Cytotoxic Hydrolyzable Tannins from *Balanophora japonica*

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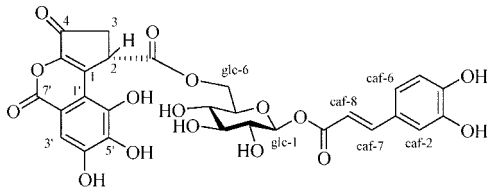
Received September 26, 2007

Four hydrolyzable tannins named balanophotannins D−G (**1**–**4**) were isolated from the aerial parts of the parasitic plant *Balanophora japonica*. Their structures were characterized on the basis of spectroscopic and chemical evidence. Balanophotannins D−G contain an oxidized hexahydroxydiphenoyl (HHDP) group. The absolute configurations of balanophotannins D (**1**) and F (**3**) were determined via the PGME method. Balanophotannin E (**2**) showed cytotoxicity to Hep G2 cancer cells with an IC_{50} value of 4.22 μM.

We have reported the isolation and structural characterization of 18 new and 16 known acyl glucoses having caffeoyl, coumaroyl, and hexahydroxydiphenoyl groups,[1] three new ellagitannins named balanophotannins A−C, and four known lignan glycosides[2] from *Balanophora japonica* M. (Balanophoraceae), a plant used as an antipyretic, antidote, and hemostatic medicine in China.[1,2] Our further investigation of the aqueous and EtOAc layers of the MeOH extract of this medicinal plant using a combination of column chromatographies led to the discovery of four new hydrolyzable tannins, named balanophotannins D−G (**1**–**4**). This paper describes the elucidation of their chemical structures by means of NMR and MS techniques and chemical methods, and the evaluation of their cytotoxicities to Hep G2 cancer cells.



Balanophotannin D (**1**)

Balanophotannin D (**1**) was isolated as a tan, amorphous powder showing a dark blue reaction with $FeCl_3$ on TLC. Its molecular formula was identified as $C_{28}H_{23}O_{16}$ on the basis of HRESIMS data. The ^{1}H NMR spectrum displayed proton signals of a 1,6-diacylated glucopyranosyl moiety with a 1β-configuration [δ 5.53 (1H, d, J = 8 Hz, glc-1), 4.49 (1H, dd, J = 2, 12 Hz, glc-6a), 4.32 (1H, dd, J = 6, 12 Hz, glc-6b)] and a caffeoyl (caf) moiety [δ 7.09 (1H, d, J = 2 Hz, caf-2), 6.79 (1H, d, J = 8 Hz, caf-5), 7.00 (1H, dd, J = 2, 8 Hz, caf-6), 7.70 (1H, d, J = 16 Hz, caf-7), 6.31 (1H, d, J = 16 Hz, caf-8)].[1] Besides glucosyl and caffeoyl moieties, the ^{13}C NMR data (Table 1) indicated the presence of an aromatic moiety comprising 13 carbon atoms. Comparison of the ^{1}H and ^{13}C NMR data (Table 1) derived from this moiety with the reference data[3] suggested that it was a brevifolincarboxylate, which was verified by the observation of HMBC correlations from H-2 to C-1 and C-6, from H-3 to C-4 and C-6, and from H-3′ to C-2′, C-5′, and C-7′ as shown in Figure 1. Furthermore, the HMBC correlations between the anomeric proton and carbonyl carbon of the caffeoyl unit, and between H-6 of the glucose moiety and the carbonyl carbon of the brevifolincarboxylate, confirmed the linkages of the two acyl groups in the glucopyranosyl moiety. In order to determine the absolute configuration of C-2 of the brevifolincarboxylate, compound **1** was methylated and then subjected to alkaline

Table 1. ^{13}C NMR (125 MHz) Data of Compounds **1**, **3**, and **4**

no.	**1**[a]	**3**[a]	**4**[b]
caffeoyl-1	127.6	127.5	127.7
2	115.4	115.3	116.7
3	146.7	146.8	147.2
4	149.8	149.9	150.6
5	116.5	116.5	117.6
6	123.4	123.3	124.3
7	148.7	148.7	149.2
8	114.1	113.9	114.6
9	167.7	167.5	168.7
glucose-1	95.6	95.6	96.4
2	73.9	74.1	74.4
3	77.8	74.7	77.7
4	71.4	76.9	71.7
5	76.1	71.1	76.6
6	65.1	67.2	64.8
4, 6-acyl-1	140.5	176.9	177.2
2	42.4	44.5	43.1
3	38.4	48.3	91.5
4	195.5	31.2	54.0
5	147.6	174.1	85.6
6	174.4	173.5	176.3
7	-	-	179.6
1′	115.0	118.1	123.2
2′	116.3	117.4	117.8
3′	109.4	114.8	114.6
4′	150.8	148.3	148.2
5′	141.3	136.5	137.4
6′	144.6	143.3	148.8
7′	162.5	165.7	168.0

[a] In C_5D_5N. [b] In D_2O+C_5D_5N (2:1).

