Bioassay-Guided Isolation of Xanthones and Polycyclic Prenylated Acylphloroglucinols from *Garcinia oblongifolia*

Sheng-Xiong Huang,[†] Chao Feng,[‡] Yan Zhou,[†] Gang Xu,[†] Quan-Bin Han,[†] Chun-Feng Qiao,[†] Donald C. Chang,[‡] Kathy Qian Luo,*[,‡] and Hong-Xi Xu*[,†]

Chinese Medicine Laboratory, Hong Kong Jockey Club Institute of Chinese Medicine, Shatin, N.T., Hong Kong, People's Republic of China, and Bioengineering Graduate Program, Department of Chemical and Biomolecular Engineering, and Department of Biology, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, People's Republic of China

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Bioassay-guided fractionation of the acetone extract of the bark of *Garcinia oblongifolia* has resulted in the isolation of three new xanthones, oblongixanthones A−C (**1**−**3**), three new polyprenylated benzoylphloroglucinols, oblongifolins E−G (**4**−**6**), and 12 known compounds. Oblongifolins I (**5**) and J (**6**) are the first natural products that have similar structural features to those of two known oxidation products of garcinol. The structures of the new compounds **1**−**6** were characterized by spectroscopic data interpretation. All isolates were assayed for their apoptosis-inducing effects against HeLa-C3 cells. Oblongifolin C (**16**) was found to be the most potent apoptotic inducer of the compounds evaluated.

The genus *Garcinia* of the family Guttiferae is well known as a prolific source of polycyclic polyprenylated acylphloroglucinols[1] and bioactive prenylated xanthones,[2] which exhibit various biological activities including antibacterial,[3] antifungal,[4] anti-inflammatory,[5] antioxidant,[6] and cytotoxic effects.[7] In our previous studies on a *Garcinia* species, a series of polyprenated xanthones with apoptotic effects was isolated and characterized.[8] In continuing efforts to discover natural anticancer agents, an acetone extract of the bark of *Garcinia oblongifolia*, which is indigenous to southern mainland China, was found to induce apoptotic activity. In a previous phytochemical study, it was shown that this plant contains polyprenylated benzoylphloroglucinols.[9] Bioactivity-directed fractionation of this plant led to the isolation and identification of three additional new polyprenylated benzoylphloroglucinols, oblongifolins E−G (**4**−**6**), three new prenylated xanthones, oblongixanthones A−C (**1**−**3**), and 12 known compounds, 1,3,5-trihydroxy-13,13-dimethyl-2*H*-pyran[7,6-*b*]xanthone (**7**),[10] nigrolineaxanthones V (**8**) and T (**11**),[10,11] parvifolixanthone B (**9**),[12] rheediaxanthone A (**10**),[13] leiaxanthone (**12**),[14] euxanthone (**13**), oblongifolins A−D (**14**−**17**),[9] and guttiferone B (**18**).[9,15] We report herein the structure elucidation of the new compounds **1**−**6** by spectroscopic data interpretation. In addition, isolates **1**−**18** were tested for their apoptotic effects against HeLa-C3 cells.

Results and Discussion

In the present study, the acetone-soluble extract of *G. oblongifolia* was found to have stronger apoptotic inducing effects against HeLa-C3 cells than the methanol-soluble extract. The combined crude acetone extract (543 g) was suspended in H_2O (3 L) to produce an aqueous solution, then partitioned in turn with $CHCl_3$ (3 × 1.5 L) and EtOAc (3 × 1.5 L) to afford dried $CHCl_3$- (108.8 g), EtOAc- (129.0 g), and H_2O-soluble (300 g) extracts. The $CHCl_3$-soluble partition was found to have significant apoptotic activity. Therefore, this fraction was selected for further purification. Altogether 18 compounds (**1**−**18**) were obtained by repeated chromatography.

Oblongixanthone A (**1**) was isolated as a yellow gum. The molecular formula, $C_{18}H_{14}O_6$, was deduced by HRESIMS. The ^{1}H NMR spectrum was similar to the known xanthone **7**, with characteristic peaks of a 1,2,3,5-tetrasubstituted benzene ring [δ_H 6.16 (1H, d, J = 1.9 Hz) and 6.40 (1H, d, J = 1.9 Hz)], a chelated hydroxy proton [δ_H 13.0 (OH-1, s)], an aromatic proton singlet at δ_H 7.33, and a dimethylchromene ring [δ_H 6.88, 5.93 (1H each, d, J = 10.0 Hz) and 1.47 (6H, s)]. As the highest field aromatic protons at δ_H 6.16 and 6.40, which coupled to each other, were attributed to H-1 and H-3 in the 1,2,3,5-tetrasubstituted benzene ring B, respectively, and the other aromatic proton at δ_H 7.33 was assigned to ring A. This was confirmed by comparing the NMR data of ring B with those of **7** and the correlation peaks observed in the HMBC spectrum (Figure 1). The HMBC correlations of the ring A aromatic proton with a ketone carbon (δ_C 178.9 s) and three oxygenated aromatic carbons at δ_C 147.3, 145.3, and 143.4 (C-6, C-7, and C-10a) indicated that this proton may be attributed to H-8. The dimethylchromene ring unit was located at C-5 and C-6 on the basis of the HMBC correlations of H-11 with C-5, C-6, and C-10a. Accordingly, oblongixanthone A was assigned as 1,3,7-trihydroxy-13,13-dimethyl-2*H*-pyran[5,6-*b*]xanthone.

