11 761 reflections were collected, 8048 of which were independent (R_{int} = 0.0089). The structure was solved by direct methods (SHELXTL-V5.0) and refined with full-matrix least-squares calculations. The final cycle of full-matrix least-squares refinement was based on 8048 observed reflections ($I > 2\sigma(I)$) and 442 variable parameters. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined.

Methyl tortuoate B (2): colorless prisms, mp 229−231 °C (EtOH); $[\alpha]^{25}_D$ +84° (*c* 0.03, CH_3OH); IR (KBr) ν_{max} 3506 (s), 1740, 1705, 1210, 1071 cm^{-1}; ^{1}H NMR and ^{13}C NMR data in Table 1; HRFABMS *m/z* 705.4377 (calcd for $C_{41}H_{62}O_8Na$, 705.4342 $[M + Na]^+$).

X-ray Crystal Data for 2. Crystal data were as follows: colorless crystal, $C_{41}H_{62}O_8 \cdot C_2H_5OH$, fw 728.97; crystal dimensions 0.50 × 0.42 × 0.18 mm; crystal system orthorhombic; space group $P2_12_12_1$; unit cell dimensions a = 9.4139(15) Å, α = 90°, b = 9.7850(16) Å, β = 90°, c = 45.436(7) Å, γ = 90°; Z = 4; V = 4185.3(12) Å^3; calculated density 1.157 g/cm^3; F_{000} = 1592. The R (R_w) value of **2** was 0.0463 (0.1218). The X-ray measurements were made on a Bruker SMART 1000 CCD X-ray diffractometer with graphite-monochromated Mo Kα (λ 0.71073 Å) radiation at 293(2) K. Data were collected up to 27.03° in θ. A total of 28 452 reflections were collected, 9159 of which were independent (R_{int} = 0.0199). The structure was solved by direct methods (SHELXTL-V5.0) and refined with full-matrix least-squares calculations. The final cycle of full-matrix least-squares refinement was based on 9159 observed reflections ($I > 2\sigma(I)$) and 469 variable parameters. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined.

Cytotoxicity Bioassays. The tetrazolium-based colorimetric assay (MTT assay) was used for the in vitro assay of cytotoxicity to human nasophyringeal carcinoma (CNE-2) and murine P-388 tumor cell lines.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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