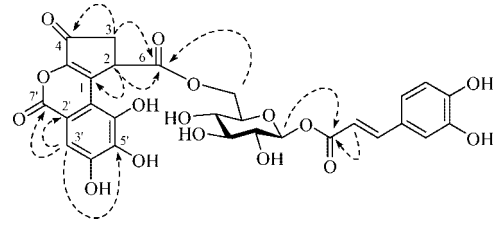


Figure 1. Important HMBC correlations (H to C) of compound **1**.

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10.1021/np070519+ CCC: $40.75
Published on Web 02/27/2008

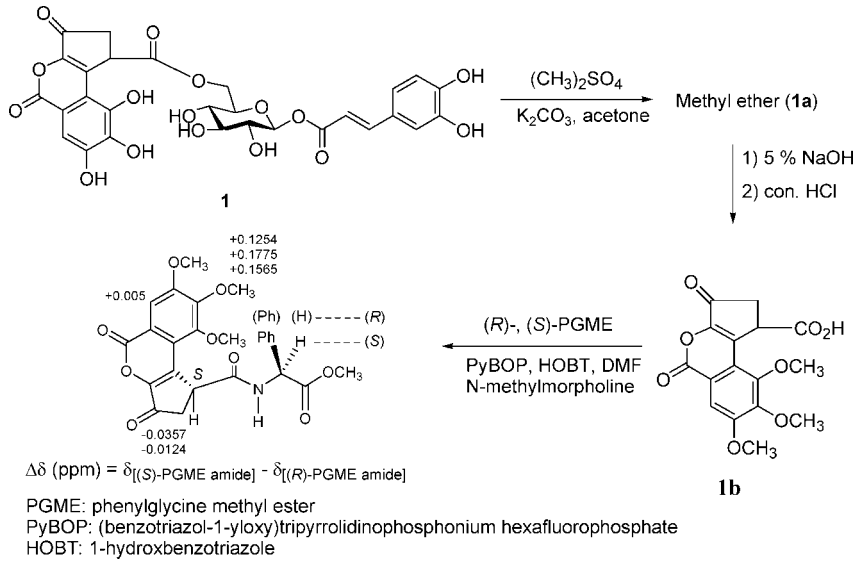


Figure 2. Determination of absolute configuration of **1** by the PGME method.

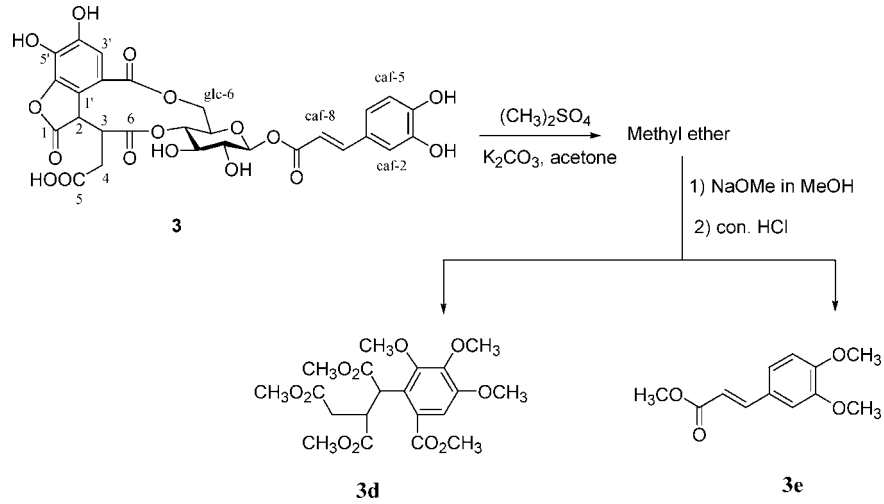
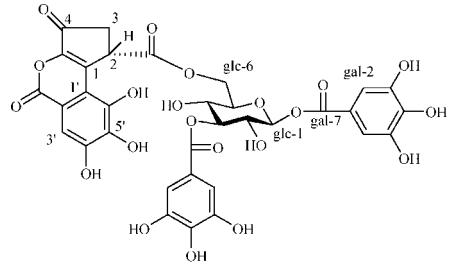


