Abietane Diterpenoids from *Clerodendrum bungei*

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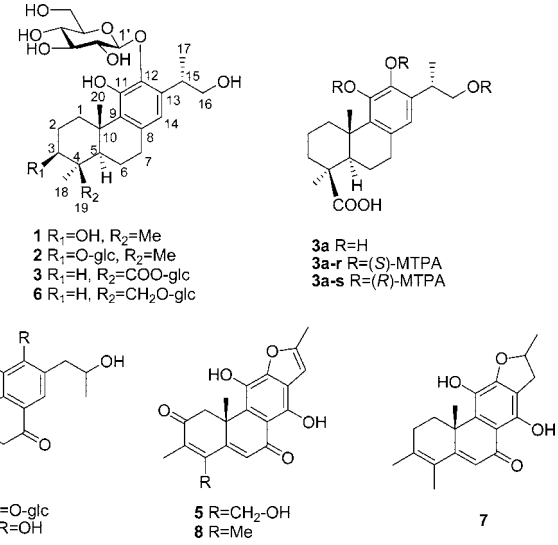
Five new naturally occurring abietane diterpenoids (**1**–**5**) along with three known diterpenoids (**6**–**8**) were isolated from an acetone-soluble extract of the roots of *Clerodendrum bungei*. The structures of the new compounds were elucidated on the basis of spectroscopic analysis and chemical methods. In addition, all compounds were evaluated for cytotoxic activity against the cultured B16 (murine melanoma), HGC-27 (human gastric), and HEK-293 (human epithelial kidney) cell lines. Uncinatone (**7**) exhibited moderate cytotoxicity, inhibited cell proliferation, and induced cell-cycle G_2/M phase arrest.

The genus *Clerodendrum* belongs to the subfamily Viticoideae and is the largest genus of the family Verbenaceae. Plants of the genus *Clerodendrum* are well known for their pesticidal properties.[1] In East Africa they are used as army-worm antifeedants,[2] and in West Africa the leaf extract is used for arresting bleeding from cuts and other wounds as well as for stopping postpartum hemorrhage. *Clerodendrum bungei* Steud. (Verbenaceae) is a small shrub native to mainland China.[3] Preparations of the leaves and branches of *C. bungei*, have been used in folk medicine to treat boils, hemorrhoids, eczema, and hypertension, and the roots are used to alleviate rheumatism, beriberi, hypertension, and prolapse of the uterus.[3,4] Several types of constituents including diterpenoids,[4] phenylethanoid glycosides,[5] and steroids and triterpenoids[6,7] have been identified from this plant. An extract of the roots of *C. bungei* has been found to inhibit the growth of sarcoma in mice,[8] but the antitumor components of this plant have not been determined. As part of an ongoing effort to discover potential anticancer agents from Chinese medicinal plants, the roots of *C. bungei*, collected in Guangxi Province, were investigated systematically. This has led to the isolation and structure elucidation of five new abietane-type diterpenoids (**1**–**5**) and the purification of three known substances (**6**–**8**). Compounds **1**–**8** were evaluated for cytotoxicity against several cancer cell lines, and uncinatone (**7**) was further tested by cell-cycle analysis.

Results and Discussion

A crude aqueous acetone (H_2O/acetone = 3:7) extract of the air-dried and powdered roots of *C. bungei* was subjected to separation over a column containing Diaion HP-20, eluting with a gradient of H_2O−MeOH. A series of diterpenoid-containing fractions was afforded and further purified by passage over MCI gel CHP20P, Sephadex LH-20, Toyopearl HW-40, and C_{18} and C_8 Cosmosil, yielding eight compounds (**1**–**8**). Compounds **6**–**8** were identified as ajugaside A,[9] uncinatone,[10] and teuvincenone F,[11] respectively, by comparison of their spectroscopic data with those reported in the literature.