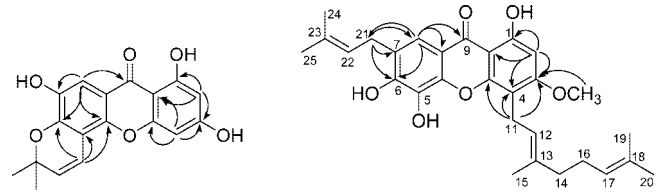

Figure 1. Key HMBC correlations of **1** and **2**.

Oblongixanthone B (**2**) was obtained as a yellow solid, with a molecular formula of $C_{29}H_{34}O_6$. The ^{1}H NMR spectrum exhibited signals of two aromatic protons at δ_H 6.40 (s, H-2) and 7.48 (s, H-8), a methoxyl group at δ_H 3.90 (s, OCH_3-3), and a geranyl unit, as evident from the following resonances: two olefinic protons at δ_H 5.24 (brt, H-12) and δ_H 4.99 (m, H-17), three pairs of methylene protons at δ_H 3.58 (d, H_2-11), 1.94 (m, H_2-14), and 2.04 (m, H_2-16), and three allylic methyls at δ_H 1.82 (s, H_3-15), 1.48 (s, H_3-19), and 1.51 (s, H_3-20). The remaining signals appeared as typical signals of a prenyl group. These signals were a doublet of the methylene protons H_2-21 at δ_H 3.37, a broad triplet of the olefinic proton H-22 at δ_H 5.36, and two singlets of methyl groups H_3-24 and H_3-25 at δ_H 1.73 and 1.77, respectively. All the assignments were further supported by the ^{13}C NMR and DEPT spectra (Table 2). The prenyl unit was located at C-8 according to the correlations of benzylic methylene protons H_2-21 to C-6, C-7, and C-8 and of H-8 to C-9. The methylene protons H_2-11 correlated to C-3, C-4, and C-4a, whereas the aromatic proton H-2 correlated to C-1, C-3, C-4, and C-9a. Consequently, the geranyl group was located at C-4.

* To whom correspondence should be addressed. Tel: +852-34062873. Fax: +852-35517333. E-mail: qluo@ust.hk; xuhongxi@hkjcicm.org.
[†] Hong Kong Jockey Club Institute of Chinese Medicine.
[‡] Hong Kong University of Science and Technology.

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Table 1. ^{1}H NMR Data of Compounds **4**−**6** (CD_3OD, 400 MHz, *J* in Hz)[a]

no.	4	5	6	no.	4	5	6
6	1.52 (m)	1.48 (m)	1.58 (m)	24	2.12 (m)	2.00 (m)	2.15 (m)
					2.08 (m)	1.77 (m)	1.76 (m)
7	2.18 (m) eq	2.18 (m)	2.02 (m)	25	4.89 (m)	4.84 (m)	4.89 (m)
	2.08 (m) ax	2.06 (m)	1.46 (t, 13.0)				
12	6.97 (t, 1.6)	7.43 (s)	7.45 (s)	27	1.97 (m)	1.93 (m)	1.94 (m)
14	6.96 (m)			28	1.46 (s)	1.39 (s)	1.49 (s)
15	7.16 (t, 7.9)	6.95 (s)	6.90 (s)	29	2.50 (m)	2.52 (d, 6.6)	2.51 (d, 6.8)
16	6.89 (m)			30	5.15 (m)	5.19 (m)	5.02 (m)
17	2.71 (m)	2.90 (m)	2.98 (m)	32	1.66 (s)	1.68 (s)	1.62 (s)
	2.58 (m)	2.83 (m)	2.89 (m)				
18	4.93 (m)	4.60 (m)	4.65 (m)	33	1.66 (s)	1.68 (s)	1.69 (s)
20	1.70 (s)	1.39 (s)	1.47 (s)	34	2.06 (m)	2.02 (m)	2.03 (m)
21	1.66 (s)	1.71 (s)	1.79 (s)	35	5.05 (m)	5.03 (m)	4.99 (m)
22	1.01 (s)	1.10 (s)	0.89 (s)	37	1.66 (s)	1.64 (s)	1.65 (s)
23	1.22 (brs)	1.24 (s)	1.21 (s)	38	1.55 (s)	1.56 (s)	1.55 (s)

[a] Assignments were based on ^{1}H−^{1}H COSY, HMQC, and ROESY experiments.