Figure 3. Methylation and alkaline hydrolysis of compound **3**.

hydrolysis to yield a trimethylated brevifolincarboxylic acid (**1b**). By applying the phenylglycine methyl ester (PGME) method[4] to this carboxylic acid (Figure 2), the configuration at C-2 was determined to be *S*. Therefore, balanophotannin D was determined as 1-*O*-caffeoyl-6-*O*-(*S*)-brevifolincarboxyl-β-D-glucopyranose (**1**).



Balanophotannin E (**2**)

Balanophotannin E (**2**) showed similar spectroscopic characteristics to balanophotannin D (**1**). Its molecular formula was identified as $C_{33}H_{26}O_{21}$ based on HRESIMS data. The ^{1}H NMR data suggested two galloyl groups and a 1,3,6-triacylated β-glucopyranosyl moiety. Comparison of the ^{13}C NMR data of **2** with those of ellagitannins having a brevifolincarboxylate group[3] further indicated the presence of a brevifolincarboxylate moiety in **2**. Enzymatic hydrolysis of **2** by tannase furnished 6-*O*-(*S*)-brevifolincarboxyl-D-glucopyranose (**2a**). Based on the above evidence, balanophotannin E was characterized as 1,3-di-*O*-galloyl-6-*O*-(*S*)-brevifolincarboxyl-β-D-glucopyranose (**2**).

Compound **3**, named balanophotannin F, was isolated as a tan, amorphous powder. Its molecular formula was determined to be $C_{28}H_{24}O_{17}$ based on HRESIMS data. The ^{1}H NMR spectrum of **3** displayed resonances typical of a caffeoyl group and a 1,4,6-triacylated β-glucopyranosyl moiety. In the ^{13}C NMR spectrum, in addition to the signals derived from the glucopyranosyl and caffeoyl groups, three ester carbonyls (δ 173.5, 165.7, 176.9), a carboxylic carbon (δ 174.1), two methines (δ 48.3, 44.5), a methylene (δ 31.2), and a pentasubstituted benzene ring were observed (Table 1). This acyl moiety was assumed to be the same as that in euphormisin M_2[3] on the basis of comparison of ^{1}H and ^{13}C NMR data of **3** with those of the reported data[5] and the observation of HMBC correlations from H-3′ to C-4′ and C-7′, from H-2 to C-1, C-3, and C-6, and from H-4 to C-3 and C-5 (Figure 4). This was supported by the formation of a heptamethyl derivative (**3d**)[3] by alkaline hydrolysis of the methylated **3** (Figure 3). The caffeoyl group was confirmed to be located at the anomeric position by the HMBC correlation of the anomeric proton and the carbonyl carbon (Figure 4). The ester linkage of another acyl group to C-4 and C-6 of the glucose moiety was similarly identified on the basis of the HMBC

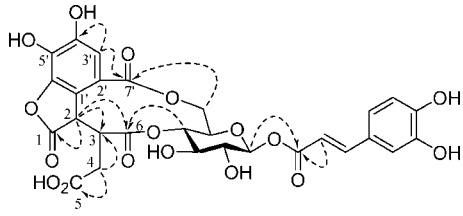
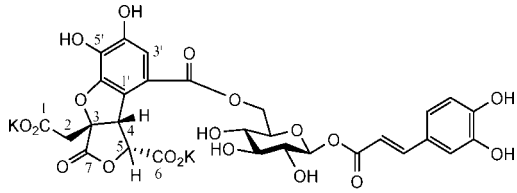


Figure 4. Important HMBC correlations (H to C) of compound **3**.

correlation between H-4 and H-6 and the ester carbonyl carbons (Figure 4). Finally, application of the PGME method[6] to compound **3** elucidated the *S* configuration at C-3 of the glc-4/glc-6 acyl moiety (Figure 5).