Compound **1** was obtained as a flaxen, amorphous powder, with the molecular formula $C_{26}H_{40}O_9$ determined from the HRESIMS (*m*/*z* 519.2565 $[M + Na]^+$), indicating seven degrees of unsaturation. The IR spectrum of **1** indicated the presence of hydroxy groups (3405 cm^{-1}) and an aromatic moiety (1637 and 1566 cm^{-1}). The ^1H NMR spectrum of **1** showed three methyl singlets at δ_H 1.05 (H_3-18), 0.87 (H_3-19), and 1.29 (H_3-20) and one methyl doublet at δ_H 1.13 (J = 6.9 Hz, H_3-17). It also displayed signals attributed to one oxygen-bearing methylene at δ_H 3.41 (m, H-16a) and 3.58 (m, H-16b), one olefinic methine at δ_H 6.37 (s, H-14), and one oxygen-bearing methine at δ_H 3.20 (dd, J = 4.7 and 11.7 Hz, H-3). The ^1H NMR spectrum also had two multiplets from a deshield shifted methine at δ_H 3.69 (H-15) and an upshield shifted methine at δ_H 1.18 (H-5), and partially overlapped multiplets due to four methylenes between δ_H 1.25 and 2.77, together with characteristic signals due to a β-glucopyranosyl unit with the anomeric proton [δ_H 4.38 (d, J = 7.5 Hz)] (Table 1). In addition to protonated carbon signals corresponding to the above protons, the ^{13}C NMR and DEPT spectra of **1** showed signals of 20 carbons of an aglycon attributable to four methyls (three tertiary), five methylenes (one oxygenated), four methines (one olefinic), and seven quaternary carbons (five olefinic), along with signals for a sugar unit. These data suggested that **1** is a glycosidic diterpene with a tricyclic system including an aromatic ring, characteristic of an abietane 8,11,13-triene.[12] The glucose unit obtained after hydrolysis gave a positive optical rotation, $[\alpha]^{22}_D$ +40 (*c* 0.11, H_2O), indicating that it was D-glucose.



The proton and protonated carbon NMR signals of **1** were assigned unambiguously by the HSQC experiment. The abietane-diterpene skeleton was further confirmed, both by the $^1H-^1H$ COSY correlations, from H-1 through H-2 to H-3 and from H-4 through H-5 to H-6, and by the HMBC correlations, from H-20 to C-1 and C-5 and from H-18/19 to C-3 and C-5, together with their chemical shift values. The HMBC correlation of the anomeric proton H-1′ (δ_H 4.38) to C-12 (δ_C 143.4) indicated that the β-D-glucopyranosyl unit is located at C-12. The $^1H-^1H$ COSY correlations from H-16 through H-15 to H-17, in combination with HMBC correlations from H-15 to C-12 and C-14, were suggestive of an isobutanol

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Table 1. ^{1}H NMR Spectroscopic Data (400 MHz) for Compounds **1**−**4**, **6**, **3a**, and **4a**[a]

position	**1**[b]	**2**[b]	**3**[b]	**4**[c]	**6**[b]	**3a**[d]	**4a**[d]
1α	1.25 m	1.23 m	1.07 m	1.39 m	1.19 m	1.62 m	1.95 m
1*β*	3.33 m	3.33 m	3.30 m	3.17 m	3.34 m	4.02 m	4.10 m
2α	1.65 m	1.99 m	1.47 m	1.94 m	1.28 m	1.78 m	2.20 m
2*β*	1.73 m	1.81 m	2.00 m,	2.09 m	1.74 m	2.61 m	2.48 m
3α			1.07 m		1.02 m	1.32 m	
3*β*	3.20 dd (4.7,11.7)	3.19 d (8.1)	2.25 m		2.01 m	2.68 m	
5	1.18 m	1.15 m	1.47 d (11.6)	2.88 m	1.31 m	1.84 m	3.68 m
6α	1.80 m	1.74 m	2.14 m		1.72 m	2.57 m	3.52 m
6*β*	1.55 m	1.51 m	1.92 m	2.48 m	1.49 m	2.48 m	3.09 m
7	2.77 m	2.70 m	2.71 m		2.73 m	3.07 m	
14	6.37 s	6.32 s	6.34 s	7.32 s	6.34 s	6.77 s	8.02 s
15	3.69 m	3.65 m	3.67 m	2.84 dd (7.3,13.8), 2.71 dd (5.9,13.6)	3.69 m	3.64 m	3.08 m
16	3.58 m, 3.41 m	3.59 m, 3.39 m	3.57 m, 3.43 m	3.96 m	3.59 m, 3.43 m	4.17 m	4.54 m
17	1.13 d (6.9)	1.10 d (6.9)	1.10 d (6.7)	1.02 d (6.2)	1.12 d (6.9)	1.55 d (6.7)	1.36 d (6.2)
18	1.05 s	1.05 s	1.29 s	1.54 s	1.07 s	1.57 s	2.10 s
19	0.87 s	0.90 s			3.89m,3.69m		
20	1.29 s	1.25 s	1.21 s	1.11 s	1.30 s	1.92 s	1.11 s
Glc-1′	4.38 d (7.5)	4.31 d (7.8)	4.36 d (7.7)	4.60 m	4.39 d (7.6)		
2′	3.42 m	3.23 m	3.39 m	3.45 m	3.41 m		
3′	3.27 m	3.34 m	3.25 m	3.26 m	3.27 m		
4′	3.27 m	3.30 m	3.25 m	3.44 m	3.26 m		
5′	3.45 m	3.18 m	3.43 m	3.51 m	3.44 m		
6′	3.90 m, 3.67 m	3.64 m	3.85 m, 3.66 m	3.70 m	3.88 m, 3.67 m		
Glc-1″		4.36 d (7.5)	5.46 d (7.9)		4.23 d (7.8)		
2″		3.43 m	3.34 m		3.26 m		
3″		3.24 m	3.36 m		3.34 m		
4″		3.25 m	3.34 m		3.26 m		
5″		3.45 m	3.36 m		3.16 m		
6″		3.84 m	3.75 m, 3.66 m		3.85 m, 3.67 m		