Table 2. ^{13}C NMR Data of Compounds **4**−**6** (CD_3OD, 100 MHz)[a]

no.	4	5	6	no.	4	5	6
1	195.6 (s)	194.3 (s)	195.3 (s)	20	26.3 (q)	26.0 (q)	25.9 (q)
2	118.0 (s)	119.6 (s)	120.6 (s)	21	18.3 (q)	18.5 (q)	18.6 (q)
3	195.2 (s)	179.3 (s)	177.9 (s)	22	27.3 (q)	26.7 (q)	16.5 (q)
4	68.7 (s)	64.3 (s)	66.7 (s)	23	23.2 (q)	22.2 (q)	24.3 (q)
5	48.9 (s)	49.5 (s)	49.3 (s)	24	30.1 (t)	30.4 (t)	29.5 (t)
6	47.7 (d)	47.6 (d)	43.6 (d)	25	125.5 (d)	124.5 (d)	123.7 (d)
7	40.8 (t)	40.1 (t)	43.6 (t)	26	137.4 (s)	137.9 (s)	138.1 (s)
8	62.0 (s)	66.8 (s)	67.1 (s)	27	40.8 (t)	41.0 (t)	40.8 (t)
9	209.4 (s)	207.2 (s)	207.0 (s)	28	16.4 (q)	16.2 (q)	16.3 (q)
10	197.9 (s)	174.3 (s)	174.1 (s)	29	32.0 (t)	30.2 (t)	31.1 (t)
11	140.0 (s)	118.1 (s)	118.3 (s)	30	120.6 (d)	120.8 (d)	121.0 (d)
12	116.2 (d)	109.6 (d)	109.8 (d)	31	135.8 (s)	135.3 (s)	135.0 (s)
13	158.5 (s)	147.1 (s)	147.1 (s)	32	26.3 (q)	26.3 (q)	26.2 (q)
14	120.7 (d)	154.8 (d)	154.8 (d)	33	18.2 (q)	18.1 (q)	18.2 (q)
15	129.9 (d)	104.0 (d)	103.9 (d)	34	27.5 (t)	27.6 (t)	27.5 (t)
16	121.3 (d)	151.3 (d)	151.2 (d)	35	125.4 (d)	125.2 (d)	125.2 (d)
17	27.0 (t)	27.7 (t)	26.1 (t)	36	132.1 (s)	132.3 (s)	132.3 (s)
18	120.6 (d)	119.6 (d)	120.4 (d)	37	26.0 (q)	25.9 (q)	26.1 (q)
19	135.6 (s)	136.1 (s)	135.7 (s)	38	17.8 (q)	17.7 (q)	17.8 (q)

[a] Assignments were based on DEPT, HMQC, and HMBC experiments; chemical shifts are given in ppm.

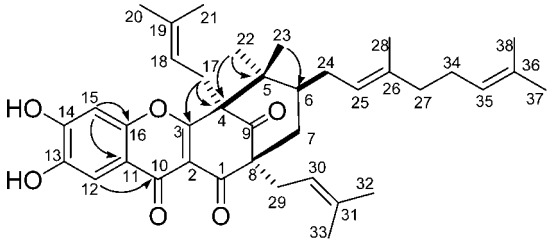


Figure 2. Key HMBC correlations of **5**.

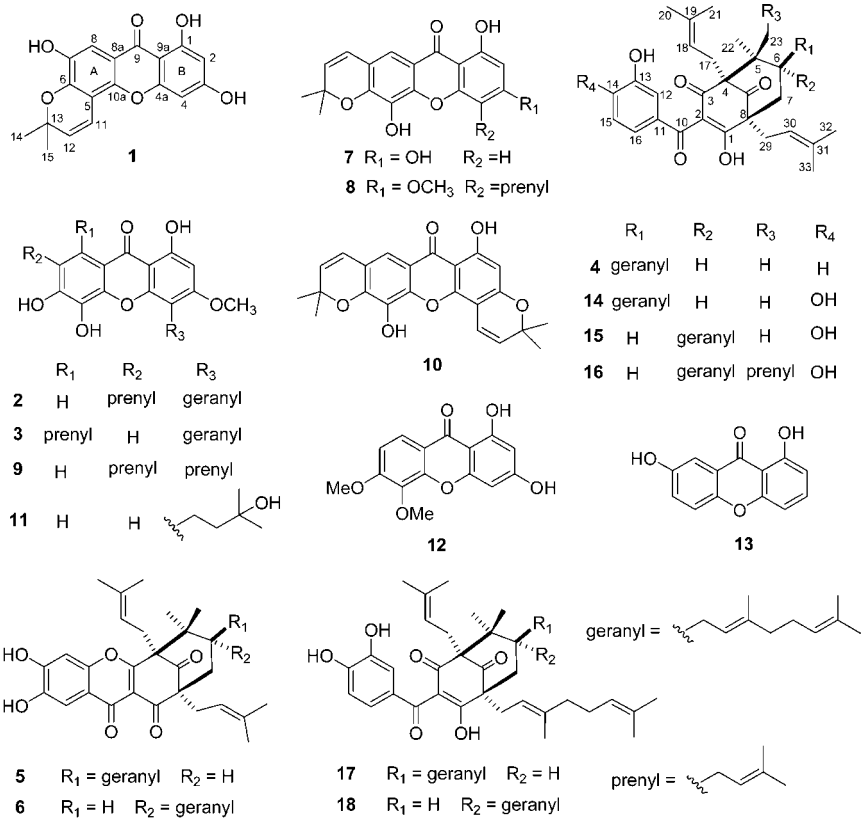
In a NOESY experiment, the OCH_3 group exhibited a strong correlation with H-2, so the methoxy group was placed at C-3. Oblongixanthone B (**2**) was therefore characterized as 1,5,6-trihydroxy-3-methoxy-7-(3-methyl-2-butenyl)-4-(3,7-dimethyl- 2,6-octadienyl)xanthone.