Balanophotannin G (**4**) was identified as an acylated glucopyranose from its similar ^{1}H NMR data to those of compound **1** because of the proton resonance of the caffeoyl and 1β,6-diacylated glucopyranosyl moieties. Although the positive and negative FABMS gave molecular ions at *m/z* 755 and 753, respectively, the negative HRESIMS gave an ion at *m/z* 677.1016 corresponding to $[M - 2K + H]^-$. Therefore, the molecular formula of **4** was determined to be $C_{29}H_{24}O_{19}K_2$, which was further supported by the result of elemental analysis. In addition to caffeoyl and β-glucosyl moieties, the ^{13}C NMR spectrum of **4** further exhibited the signals derived from an aromatic lactone structural unit having 14 carbons including a pentasubstituted benzene ring, two carboxylic carbonyls (δ 177.2, 176.3), a lactone carbonyl (δ 179.6), an ester carbonyl (δ 168.0), a methylene (δ 43.0), two methines (δ 85.6, 54.0), and a quaternary oxygenated carbon (δ 91.5) (Table 1). The structure of this acyl moiety was determined on the basis of the HMBC correlations (H-2 to C-1, C-7, and C-4; H-4 to C-6; H-5 to C-6, C-7, and C-1′; H-3′ to C-1′, C-4′, C-5′, and C-7′) as shown in Figure 6. The linkage of this acyl group at C-6 of the glucosyl unit was confirmed by the HMBC correlation of H-6 of glucosyl and the carbonyl carbon (C-7′ δ 168.0) (Figure 6). The caffeoyl group was located at the anomeric position on the basis of the HMBC correlation between the anomeric proton and the carbonyl carbon of the caffeoyl moiety (Figure 6). Finally, the relative configurations of C-3, C-4, and C-5 of the aromatic lactone were elucidated on the basis of the NOE correlations between H-2 and H-4, H-2 and H-5, and H-4 and H-5 (Figure 7).

The cytotoxicities of balanophotannins D−G (**1**–**4**) to HepG2 cancer cells were assayed using the Cell Counting Kit-8.[7] Their IC_{50} values summarized in Table 2 revealed that balanophotannin E (**2**) exhibits the strongest cytotoxicity to HepG2 cells, which is equivalent to that of cisplatin.



Balanophotannin G (**4**)

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Unity plus 500 and Varian Gemini 300 spectrometers. Coupling constants (*J*) are expressed in Hz, and chemical shifts are given on the δ (ppm) scale with TMS as an internal standard. HRESIMS were recorded on a Q-TOF mass spectrometer (Bruker Daltonics, MA). The calibration of accurate mass was carried out using ESI-TOF tuning mix (Part No.: G1969-85000, Agilent, CA). EI- and FABMS were recorded on a JEOL JMS DX-303 spectrometer, and glycerol was used as a matrix for FABMS measurement. Column chromatographies were performed with Kieselgel 60 (70–230 mesh, Merck), Sephadex LH-20 (25–100 mm, Pharmacia Fine Chemical Co. Ltd.), MCI-gel CHP 20P (75–150 mm, Mitsubishi Chemical Co. Ltd.), TSK gel Toyopearl HW-40F (Tosoh), Chromatorex ODS (100–200 mesh, Fuji Silysia Chemical), and Bondapak C18 (125 um, Waters). Thin-layer chromatography (TLC) was performed on precoated Kieselgel 60 F_{254} plates (0.2 mm, Merck), and spots were detected by ultraviolet (UV) illumination and by spraying with 2% $FeCl_3$ and 10% sulfuric acid reagents.

Extraction and Isolation. The MeOH extract (223.7 g) of the aerial parts of fresh *B. japonica* (1.73 kg) collected in Nagasaki Prefecture was divided into Et_2O, EtOAc, and H_2O layers as described in our previous paper.[1] The H_2O layer was subjected to MCI-gel CHP20P column chromatography (CC) (H_2O−MeOH) to give three fractions. The first fraction (140 g), containing a large amount of sugars, was sequentially chromatographed over Chromatorex ODS (H_2O−MeOH) and MCI-gel CHP 20P (H_2O-MeOH) to furnish compound **4** (278 mg). The second fraction (19.5 g) was repeatedly chromatographed over Chromatorex ODS, MCI-gel CHP20P, Sephadex LH-20, and TSK gel Toyopearl HW-40F (all eluted with H_2O containing an increasing amount of MeOH) to yield compound **1** (750 mg) and compound **3** (270 mg). Another 110 mg of compound **1** was also isolated from the third fraction (13.75 g) by a combination of the chromatographies applied for the second fraction. The second fraction (58.0 g) of the EtOAc layer described in our previous paper was subjected to Chromatorex ODS, MCI-gel CHP 20P, Sephadex LH-20, and Bondapak C18 CC to afford compound **2** (22.5 mg).