[a] Values in *δ* (ppm); coupling constants (Hz) in parentheses. [b] Spectra obtained in CD_3OD. [c] Spectra obtained in acetone-d_6 and D_2O. [d] Spectra obtained in pyridine-d_5.

moiety in **1**. Therefore, the structure of **1** was elucidated as 12-*O*-*β*-D-glucopyranosyl-3,11,16-trihydroxyabieta-8,11,13-triene.

Compound **2**, a colorless, amorphous powder, was shown to have a molecular formula of $C_{32}H_{50}O_{14}$ by HRESIMS (*m*/*z* 681.3079, $[M + Na]^+$). Its IR spectrum revealed similar absorption bands to **1**. Comparison of the NMR data of **2** with those of **1** showed that both compounds are based on the same aglycon. The NMR data of **2** were similar to those of **1**, except for resonances of one additional sugar unit. On the basis of the $^1H-^1H$ COSY and HSQC spectra, all sugar protons and carbons could be assigned, from which the extra sugar unit was also concluded to be a *β*-glucopyranose unit. The glycosidic linkages were determined from the following HMBC correlations: H-1′ (δ_H 4.36)/C-12 (δ_C 143.3) and H-1″ (δ_H 4.31)/C-3 (δ_C 91.1). On the basis of the above evidence, compound **2** was established as 3,12-*O*-*β*-D-diglucopyranosyl-11,16-dihydroxyabieta-8,11,13-triene.

The HRESIMS of compound **3** showed a sodiated molecular ion at *m*/*z* 695.2924 $[M + Na]^+$, which, in conjunction with the ^{13}C NMR data, was used to establish a molecular formula of $C_{32}H_{48}O_{15}$. It also had similar IR absorptions to **1** except for a carbonyl absorption at 1726 cm^{-1}. On comparing the ^{13}C NMR data of **3** with those of **1**, compound **3** showed a signal attributed to a carboxy carbon (δ_C 178.5), while the hydroxyl group at C-3 (δ_C 79.9) in **1** was absent in **3**. Accordingly, the carboxylic group was placed at C-19, to which a sugar unit was linked directly, as determined from the following HMBC correlations: H-3 (δ_H 2.25), H-5 (δ_H 1.47) and H-18 (δ_H 1.29)/C-19 (δ_C 178.5), and H-1″ (δ_H 5.46)/C-19 (δ_C 178.5). Enzymatic hydrolysis of **3** yielded glucose as the sugar component, having a *β*-D-configuration from the ^1H and ^{13}C NMR data and optical rotation analysis. Thus, compound **3** was characterized as 19-*O*-*β*-D-carboxyglucopyranosyl-12-*O*-*β*-D-glucopyranosyl-11,16-dihydroxyabieta-8,11,13-triene.

The stereochemistry including the absolute configuration of **1**−**3** was elucidated by a combination of analyses, including correlations in the NOESY experiment and the modified Mosher's method. Since the biogenetically related diterpenoids have a trans-chair

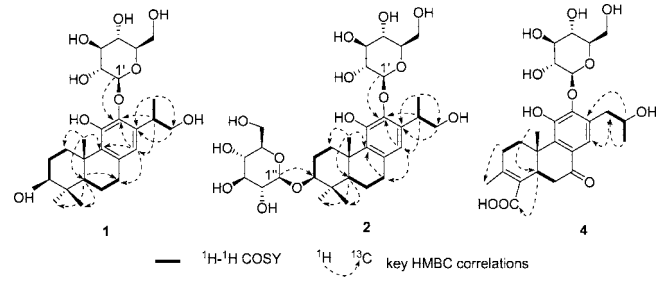

Figure 1. $^1H-^1H$ correlations and $^1H-^{13}C$ long-range correlations of compounds **1**, **2**, and **4**.