Oblongixanthone C (**3**) gave the same molecular formula as that of **2**, as determined by HRESIMS. Comparison of the spectroscopic data of **3** with those of **2** showed similarities in the B ring. The aromatic proton signal in ring A was shifted upfield from δ_H 7.48 in **2** to δ_H 6.69 in **3**, and the ketone was shifted downfield in the ^{13}C NMR spectrum. Thus, it was determined that the prenyl group is affixed to the C-8 position. This deduction was supported by the HMBC correlations of H_2-21 with C-7, C-8, and C-8a as well as of H-7 with C-5, C-8a, and C-21. Therefore, **3** was determined as 1,5,6-trihydroxy-3-methoxy-8-(3-methyl-2-butenyl)-4-(3,7-dimethyl-2,6-octadienyl)xanthone.

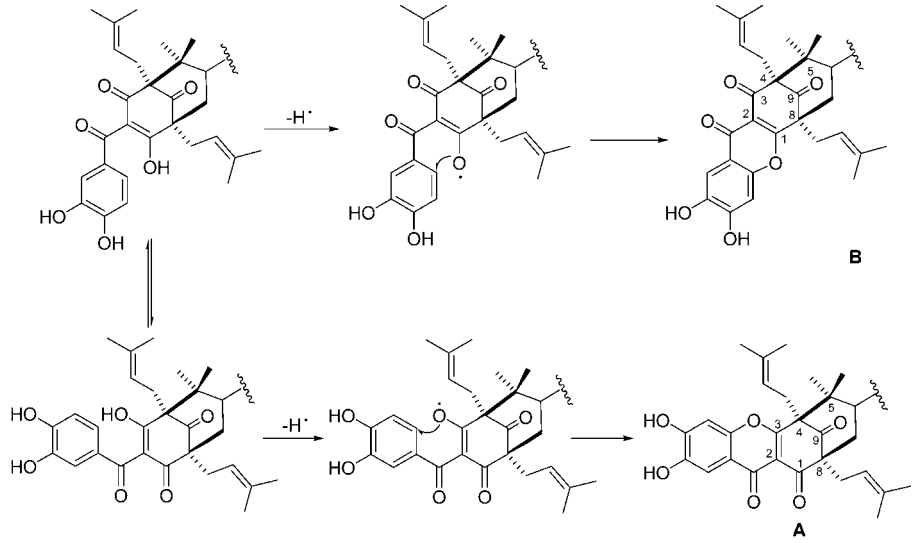
The HRMS of compound **4** showed a pseudomolecular ion at *m/z* 587.3733 $[M + H]^+$, which was used to establish its molecular formula as $C_{38}H_{50}O_5$. The IR spectrum exhibited bands for hydroxyl (3446 cm^{-1}) and both nonconjugated (1732 cm^{-1}) and conjugated (1670 cm^{-1}) carbonyl groups. Four vinyl protons, seven vinylic methyl groups, and 10 allylic protons apparent in the ^{1}H NMR spectrum (Table 1) of **4** indicated the presence of two prenyl and one geranyl group. A 1,3-disubstituted benzene ring was evident from proton resonances at δ_H 6.97 (t, $J = 1.6$ Hz), 6.96 (m), 6.89 (m), and 7.16 (d, $J = 7.9$ Hz). Characteristic ^{13}C NMR resonances, including those for substituted aromatic carbons at δ_C 140.0 and 158.5 and a conjugated carbonyl at δ_C 197.9, were indicative of a 3-hydroxybenzoyl group. Resonances for a six-membered ring consisting of a nonconjugated ketone (δ_C 209.4) flanked by two quaternary carbons (δ_C 62.0, 68.7) and an enolized 1,3-diketone (δ_C 118.0, 195.6, 195.2) were observed. These data suggested that oblongifolin E (**4**) is a polyisoprenyl benzophenone derivative related to oblongifolins A−D.[9] Comparison of the NMR data with those of oblongifolins A−D (**14**−**17**) led to the conclusion that the structure of **4** is closely comparable to that of oblongifolin A (**14**), except for the 3-hydroxybenzoyl group. All these assignments were further confirmed by the analysis of HMQC, HMBC, and ROESY NMR experiments.