Balanophotannin D (1), 1-*O*-(*E*)-caffeoyl-6-*O*-(*S*)-brevifolincarboxyl-β-D-glucopyranose: tan, amorphous powder, $[\alpha]^{16}_D$ −62.5 (*c* 0.2, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.09 (1H, d, *J* = 2 Hz, caf-2), 6.79 (1H, d, *J* = 8 Hz, caf-5), 7.00 (1H, dd, *J* = 2, 8 Hz, caf-6), 7.70 (1H, d, *J* = 16 Hz, caf-7), 6.31 (1H, d, *J* = 16 Hz, caf-8), 5.53 (1H, d, *J* = 8 Hz, glc-1), 3.40 (1H, t, *J* = 8 Hz, glc-2), 3.46 (1H, t, *J* = 9 Hz, glc-3), 3.27 (1H, t,*J* = 9 Hz, glc-4), 3.68 (1H, m, glc-5), 4.49 (1H, dd, *J* = 2, 12 Hz, glc-6a), 4.32 (1H, dd, *J* = 6, 12 Hz, glc-6b), 7.29 [1H, s, brevifolin (brev)-3′], 4.56 (1H, dd, *J* = 2, 8 Hz, brev-2), 3.02 (1H, dd, *J* = 8, 19 Hz, brev-3a), 2.60 (1H, dd, *J* = 2, 19 Hz, brev-3b); ^{13}C NMR data, Table 1; *anal.* calcd for $C_{28}H_{24}O_{16}\cdot 5/4H_2O$ C, 52.63; H, 4.18, found C, 52.43; H, 4.19; negative FABMS *m/z* 615 $[M - H]^-$; negative HRESIMS *m/z* 615.1002 (calcd for $C_{28}H_{23}O_{16}$, 615.0986).

Methylation and Alkaline Hydrolysis of 1. Compound **1** (300 mg) was dissolved in acetone (30 mL) containing K_2CO_3 (3.0 g). Dimethylsulfate (2.0 mL) was added to the solution, which was refluxed for 3.5 h. The residue of the reaction was purified by Si gel CC (10% MeOH in $CHCl_3$) to afford a methylate of **1** (90.2 mg), which was hydrolyzed by 5% NaOH. After neutralization with concentrated HCl, the mixture was subjected to Si gel CC ($CHCl_3$−MeOH−H_2O, 9:1:0.1–8:2:0.2) to yield compound **1a** (30.8 mg): tan, amorphous powder, $[\alpha]^{23}_D$ −507.6 (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.66 (1H, s, H-3′), 4.37 (1H, dd, *J* = 2, 8 Hz, H-2), 3.03 (1H, dd, *J* = 8, 19 Hz, H-3a), 2.67 (1H, dd, *J* = 2, 19 Hz, H-3b), 4.03, 4.02, 3.97 (each 3H, s, OMe); EIMS *m/z* 334 $[M^+]$.

(*S*)-PGME Amide (1b) of 1a. A solution of **1a** (25.8 mg), (*S*)-PGME (13 mg), PyBOP (30 mg), HOBT (8 mg), and methylmorpholine (20 uL) in DMF (1 mg) was stirred for 1.5 h at room temperature. Then EtOAc (15 mL) was added and the solution was successively washed with 5% HCl (15 mL), saturated $NaHCO_3$ (15 mL), and brine (15 mL). The organic layer was dried with anhydrous Na_2SO_4 and concentrated to afford a residue, which was purified over Si gel CC eluted with 50% EtOAc in *n*-hexane to 100% EtOAc to give (*S*)-PGME amide (**1b**, 4.6 mg). The use of (*R*)-PGME gave (*R*)-PGME amide (**1c**, 6.2 mg).