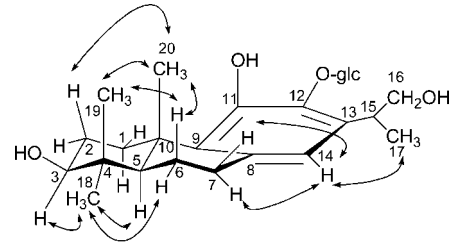

Figure 2. Key NOE correlations in the NOESY experiment of compound **1**.

conformation, the A and B rings, the C-20 methyl group, and the C-5 proton were presumed to be trans-diaxial.[13] On the basis of the NOESY NMR data, Me-20 and Me-19 were assigned as *β*-axial, Me-18 was assigned as α-equatorial, and the orientation of OH-3 of compound **1** and **2** was determined as *β*, from the NOESY correlations observed from Me-18 to H-3 and from Me-19 to Me-20 (Figure 2).

On acidic hydrolysis of **3** with 5% H_2SO_4 in EtOH, a new diterpene, 19-carboxy-11,12,16-trihydroxyabieta-8,11,13-triene (**3a**), was obtained. The absolute configuration of **3a** was determined using the method first described by Mosher and colleagues and

Table 2. ^{13}C NMR Spectroscopic Data (100 MHz) for Compounds **1**−**6**, **3a**, and **4a**[a]

position	**1**[b]	**2**[b]	**3**[b]	**4**[c]	**5**[b]	**6**[b]	**3a**[d]	**4a**[d]
1	36.2, t	36.2, t	37.5, t	31.1, t	48.0, t	38.1, t	37.1, t	31.9, t
2	29.1, t	28.3, t	21.1, t	28.2, t	200.3, s	31.0, t	20.9, t	29.9, t
3	79.9, d	91.1,d	39.1, t	127.7, s	138.6, s	37.3, t	38.8, t	125.7, s
4	40.8, s	41.0, s	45.8, s	132.5, s	149.4, s	39.9, s	44.4, s	130.0, s
5	54.7, d	54.9, d	57.4, d	41.6, d	161.7, s	56.2, d	56.2, d	43.1, d
6	20.4, t	20.2, t	22.6, t	37.8, t	125.3, d	20.5, t	22.5, t	38.7, t
7	34.3, t	34.2, t	35.0, t	202.1, s	191.9, s	34.7, t	34.2, t	197.1, s
8	135.4, s	135.4, s	135.8, s	129.3, s	109.9, s	135.5, s	129.4, s	125.2, s
9	134.8, s	134.8, s	133.7, s	139.4, s	136.5, s	135.3, s	132.9, s	137.6, s
10	40.8, s	40.5, s	41.5, s	37.5, s	44.2, s	40.9, s	40.7, s	38.1, s
11	149.4, s	149.4, s	149.6, s	148.5, s	131.3, s	149.4, s	146.6, s	151.1, s
12	143.4, s	143.3, s	143.5, s	149.3, s	156.3, s	143.5, s	142.2, s	145.6, s
13	136.7, s	136.7, s	136.8, s	131.6, s	120.6, s	136.7, s	130.6, s	130.0, s
14	118.5, d	118.5, d	118.6, d	121.8, d	158.2, s	118.5, s	119.3, d	123.3, d
15	35.1, d	35.1, d	35.1, d	38.9, t	101.5, d	35.2, d	38.2, d	41.8, t
16	69.4, t	69.4, t	69.4, t	67.7, d	154.5, s	69.4, t	68.6, t	69.1, d
17	18.6, q	18.6, q	18.7, q	22.1,q	14.1, q	18.7, q	16.9, q	23.5,q
18	17.0, q	17.7, q	29.8, q	19.8, q	11.6, q	28.9, q	29.8, q	16.6, q
19	29.5, q	29.3, q	178.5, s	178.6, s	60.2, t	74.6, t	180.5, s	171.9, s
20	20.1, q	20.1, q	18.1, q	15.2, q	27.8, q	21.0, q	18.1, q	20.8, q
Glc-1′	108.0, d	107.9, d	108.0, d	105.7, d		108.1, d		
2′	75.9, d	75.8, d	75.9, d	73.8, d		78.3, d		
3′	79.2, d	79.2, d	79.2, d	76.8, d		79.3, d		
4′	71.8, d	71.7, d	71.8, d	69.3, d		72.1, d		
5′	78.2, d	78.1, d	78.2, d	76.1, d		75.9, d		
6′	63.2, t	63.2, t	63.3, t	60.5, t		63.3, t		
Glc-1″		107.0, d	95.8, d			105.8, d		
2″		75.9, d	74.5, d			78.1, d		
3″		78.5, d	78.9, d			78.5, d		
4″		71.8, d	71.5, d			71.8, d		
5″		77.8, d	78.7, d			75.7, d		
6″		63.0, t	62.8, t			63.1, t		