Oblongifolins F (**5**) and G (**6**) were obtained initially as a mixture by silica gel column chromatography and then separated by semipreparative HPLC. Analysis of their ^{1}H and ^{13}C NMR data indicated that **5** and **6** have very similar structure and might be a pair of epimers with a polyprenylated benzoylphloroglucinol backbone. The NMR data indicated that both of these substances have two prenyl groups and one geranyl group, like **4**. Nonconjugated and conjugated ketones and two quaternary carbons were also

Chart 1



Scheme 1. Proposal for the Formation of Two Possible Structures, **A** and **B**



observed (Table 2). Compounds **5** and **6** gave the same molecular formula, $C_{38}H_{48}O_6$, as determined by HRESIMS. This elemental composition indicated 15 degrees of unsaturation, which showed **5** and **6** to have one more unit of unsaturation than **14** and **15**. Two singlet aromatic protons in the ^{1}H NMR spectra indicated the presence of a 1,2,4,5-tetrasubstituted benzene ring rather than the trisubstituted ring in **14** and **15**. In addition, there were three oxygen-substituted aromatic carbons in the ^{13}C NMR spectra. Based on the information mentioned above, it was inferred that H-16 is substituted by oxygen and formed another ring with C-1 or C-3. Because the enolized 1,3-diketone can undergo keto−enol tautomerism,[16] the formation of the ring could occur via two regioisomeric options (Scheme 1), that is, cycloaddition to form a bond between O-16 and C-3 (**A** in Scheme 1) or between O-16 and C-1 (**B** in Scheme 1). Obviously, the key differences between the ^{1}H NMR spectra of **5** and oblongifolin A (**14**) are the downfield-shifted resonance of carbon C-8 and upfield-shifted resonance of carbon C-4 in **5**, which indicated that both ketones are proximate to C-8. Further evidence was provided by the HMBC correlations of Me-22 and Me-23 with C-4, C-5, and C-6 as well as of H-17 with C-4 and C-3 (δ_C 179.3, s) in **5**. Hence, the structure of **5** was confirmed as **A** (Scheme 1).

The only structural difference between **5** and **6** was found to be in the opposite configuration of C-6. This was deduced by the different ^{13}C NMR chemical shift of Me-22, which was due to the γ-gauche interaction shielding of the axial methyl by the C-6 substituent when this group is equatorial.[9] Therefore, the structure of **6** was determined as shown.

Oblongifolins F (**5**) and G (**6**) are the first naturally polyprenylated acylphloroglucinols in which the enol hydroxyl forms a six-

Table 3. Apoptosis-Inducing Effects of Test Compounds at 20 μM[a]

compound	apoptotic effect at 24 h	48 h	72 h
3	−	+	+
5	−	+	+
6	−	+	+
7	−	−	+
11	−	−	+
15	−	+	+
16	+	+	+
17	−	−	+
paclitaxel(500nM)	−	+	+

[a] "+" means the YFP/CFP emission ratio of HeLa-C3 cells treated with that compound was below 3 at the indicated time point. From this table, it could be found that compound **16** at 20 μM is better than the positive control (500 nM paclitaxel) in inducing apoptosis.

membered ring with the benzene ring carbon and are analogues of two oxidation products of garcinol, GDPPH-1 and GDPPH-2.[17] The mechanism for their formation is proposed in Scheme 1. The reaction of garcinol with azobisisobutyronitrile (AIBN) or the stable free diphenylpicryl hydrazyl (DPPH) radical generates a conjugated radical. Cyclization then occurs, involving the catechol ring or a pendant alkenyl group to generate both compounds. Therefore, we presume that **5** and **6** originated from **14** and **15** through a similar radical pathway.

The polycyclic prenylated acylphloroglucinols for which absolute configurations have been determined experimentally are xanthochymol,[18] guttiferone E,[19] and garcinol.[19] Certain compounds, however, have been isolated in both enantiomeric forms. In this regard, some authors have suggested that the specific rotation (positive or negative) of a compound can reveal its absolute configuration. However, caution should be exercised because of the possibility of the presence of impurities at high optical rotation and also because comparing the optical rotation of a natural product to that of a chemically related molecule can be unreliable and should not be assumed to be definitive.[20] Therefore, the absolute configurations for **4**−**6** remain undetermined in the present study.

The biological activity of compounds **1**−**11** and **13**−**17** was evaluated for apoptosis-inducing effects using genetically engineered HeLa-C3 cells that can produce a fluorescent biosensor capable of detecting caspase-3 activation.[21] These cells emit a green light under normal growth conditions and change to a blue light when caspase-3 is activated during apoptosis to cleave the sensor protein inside the cells. This color change allows one to use a fluorescent plate reader to directly detect the activation level of caspase-3 in HeLa-C3 cells during the course of the compound treatment in a noninvasive way.[22] Based on our previous test results, the emission ratio of YFP (yellow fluorescent protein)/CFP (cyan fluorescent proteins) is usually between 6 and 8 in normal cells, and this ratio will decrease to a value below 3 if a compound can activate caspase-3 and kill cancer cells. Therefore, any compound that can reduce the YFP/CFP emission ratio to a value below 3 is considered positive in activating apoptosis.