Balanophotannin E (2), 1,3-di-*O*-galloyl-6-*O*-(*S*)-brevifolincarboxyl-β-D-glucopyranose: tan, amorphous powder; $[\alpha]^{20}_D$ −39.8 (*c* 0.3, MeOH); ^{1}H NMR (acetone-d_6, 300 MHz) δ 7.24, 7.17 (each 2H, s, galloyl-2, 6), 5.85 (1H, d, *J* = 8 Hz, glc-1), 3.86 (1H, t, *J* = 8 Hz, glc-2), 5.30 (1H, t, *J* = 9 Hz, glc-3), 3.95 (1H, t, *J* = 9 Hz, glc-4), 3.71 (1H, m, glc-5), 4.61 (1H, dd, *J* = 2, 12 Hz, glc-6a), 4.32 (1H, dd, *J* = 6, 12 Hz, glc-6b), 7.44 (1H, s, brev-3′), 4.58 (1H, dd, *J* = 2, 8 Hz, brev-2), 3.01 (1H, dd, *J* = 8, 19 Hz, brev-3a), 2.57 (1H, dd, *J* = 2, 19 Hz, brev-3b); ^{13}C NMR (acetone-d_6, 75 MHz) δ brevofolin moiety, 193.7 (C-4), 173.1 (C-6), 161.0 (C-7′), 149.7 (C-4′), 147.8 (C-5), 143.9 (C-6′), 140.3 (C-5′), 139.6 (C-1), 116.4 (C-2′), 115.1 (C-1′), 109.3 (C-3′), 41.6 (C-2), 38.0 (C-3); glc: 95.6 (C-1), 78.6, 75.7, 72.0, 69.6 (C-2,

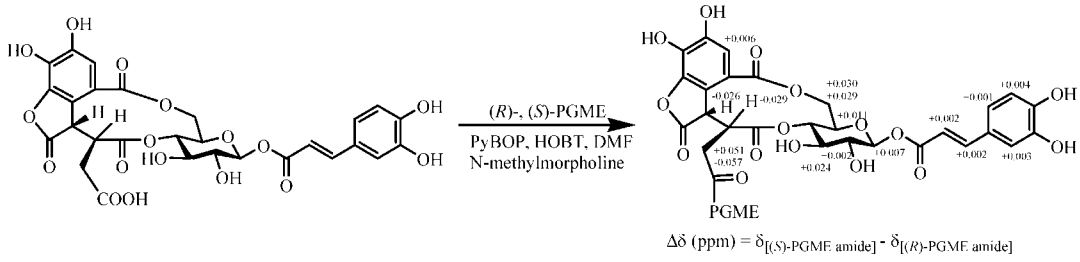


Figure 5. Determination of absolute configuration of **3** by the PGME method.

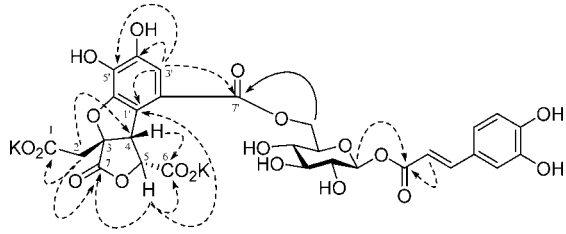


Figure 6. Important HMBC correlations (H to C) of compound **4**.

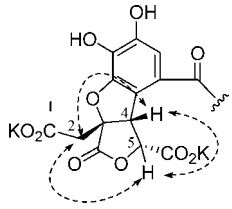


Figure 7. Key NOE correlations observed in compound **4**.

Table 2. IC_{50} Values of Balanophotannins D–G (**1**–**4**) in HepG2 Cells

compound	IC_{50} (μM) $n = 5$
balanophotannin D (**1**)	17.41 ± 2.63
balanophotannin E (**2**)	4.22 ± 0.73
balanophotannin F (**3**)	48.42 ± 6.51
balanophotannin G (**4**)	18.04 ± 2.74
5-fluorouracil	18.70 ± 2.76
cisplatin	4.00 ± 0.61

3, 4, 5), 64.6 (C-6); galloyls, 166.5, 165.8 (C-7), 146.0, 145.8 (C-3, 5), 138.7, 138.5 (C-4), 121.9, 120.6 (C-1), 110.5, 110.2 (C-2, 6); *anal.* calcd for $C_{33}H_{26}O_{21}\cdot 5H_2O$ C, 46.71; H, 4.28, found C, 46.94; H, 4.18; negative FABMS *m/z* 757 $[M - H]^-$; negative HRESIMS *m/z* 757.0881 (calcd for $C_{33}H_{25}O_{21}$, 757.0888).