[a] Values in δ (ppm). [b] Spectra obtained in CD_3OD. [c] Spectra obtained in acetone-d_6 and D_2O. [d] Spectra obtained in pyridine-d_5.

further elaborated by other research groups.[14] Compound **3a** was treated with (+)- and (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chlorides (MTPA chlorides) in NMR tubes[15] to afford the 16-*O*-(+)- and 16-*O*-(−)-MTPA esters **3a**−**r** and **3a**−**s**, respectively. Comparison of the chemical shift difference (Δ 0.19 ppm) of the methylene proton signals at C-16 of **3a**−**s** at δ_H 4.58 and 4.62 with that (Δ 0.04 ppm) of **3a**−**r** at δ_H 4.48 and 4.52 indicated the 15*S* configuration of **3a**; therefore, the C-15 configuration of compounds **1**−**3** was inferred as being 15*S*.

Compound **4** exhibited a molecular $[M + Na]^+$ ion peak at *m/z* 545.2043 in the positive HRESIMS, indicative of a molecular formula of $C_{26}H_{34}O_{11}$. The IR spectrum showed absorptions at 3396 (OH), 1681 and 1668 (C=O), and 1601 and 1541 (aromatic) cm^{-1}. The assignments of ^{1}H and ^{13}C NMR data (Tables 1 and 2) were based on HSQC, HMBC, and ^{1}H−^{1}H COSY spectra. The ^{13}C NMR spectrum showed signals for a ketone group at δ_C 202.1, a carboxylic group at δ_C 178.6, two tertiary methyl groups, and four double bonds including an aromatic ring characteristic of an abieta-8,11,13-triene. Enzymatic hydrolysis of **4** yielded β-D-glucose as its sugar component. The ^{1}H NMR spectrum of **4** allowed the assignment of one aromatic proton [δ_H 7.32 (s)], three methyl protons [δ_H 1.02 (d, J = 6.2 Hz), 1.12 (s), 1.77 (s)], and one anomeric proton [δ_H 4.65 (d, J = 7.8 Hz)], indicating a β-configuration. The HMBC spectrum showed cross-peaks from H-2 (δ_H 2.09 and 1.94) to C-1 (δ_C 31.1), C-3 (δ_C 127.7), C-4 (δ_C 132.5), C-10 (δ_C 37.5), and C-18 (δ_C 19.8) and from H-18 (δ_H 1.55) to C-2 (δ_C 28.2), C-3 (δ_C 127.7), C-4 (δ_C 132.5), and C-19 (δ_C 178.6). These interactions indicated the presence of a ring A structural unit with a double bond at C-3 and C-4, and one methyl group linked to C-3, with one carboxyl at C-4. The ketone group and the sugar unit were placed at C-7 and C-12, respectively, from the HMBC correlations: H-14 (δ_H 7.32) and H-6 (δ_H 2.88)/C-7 (δ_C 202.1); and H-1′ (δ_H 4.60)/C-12 (δ_C 149.3). On enzymatic hydrolysis of **4** with β-cellulase in H_2O for 48 h, the new compound 11,12,16-trihydroxy-17(15→16),18(4→3)-*abeo*-4-carboxy-3,8,11,13-abietatetraen-7-one (**4a**) was produced and characterized from its ^{1}H and ^{13}C NMR and EIMS data. Thus, the structure of compound **4** was elucidated as 11,16-dihydroxy-12-*O*-β-D-glucopyranosyl-17(15→16), 18(4→3)-*abeo*-4-carboxy- 3,8,11,13-abietatetraen-7-one.

HREIMS and NMR data of compound **5** were consistent with the molecular formula $C_{20}H_{18}O_6$. Its ^{1}H and ^{13}C NMR spectra were almost identical with those of teuvincenone F (**8**).[11] In fact, the only difference was an oxygenated methylene (δ_C 60.2) in **5** instead of a methyl group (δ_C 17.5) in **8**. Moreover, in the HMBC spectrum, the oxygenated methylene signals at δ_H 4.67 and 4.72 showed correlations with the signals at C-3 (δ_C 138.6), C-4 (δ_C 149.4), and C-5 (δ_C 161.3). Consequently, compound **5** was assigned as 19-hydroxyteuvincenone F.