As shown in Table 3, compounds were tested at a concentration of 20 μM. **3**, **5**, **6**, **7**, **11**, **15**, **16**, and **17** were found to activate caspase-3 in HeLa-C3 cells within 72 h. Among these eight compounds, **3**, **5**, **6**, **15**, and **16** reduced the YFP/CFP emission ratio below 3 within 48 h. In particular, compound **16** required less than 24 h to achieve this, indicating that it is the most potent apoptotic inducer among the compounds tested and even better than the anticancer drug paclitaxel (500 nM), which needed 48 h to produce the same apoptotic effect.

Other than determining the caspase activation using our HeLa-C3 cells, which contain a caspase sensor, we also examined the cell morphology changes after the treatment of compound **16**. As shown in Figure 3, in the control sample, without the compound

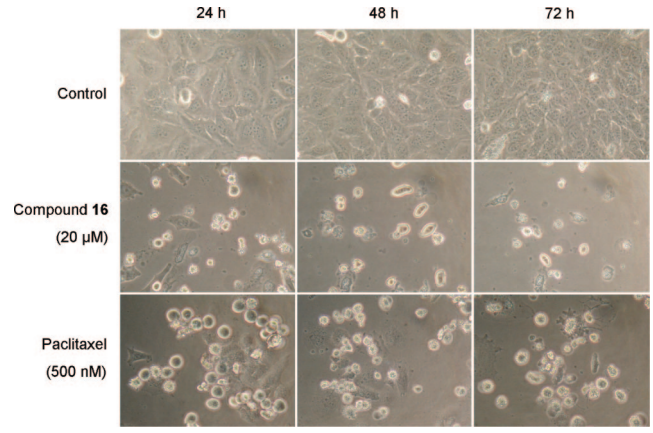


Figure 3. Cell morphology changes during the course of compound treatment. HeLa-C3 cells were treated with compound **16** at 20 μM, with an anticancer drug, pacilitaxel, at 500 nM (serving as a positive control), or without any drug (control) for 24, 48, and 72 h. Various cell morphologies were recorded at the indicated time points. The control cells have normal attached cell morphology through the course of the experiment. Cells have shrinkage and detached morphology when they were treated with either the anticancer drug paclitaxel or compound **16**, confirming its ability to kill cancer cells.

treatment, the cells attached to the culturing surface with normal cell morphology. When cells were treated with a clinically used anticancer drug, paclitaxel (500 nM), which is known to induce apoptotic cell death, the cells first rounded up and then appeared with typical cell shrinkage morphology. Similar cell shrinkage was observed in HeLa-C3 cells treated with compound **16** (20 μM) for only 24 h. Since both caspase activation and cell shrinkage are landmark events only occurring in apoptotic cells, we can conclude that compound **16** is capable of inducing apoptotic cell death in HeLa-C3 cells.

Experimental Section

General Experimental Procedures. Ultraviolet absorption spectra were recorded using a Perkin-Elmer Lambda L14 spectrometer, whereas infrared spectra were obtained with a Perkin-Elmer 577 spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AV-400 spectrometer with TMS as the internal standard. HRMS were obtained using a nanoLC-MS/MS system, with a nanoAcquity UPLC module and a Q-TOF spectrometer equipped with a nanoelectrospray ion source (Waters, Milford, MA) and provided by a lock-mass apparatus to perform a real-time calibration correction. Column chromatography was performed with silica gel 60 (200−300 mesh, Merck), Sephadex LH-20 (Pharmacia), and reversed-phase C_{18} silica gel (250 mesh, Merck). Precoated TLC sheets of silica gel 60 GF254 were used. An Agilent 1100 series equipped with an Alltima C_{18} or Zorbax SB-C_{18} column (4.6 × 250 mm) was used for HPLC analysis, and semipreparative and preparative Alltima C_{18} columns or Zorbax SB-C_{18} columns (9.4 × 250 mm and 22 × 250 mm) were used in sample preparation. Spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Plant Material. The bark of *G. oblongifolia* was collected at Bobai, Guangxi Province, People's Republic of China, in December 2005. The sample was identified by Dr. Chunfeng Qiao, and a voucher specimen (CMED-0469) has been deposited at Hong Kong Jockey Club Institute of Chinese Medicine.

Extraction and Isolation. Air-dried and powdered bark of *G. oblongifolia* (5.4 kg) was extracted with acetone (3 × 10 L, each 2 days) at room temperature. After evaporating the solvents in vacuo at 45 °C, a residue (543 g) was obtained. The combined crude acetone extract (543 g) was suspended in H_2O (3 L) to produce an aqueous solution, then partitioned in turn with $CHCl_3$ (3 × 1.5 L) and EtOAc (3 × 1.5 L) to afford dried $CHCl_3$- (108.8 g), EtOAc- (129.0 g), and H_2O-soluble (300 g) extracts. The $CHCl_3$ extract was subjected to column chromatography over silica gel (200−300 mesh) and eluted