Compound 2a. A 10 mg amount of **2** was incubated with tannase (3 mg) in water at 37 °C for 2 h. It was purified through an MCI-gel CHP 20P CC to afford 6-*O*-brevifolincarboxyl-D-glucopyranose (6 mg): tan, amorphous powder; $[\alpha]^{24}_D$ −17.7 (*c* 0.3, MeOH); ^{1}H NMR (acetone-d_6, 300 MHz) δ 7.44 (2H, s, brev-3′), 4.61 (2H, dd, $J = 2$, 8 Hz, brev-2), 3.06 (2H, dd, $J = 8$, 19 Hz, brev-3a), 2.62, 2.60 (each 1H, dd, $J = 2$, 19 Hz, brev-3b), 5.14 (1H, d, $J = 4$ Hz, α-glc-1), 4.58 (1H, d, $J = 8$ Hz, β-glc-1), 4.47 (1H, dd, $J = 2$, 12 Hz, α-glc-6a), 4.44 (1H, dd, $J = 2$, 12 Hz, β-glc-6a), 4.28 (2H, dd, $J = 5$, 12 Hz, α-glc-6b, β-glc-6b), 4.04 (1H, m, α-glc-5), 3.74 (1H, t, $J = 9$ Hz, α-glc-3), 3.58 (1H, m, β-glc-5), 3.45 (1H, t, $J = 9$ Hz, β-glc-3), 3.39 (1H, dd, $J = 4$, 9 Hz, α-glc-2), 3.36 (1H, t, $J = 9$ Hz, α-glc-4), 3.29 (1H, t, $J = 9$ Hz, β-glc-4), 3.21 (1H, t, $J = 9$ Hz, β-glc-2); negative FABMS *m/z* 453 $[M - H]^-$.

Balanophotannin F (3): tan, amorphous powder; $[\alpha]^{16}_D$ −219.3 (*c* 0.6, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 7.07 (1H, d, $J = 2$ Hz, caf-2), 6.78 (1H, d, $J = 8$ Hz, caf-5), 6.97 (1H, dd, $J = 2$, 8 Hz, caf-6), 7.67 (1H, d, $J = 16$ Hz, caf-7), 6.31 (1H, d, $J = 16$ Hz, caf-8), 5.70 (1H, d, $J = 8$ Hz, glc-1), 3.64 (1H, t, $J = 8$ Hz, glc-2), 3.90 (1H, t, $J = 9$ Hz, glc-3), 4.93 (1H, t, $J = 9$ Hz, glc-4), 4.05 (1H, m, glc-5), 4.16 (1H, t, $J = 11$ Hz, glc-6a), 4.08 (1H, dd, $J = 3$, 11 Hz, glc-6b), 7.24 (1H, s, 4, 6-acyl-3′), 4.70 (1H, d, $J = 2$ Hz, 4, 6-acyl-2), 3.66 (1H, ddd, $J = 2$, 3, 11 Hz, 4, 6-acyl-3), 2.18 (1H, dd, $J = 11$, 17 Hz, 4, 6-acyl-4a), 1.81 (1H, dd, $J = 3$, 17 Hz, 4, 6-acyl-4b); ^{13}C NMR data, Table 1; *anal.* calcd for $C_{28}H_{24}O_{17}\cdot 5/4H_2O$ C, 51.34; H, 4.08, found C, 51.39; H, 4.22; negative FABMS *m/z* 631; negative HRESIMS *m/z* 631.0970 (calcd for $C_{28}H_{23}O_{17}$, 631.0935).

Synthesis of PGME Amide of 3. A solution of **3** (10.2 mg), (*S*)-PGME (10 mg), PyBOP (18 mg), HOBT (9 mg), and methylmorpholine (20 uL) in DMF (1 mg) was stirred for 2 h at room temperature, H_2O (15 mL) was added, and the solution was extracted with *n*-butanol (15 mL). The organic layer was dried with anhydrous Na_2SO_4 and concentrated to afford a residue, which was purified over Si gel CC eluted with $CHCl_3$−MeOH−H_2O (8:2:0.2) to give (*S*)-PGME amide (**3a**, 4.2 mg). The use of (*R*)-PGME gave (*R*)-PGME amide (**3b**, 3.8 mg).