The cytotoxic activities of the isolated compounds **1**−**8** were evaluated against three cell lines, B16 murine melanoma, HGC-27 human gastric, and HEK-293 human epithelial kidney, using the MTT [3-(4,5-dimethylythiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] assay.[16] However, all compounds were inactive (IC_{50} > 10 μM), except for compound **7**, which showed cytotoxicity against the B16, HGC-27, and HEK-293 cell lines, with IC_{50} values of 6.4, 5.3, and 1.2 μM, respectively.

To evaluate uncinatone (**7**) further, cell-cycle analysis of the HEK-293 cell line was conducted by staining with propidium iodide and using flow cytometry.[17] Compared with untreated HEK-293 cells in vehicle (0.1% DMSO), treatment with 2.5, 5, and 10 μM uncinatone (**7**) for 24 h resulted in a reduction of cells in the G_1 phase with an accumulation of cells at the G_2/M phase, indicating a G_2/M cell-cycle arrest (Figure S1, Supporting Information). In addition, concurrent paclitaxel and uncinatone (**7**) exposure caused an increase in the hypodiploid fraction of the HEK-293 cell line. Pretreatment with paclitaxel also increased the percentage of G_2/M arrest, 29.02% [paclitaxel + uncinatone (**7**)] compared with 16.14% [(only uncinatone (**7**)] and 23.57% (only paclitaxel) (Figure S2, Supporting Information).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV and IR spectra were recorded on a Shimadzu UV-2450 and a Perkin-Elmer 577 spectrophotometer, respectively. NMR spectra were taken on a Varian Mercury NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. ESIMS were measured using a Bruker Esquire-3000 mass spectrometer, and EIMS were obtained on a Finnigan/MAT-95 spectrometer. Reversed-phase HPLC purification was performed on an Agilent 1100 series system equipped with an Eclipse XDB-C18 column (9.4 m × 25 cm, Zorbax columns). Thin-layer chromatography (TLC): precoated silica-gel GF_{254} plates (Yantai, People's Republic of China). Column chromatography: silica gel (200–300 mesh; Qingdao), Cosmosil 75 C_{18}-OPN (40–105 μm; Nacalai Tesque, Inc.), C_8-OPN (140 mesh; Nacalai Tesque, Inc.), MCI gel CHP-20P (75–150 μm, Mitsubishi Chemical Industries Co., Ltd.), TSK gel Toyopearl HW-40F (30–60 μm; Toso Co., Ltd.), Sephadex LH-20 (20–80 μm, Pharmacia), and Diaion HP 20 (Mitsubishi Chemical Industries Co., Ltd.). β-Cellulase was manufactured by Lizhu Dongfeng BioTech Co. Ltd., Shanghai, People's Republic of China.

Plant Material. The roots of *Clerodendrum bungei* Steud. (Verbenaceae) were collected from Nanning, in Guangxi Province, People's Republic of China, in March 2006, and authenticated by Prof. He-Ming Yang. A voucher specimen (No. SIMMCB06) is deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

Extraction and Isolation. Dried roots of *C. bungei* (6.0 kg) were extracted with 70% aqueous acetone at room temperature. The solvent was removed in vacuo to yield 208 g of a gummy residue. The crude extract was subjected to a column of Diaion HP 20 and eluted with H_2O and 25%, 50%, 75%, and 100% MeOH. The 75% fraction (13 g) was further separated by passage over a LH-20 column (20% to 100% MeOH) to give five subfractions (A−E). Fraction A was chromatographed on a C_{18} column eluted with MeOH−H_2O (10% to 100%) to give four subfractions (A_1−A_4). Fraction A_1 was separated over a silica gel column using $CHCl_3$−MeOH−H_2O (8:1:0, 6:1:0, 3:1:0.1, 7:3:0.5) to give five subfractions, A_{11}−A_{15}. Fraction A_{15} was further subjected to passage over a C_{18} column with MeOH−H_2O (5% to 75%) to afford **3** (28 mg) and **4** (15 mg). Compound **5** (12 mg) was obtained from fraction A_3 using column chromatography over HW-40F with MeOH−H_2O. Fraction B was passaged over a C_8 column with MeOH−H_2O (10% to 100%) to give five subfractions (B_1−B_5). Further purification of subfraction B_3 using a C_{18} column with MeOH−H_2O (5% to 40%) as eluants afforded **1** (45 mg) and **6** (11 mg). Fraction D was subjected to C_{18} column chromatography eluted with MeOH−H_2O (10% to 100%) to afford four subfractions (D_1−D_4). Fraction D_3 was purified using silica gel column chromatography [$CHCl_3$−MeOH (40:1, 30:1, 10:1, 5:1)] and C_{18} column chromatography (MeOH−H_2O, 60% to 70%), resulting in the purification of compounds **2** (21 mg) and **8** (12 mg). Compound **7** (35 mg) was obtained from fraction E by MCI column chromatography using MeOH−H_2O (30% to 100%) as solvents.