with hexane−acetone (from 1:0 to 0:1) to give fractions A−J. Fractions C and D showed the most potent apoptotic activity. Fraction C was subjected to silica gel column chromatography and eluted with $CHCl_3$−MeOH (50:1 and 20:1) to produce subfractions C1−C4. Fractions C2 and C3 was combined and subjected to passage over a Sephadex LH-20 column, eluted with methanol, to yield three fractions. These fractions were chromatographed separately on RP-18 gel eluted in a step gradient manner with methanol−water (from 20:80 to 100:0) to afford compound **8** (12 mg) as yellow crystals and several subfractions. These subfractions were finally purified by preparative and semipreparative HPLC to afford compounds **2** (8 mg), **3** (5 mg), **4** (14 mg), **9** (6 mg), and **10** (3 mg). Compound **11** (7 mg) was obtained from fraction C4 by recrystallization in methanol. Fraction D (20.4 g) was separated in a preliminary fashion using a RP-18 gel column eluted with MeOH−H_2O (40%−100%) as a gradient system to afford four main fractions, D1−D4. Compounds **1** (5 mg), **7** (3 mg), **12** (300 mg), and **13** (2 mg) were obtained from fraction D2 (3.8 g) by Sephadex LH-20 column chromatography, by elution with methanol, and by semipreparative HPLC (3 mL/min) (MeOH−H_2O, 75% + 0.1% formic acid in both solvents). Fraction D4 (10.5 g) was divided into subfractions D4A−D4c by passage over a Sephadex LH-20 column eluted with methanol. Separation of fraction D4b (4.8 g) over silica gel and by preparative and semipreparative HPLC (3 mL/min), with MeOH−H_2O (92% + 0.1% formic acid in both solvents), afforded compounds **5** (5 mg), **6** (2 mg), **14** (610 mg), and **15** (289 mg). Fraction D4c (3.5 g) was separated by preparative HPLC (3 mL/min), eluting with CH_3CN−H_2O (95:5) + 0.3% formic acid in both solvents, to give compounds **16** (140 mg), **17** (259 mg), and **18** (155 mg).

Oblongixanthone A (1): yellow gum; UV (MeOH) λ_{max} (log ϵ) 329 (4.70) nm; IR (KBr) ν_{max} 3395, 2926, 1648, 1613, 1601, 1464, 1377, 1305, 1264, 1165, 1125 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ_H 13.0 (1H, brs, OH-1), 10.9 (1H, brs, OH-7), 9.70 (1H, brs, OH-3), 7.33 (1H, s, H-8), 6.88 (1H, d, J = 10.0 Hz, H-11), 6.40 (1H, d, J = 1.9 Hz, H-4), 6.16 (1H, d, J = 1.9 Hz, H-2), 5.93 (1H, d, J = 10.0 Hz, H-12), 1.47 (6H, s, H-14 and H-15); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 178.9 (C-9), 164.9 (C-3), 162.5 (C-1), 157.1 (C-4a), 147.3 (C-10a), 145.3 (C-6), 143.4 (C-7), 130.8 (C-12), 114.7 (C-11), 112.6 (C-8a), 109.4 (C-5), 101.6 (C-9a), 97.8 (C-2), 93.8 (C-4), 77.8 (C-13), 27.6 (C-14 and C-15); HRESIMS (positive ion) m/z 327.0865 (calcd for $C_{18}H_{15}O_6$ $[M + H]^+$, 327.0869).

Oblongixanthone B (2): amorphous, yellow solid; UV (MeOH) λ_{max} (log ϵ) 327 (4.50) nm; IR (KBr) ν_{max} 3331, 2969, 1646, 1585, 1476, 1373, 1270, 1206, 821 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ_H 7.48 (1H, s, H-8), 6.40 (1H, s, H-2), 5.36 (1H, m, H-22), 5.24 (1H, t-like, J = 7.0 Hz, H-12), 4.99 (1H, m, H-17), 3.58 (2H, d, J = 7.0 Hz H-11), 3.37 (2H, d, J = 7.2 Hz, H-21), 2.04 (2H, m, H-16), 1.94 (2H, m, H-14), 1.82 (3H, s, H-15), 1.77 (3H, brs, H-25), 1.73 (3H, brs, H-24), 1.51 (3H, brs, H-20) 1.48 (3H, brs, H-19), 3.90 (3H, s, OMe); ^{13}C NMR (CD_3OD, 100 MHz) δ_C 182.4 (C-9), 165.3 (C-3), 163.0 (C-1), 155.2 (C-4a), 152.8 (C-6), 147.6 (C-10a), 135.9 (C-13), 134.1 (C-23), 132.7 (C-5), 132.0 (C-18), 127.4 (C-7), 125.4 (C-17), 123.9 (C-12), 122.9 (C-22), 117.3 (C-8), 113.8 (C-8a), 109.4 (C-4), 103.7 (C-9a), 94.9 (C-2), 40.8 (C-14), 29.1 (C-21), 27.6 (C-16), 26.0 (C-25), 25.7 (C-20), 22.2 (C-11), 17.9 (C-24), 17.6 (C-19), 16.4 (C-15), 56.6 (OCH_3); HRESIMS (positive ion) m/z 479.2425 (calcd for $C_{29}H_{35}O_6$ $[M + H]^+$, 479.2434).