Methylation and Subsequent Alkaline Hydrolysis of 3. A solution containing compound **3** (54 mg), dry acetone (15 mL), dimethylsulfate (1.5 mL), and K_2CO_3 (2 g) was refluxed for 1.5 h. After filtration, the solution was concentrated and subjected to Si gel CC (2–20% MeOH in $CHCl_3$) to afford methylate (**3c**, 28.8 mg). A 2% NaOMe in MeOH (2 mL) solution was added to compound **3c** and stirred for 2 h. Then, the solution was neutralized by adding concentrated HCl. The Et_2O extract of the aqueous layer was methylated by CH_2N_2 to furnish compounds **3d** (5.1 mg) and **3e** (7.5 mg). Compound **3d**: white, amorphous powder; $[\alpha]^{24}_D$ −88.3 (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.26 (1H, s, H-3′), 5.29 (1H, d, $J = 10$ Hz, H-1), 3.902 (×2), 3.896, 3.87, 3.78, 3.62, 3.47 (3H, s, OMe), 3.72 (1H, ddd, $J = 6$, 9, 10 Hz, H-2), 2.38 (1H, dd, $J = 9$, 16 Hz, H-3a), 2.22 (1H, dd, $J = 6$, 16 Hz, H-3b); EIMS *m/z* 442 $[M^+]$. Methyl *trans*-3,4-dimethoxycinnamate (**3e**): white powder; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.64 (1H, d, $J = 16$ Hz, H-7), 7.11 (1H, dd, $J = 2$, 8 Hz, H-6), 7.05 (1H, d, $J = 2$ Hz, H-2), 6.87 (1H, d, $J = 8$ Hz, H-5), 6.32 (1H, d, $J = 16$ Hz, H-8), 3.92, 3.92, 3.91 (each 3H, s, OMe); EIMS *m/z* 222 $[M^+]$.

Balanophotannin G (4): tan, amorphous powder; $[\alpha]^{16}_D$ −47.7 (*c* 0.2, MeOH); ^{1}H NMR [$D_2O + C_5D_5N$ (2:1), 500 MHz] δ 7.24 (1H, d, $J = 2$ Hz, caf-2), 7.14 (1H, d, $J = 8$ Hz, caf-5), 6.90 (1H, dd, $J = 2$, 8 Hz, caf-6), 7.66 (1H, d, $J = 16$ Hz, caf-7), 6.26 (1H, d, $J = 16$ Hz, caf-8), 6.19 (1H, d, $J = 8$ Hz, glc-1), 4.15 (1H, dd, $J = 8$, 9 Hz, glc-2), 4.25 (1H, t, $J = 9$ Hz, glc-3), 4.20 (1H, t, $J = 9$ Hz, glc-4), 4.34 (1H, m, glc-5), 5.18 (1H, d, $J = 12$ Hz, glc-6a), 4.94 (1H, dd, $J = 4$, 12 Hz, glc-6b), 7.43 (1H, s, 6-acyl-3′), 5.47 (1H, d, $J = 2$ Hz, 6-acyl-4), 5.24 (1H, d, $J = 2$ Hz, 6-acyl-5), 3.54 (1H, d, $J = 16$ Hz, 6-acyl-2a), 3.48 (1H, d, $J = 16$ Hz, 6-acyl-2b); ^{13}C NMR data, Table 1; *anal.* calcd for $C_{29}H_{24}O_{19}K_2\cdot 2H_2O$ C, 44.06; H, 3.57, found C, 44.08; H, 3.52; negative FABMS *m/z* 753 $[M - H]^-$; positive FABMS *m/z* 755 $[M + H]^+$; negative HRESIMS *m/z* 677.1016 $[M - 2K + H]^-$ (calcd for $C_{29}H_{25}O_{19}$, 677.1000).

Cell Counting Kit-8 (CCK-8) Assay of Compounds 1–4. Hep G2 (human hepatocarcinoma) cells were cultured on 96-well plates (6×10^3 cells/well) with Dulbecco's modified Eagle medium (DMEM, Gibco Life Technologies) growth medium with 10% fetal bovine serum (FBS, Gibco Life Technologies). The cells were maintained at 37 °C in a humidified 5% CO_2 environment for 24 h prior to the cytotoxicity tests. According to the CCK-8 technical manual, the cell viability assessment was performed by using the kit, which was purchased from Beyotime Institute of Biotechnology, China (http://www.beytime.com/c0038.htm#protocol). The IC_{50} (Table 2) values of the compounds of interest were calculated by the Compusyn software.[8]

Acknowledgment. Financial support from the Department of Science and Technology of Guangzhou in China (Grant No. 2006Z1-E6021) is gratefully acknowledged.

Supporting Information Available: HMBC spectra of compounds **1**, **3**, and **4** and diff. NOE spectra of compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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NP070519+