Compound 1: amorphous powder; $[\alpha]^{22}_D$ +2.0 (*c* 0.19, MeOH); UV (MeOH) (log ϵ) λ_{max} 206 (4.59), 280 (3.39) nm; IR (KBr) ν_{max} 3405, 2929, 2874, 1637, 1566, 1423, 1329, 1246, 1072, 594 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 519.3 [M + Na]$^+$; HRESIMS *m/z* 519.2565 [M + Na]$^+$ (calcd for $C_{26}H_{40}O_9Na$, 519.2570).

Compound 2: amorphous powder; $[\alpha]^{21}_D$ +21.5 (*c* 0.33, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.75), 280 (3.67) nm; IR (KBr) ν_{max} 3405, 2966, 2875, 1637, 1566, 1423, 1369, 1329, 1248, 1155, 1074, 635 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 681.2 [M + Na]$^+$; HRESIMS *m/z* 681.3079 [M + Na]$^+$ (calcd for $C_{32}H_{50}O_{14}Na$, 681.3098).

Compound 3: amorphous powder; $[\alpha]^{22}_D$ +26.5 (*c* 0.37, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.59), 280 (3.16) nm; IR (KBr) ν_{max} 3403, 2929, 2874, 1726, 1637, 1423, 1385, 1331, 1229, 1074, 585 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 695.3 [M + Na]$^+$; HRESIMS *m/z* 695.2924 [M + Na]$^+$ (calcd for $C_{32}H_{50}O_{14}Na$, 695.2891).

Compound 4: amorphous powder; $[\alpha]^{22}_D$ +5.0 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.33), 271 (3.91) nm; IR (KBr) ν_{max} 3396, 2931, 1668, 1601, 1541, 1425, 1327, 1236, 1117, 1068, 658 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS (positive-ion mode) *m/z* 545.0 [M + Na]$^+$; HRESIMS *m/z* 545.2025 [M + Na]$^+$ (calcd for $C_{32}H_{48}O_{15}Na$, 545.1999).

Compound 5: amorphous powder; $[\alpha]^{21}_D$ +7.0 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (4.68), 292 (4.35) nm; IR (KBr) ν_{max} 3431, 1651, 1620, 1560, 1466, 1371, 1209, 1041, 978, 717 cm^{-1}; ^{1}H NMR (CD_3OD, 400 MHz) δ 4.37 (1H, d, J = 17.1, H-1a), 2.54 (1H, d, J = 17.2, H-1b), 6.80 (1H, s, H-6), 6.58 (1H, s, H-15), 2.48 (3H, s, H-17), 2.05 (3H, s, H-18), 4.70 (1H, d, J = 12.8, H-19a), 4.59 (1H, d, J = 12.7, H-19b), 1.75 (3H, s, H-20); ^{13}C NMR data see Table 2; EIMS *m/z* 354 [M]$^+$ (2), 321 (5), 92 (59), 91 (100), 85 (12), 83 (16), 65 (9), 64 (21), 63 (5), 51 (4); HREIMS *m/z* 354.1095 (calcd for $C_{20}H_{18}O_6$, 354.1086).

Enzymatic Hydrolysis of Compounds 1, 2, 4, and 6. Compounds **1**, **2**, **4**, and **6** (10 mg each) were dissolved in H_2O (5 mL), and β-cellulase (15 mg) was added to the solution and kept at 37 °C for 2 days, in each case. The reaction mixtures were extracted with EtOAc, and the aqueous phases compared with an authentic sugar sample by co-TLC (EtOAc−MeOH−H_2O−HOAc, 13:3:3:4, R_f 0.46 for glucose). Identification of D-glucose in each aqueous layer was carried out by comparing the optical rotation of the liberated glucose with that of an authentic sample of D-glucose ($[\alpha]^{22}_D$ +52). The EtOAc layer of compound **4** was followed by purification on HPLC (60% MeOH in H_2O, 3 mL/min, t_R = 5.2 min) to afford compound **4a** (3.1 mg).

Acidic Hydrolysis of Compound 3. A solution of compound **3** (15 mg) in 5% H_2SO_4−EtOH was refluxed for 3 h. The reaction mixture was neutralized and concentrated in vacuo to remove the alcohol and extracted with $CHCl_3$ twice. The $CHCl_3$ extract of **3** afforded **3a** as an aglycon after purification by HPLC (80% MeOH in H_2O, 2 mL/min, t_R = 7.9 min). The aqueous layer was subjected to co-TLC analysis and optical rotation measurement with an authentic sugar sample.