Oblongixanthone C (3): amorphous, yellow solid; UV (MeOH) λ_{max} (log ϵ) 331 (4.70) nm; IR (KBr) ν_{max} 3389, 2913, 1648, 1612, 1583, 1446, 1376, 1302, 1112, 887, 817 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ_H 6.69 (1H, s, H-7), 6.34 (1H, s, H-2), 5.36 (1H, m, H-22), 5.26 (1H, brt, J = 7.0 Hz, H-12), 4.98 (1H, m, H-17), 3.91 (2H, d, J = 7.2 Hz, H-21), 3.52 (2H, d, J = 7.0 Hz H-11), 2.02 (2H, m, H-16), 1.94 (2H, m, H-14), 1.84 (3H, s, H-15), 1.74 (3H, brs, H-25), 1.72 (3H, brs, H-24), 1.51 (3H, brs, H-20) 1.48 (3H, brs, H-19), 3.89 (3H, s, OMe); ^{13}C NMR (CD_3OD, 100 MHz) δ_C 184.3 (C-9), 164.8 (C-3), 163.2 (C-1), 154.5 (C-4a), 152.2 (C-6), 149.5 (C-10a), 137.1 (C-8), 135.8 (C-13), 133.3 (C-18), 132.1 (C-5), 132.0 (C-23), 124.5 (C-22), 125.4 (C-17), 123.9 (C-12), 114.4 (C-7), 112.4 (C-8a), 108.7 (C-4), 104.3 (C-9a), 94.7 (C-2), 40.9 (C-14), 34.0 (C-21), 28.4 (C-16), 26.0 (C-25), 25.7 (C-20), 22.2 (C-11), 18.0 (C-24), 17.6 (C-19), 16.4 (C-15), 56.6 (OCH_3); HRESIMS (positive ion) m/z 479.2430 (calcd for $C_{29}H_{35}O_6$ $[M + H]^+$, 479.2434).

Oblongifolin E (4): yellow gum; $[\alpha]^{19}_D$ +65.1 (c 0.40, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 283 (4.40) nm; IR (KBr) ν_{max} 3446, 2972, 2911, 1732, 1670, 1653, 1542, 1448, 1375, 1287, 1208, 768 cm^{-1}; ^1H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data, see Tables 1 and 2; HRESIMS (positive ion) m/z 587.3733 (calcd for $C_{38}H_{51}O_5$ $[M + H]^+$, 587.3736).

Oblongifolin F (5): yellow gum; $[\alpha]^{19}_D$ −85.6 (c 0.41, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 338 (4.39) nm; IR (KBr) ν_{max} 3439, 2973, 2910, 1736, 1687, 1620, 1514, 1466, 1387, 1291, 1187, 1143, 842 cm^{-1}; ^1H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data, see Tables 1 and 2; HRESIMS (positive ion) m/z 601.3531 (calcd for $C_{38}H_{49}O_6$ $[M + H]^+$, 601.3529).

Oblongifolin G (6): yellow gum; $[\alpha]^{19}_D$ +5.9 (c 0.41, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 331 (4.30) nm; IR (KBr) ν_{max} 3446, 2977, 2915, 1734, 1683, 1621, 1539, 1466, 1387, 1294, 1186, 1142, 834 cm^{-1}; ^1H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data, see Tables 1 and 2; HRESIMS (positive ion) m/z 601.3539 (calcd for $C_{38}H_{49}O_6$ $[M + H]^+$, 601.3529).

Bioassay. All the test samples were dissolved in DMSO to make stock solutions. The concentration of each stock was at least 1000 times higher than the working concentration. All stocks were stored at 4 °C and used within 2−3 weeks after their preparations. HeLa-C3 cells, which can detect apoptotic cell death involving caspase activation, were cultured in minimum essential medium (MEM) containing 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin, in a 5% CO_2 humidity incubator at 37 °C. In order to test a sample with apoptotic activity, a well of a 96-well plate was seeded with 7500 HeLa-C3 cells suspended in 100 μL of culture medium as sample well. Another well of the plate was filled with only 100 μL of culture medium as background well. After 12−16 h incubation, the plate was read using a Perkin-Elmer Victor plate reader with excitation wavelength at 440 ± 10 nm and emission wavelength at 486 ± 8 nm for CFP and 535 ± 8 nm for YFP, to obtain the data for time point "0 h". Then immediately, the old medium was removed and 100 μL of freshly prepared culture medium containing the test sample at a designed working concentration was added to both the sample well and the corresponding background well. Culture medium containing 0.1% DMSO was used as a negative control, while 500 nM paclitaxel was used as a positive control. After this, the plate was read repeatedly at the time points indicated. The data acquisition period was up to 72 h. The YFP/CFP emission ratio was then calculated. If the YFP/CFP emission ratio was decreased to below 3, then a significant apoptotic cell death was considered as having occurred. All screening of samples was conducted in triplicate, and all experiments were repeated three times.

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