Preparation of the Mosher Ester Derivatives of 3a. Compound **3a** (2.5 mg) was transferred to a clean and completely dry NMR tube. Deuterated pyridine (0.5 mL) and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 μL) were added to the NMR tube immediately, and then the NMR tube was shaken carefully to evenly mix the sample and MTPA chloride. The reaction mixture was then permitted to stand at room temperature and monitored every 4 h by ^{1}H NMR spectroscopic analysis. The reaction was found to be complete after 24 h. ^{1}H NMR spectroscopic data of a (*R*)-MTPA ester derivative **3a-r** (400 MHz, C_5D_5N; obtained from the reaction NMR tube directly and assigned on the basis of the correlations of the ^{1}H−^{1}H COSY spectrum): δ 1.37 (1H, m), 4.03 (1H, m), 1.53 (1H, m), 2.23 (1H, m), 1.28 (1H, m), 2.34 (1H, m), 1.69 (1H, m), 1.55 (2H, m), 2.73 (2H, m), 3.39 (1H, m), 4.52 (1H, m), 4.48(1H,m), 1.32 (3H, s), 1.29 (3H, s), 1.20 (3H, s), 6.82 (1H, s). In the manner described for **3a-r**, another portion of compound **3a** (2.5 mg) was reacted in a second NMR tube with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (10 μL) at room temperature for 24 h using deuterated pyridine (0.5 mL) as solvent, to afford the (*S*)-MTPA ester derivative **3a-s** of **3a**. ^{1}H NMR spectroscopic data of **3a-s** (400 MHz, C_5D_5N): δ 1.52 (1H, m), 4.01 (1H, m), 1.19 (1H, m), 2.52 (1H, m), 2.70 (1H, m), 1.43 (1H, m), 1.84 (1H, m), 1.25 (1H, m), 1.49 (1H, m), 2.62 (1H, m), 3.07 (1H, m), 3.34 (1H, m), 4.72 (1H, m), 4.53 (1H, m), 1.23 (3H, d), 1.35 (3H, s), 1.40 (3H, s), 6.84 (1H, s). To further confirm the above procedure, the reaction mixtures were transferred from the NMR tubes and purified over preparative TLC (petroleum ether−EtOAc, 1:2, R_f = 0.6) to afford **3a-r** and **3a-s**: ESIMS *m/z* 1019.2 [M + Na]$^+$ and 994.9 [M − H]$^-$ (**3a-r**); 1019.2 [M + Na]$^+$ and 994.8 [M − H]$^-$ (**3a-s**).

Cytotoxicity Assay. Antiproliferative activity was evaluated as IC_{50} against the B16 murine melanoma cell line, the HGC-27 human gastric carcinoma cell line, and the HEK-293 human embryonic kidney cell line, using the MTT assay. Cells were seeded in a 96-well plate (5×10^3 cells/well) and cultured overnight. Then, 100 μL of vehicle (0.1% DMSO) or various concentrations of test compounds solubilized in DMSO were added, and the mixture was incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 48 h. The supernatants were discarded, and MTT (methyl thiazole tetrazolium, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (5 mg/mL in PBS) was added to each well. Following a 4 h incubation, 100 μL of 50% DMF−20% SDS was added. After the formazan was dissolved completely, the plates were read on a Bio-Tek Elx 800 ELISA reader at 570 nm. The concentrations required to inhibit growth by 50% (IC_{50} values) were calculated from survival curves. Paclitaxel was used as positive control (IC_{50} values: B16, 24.5 μM; HGC-27, 775 nM; HEK-293, 1.4 μM).

Cell-Cycle Analysis. HEK-293 cell proliferation was evaluated by measuring DNA content with propidium iodide. In brief, 3×10^4 cells/mL were incubated overnight and then treated with vehicle (0.1% DMSO) or different concentrations of the test compounds for 24 h. Cells were trypsinized, fixed in 70% EtOH for at least 20 min, and stained with propidium iodide (10 μg/mL) in a PBS solution containing 1 μg/mL RNase A for 30 min before DNA evaluation. Cell-cycle analysis was performed using the Becton-Dickinson FACScan and the CellQuest program.

Supporting Information Available: Figures S1 and S2, showing the effect of uncinatone (**7**) on cell cycle and evaluation of the combination of uncinatone (**7**) and paclitaxel in the HEK-293 cell line. This information is available free of charge via the Internet at http://pubs.acs.org.

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