Cytotoxic Acylphloroglucinol Derivatives from the Twigs of *Garcinia cowa*

Gang Xu,[†] Winnie L. T. Kan,[‡] Yan Zhou,[†] Jing-Zheng Song,[†] Quan-Bin Han,[†] Chun-Feng Qiao,[†] Chi-Hin Cho,[‡] John A. Rudd,[‡] Ge Lin,*[,‡] and Hong-Xi Xu*[,†]

Chinese Medicine Laboratory, Hong Kong Jockey Club Institute of Chinese Medicine, Shatin, Hong Kong, People's Republic of China, and School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong, People's Republic of China

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An unusual polyprenylated acylphloroglucinol derivative unsubstituted at C-2 and C-6, garcicowin A (**1**), together with three other new (garcicowins B−D, **2**−**4**) and nine known analogues, was isolated and characterized from the twigs of *Garcinia cowa*. The structures of **1**−**4** were elucidated by interpretation of their spectroscopic data. The compounds isolated were evaluated for their cytotoxicity against two cancer cell lines (HT-29 and HCT116) and against normal colon cells (CCD-18Co), and the results demonstrated their selective toxicity toward the cancer cells.

Polyprenylated acylphloroglucinol derivatives, with a highly oxygenated bicyclo[3.3.1]nonane-2,4,9-trione or bicyclo[3.2.1]octane-2,4,8-trione core substituted with one or more prenyl or geranyl side chains, occur in the plant family Guttiferae.[1,2] Many compounds of this type have been identified from different plants in the genus *Garcinia*.[1,2] *Garcinia* is a genus native to Asia, southern Africa, and Polynesia, with a total of 21 species distributed in mainland China.[3] The chemical constituents of this genus have been reported to possess a wide range of biological activities, such as antimicrobial, antidepressant, anti-HIV, antitumor, antioxidant, and cytotoxic effects.[1,4,5] The most extensively studied such compound is garcinol, which can induce apoptosis and inhibit cell survival and proliferation pathways such as MAPK and PI3K/Akt.[6] *Garcinia cowa* Roxb. is a tree with edible fruits and is distributed in the southern and western parts of Yunnan Province, People's Republic of China.[3] Its chemical constituents have been investigated, and xanthones,[7] flavanone glycosides,[8] and an acylphloroglucinol derivative have been isolated.[9] In the present study, four new acylphloroglucinol derivatives (garcicowins A−D, **1**−**4**) and nine known analogues, 30-epicambogin (**5**),[10] cambogin (**6**),[11] guttiferone B (**7**),[12] guttiferone K (**8**),[13] guttiferone F,[10] and oblongifolins A, B (**9**), C (**10**), and D (**11**) were isolated.[14] Reported herein are the isolation, structure elucidation, and cytotoxic activity evaluation of these compounds.

Results and Discussion

The acetone-soluble extract of the twigs of *G. cowa* was partitioned between H_2O and $CHCl_3$ to afford a $CHCl_3$-soluble fraction (113 g). This $CHCl_3$ fraction was purified by column chromatography and HPLC to afford compounds **1**−**11**.

Compound **1** was obtained as a yellow gum, and its molecular formula was determined to be $C_{36}H_{54}O_3$ by HRESIMS and from the ^{13}C NMR spectrum (Table 1). The IR spectrum displayed bands at 1725, 1657, and 1646 cm^{-1} for carbonyl groups. The ^1H NMR spectrum (Table 1) indicated that **1** possesses five olefinic protons (with four characteristic signals ascribable to isoprenyl or geranyl olefinic protons), three methyl groups on sp^3 carbons, and seven vinyl methyl groups. The ^{13}C NMR spectrum of **1** (Table 1) exhibited the presence of a nonconjugated carbonyl at δ_C 208.6 (C-9), an enolized 1,3-diketone group (δ_C 177.8, C-1; δ_C 120.3, C-2; δ_C 200.9, C-3), three quaternary carbons at δ_C 64.2 (C-4), 49.8 (C-5), and 63.9 (C-8), three methylenes (δ_C 37.8, 19.0, and 40.8), an angular methyl at δ_C 16.5 (C-15), and 25 other signals assignable to two isoprenyl groups, a geranyl group, and another C_5 unit. On comparison with data for other analogues that have been isolated from *Garcinia* species,[4,10−14] **1** was ascribed as being a substituted acylphloroglucinol derivative. Further analysis of the 1D and 2D NMR data of **1** indicated that the characteristic olefinic quaternary carbon for C-2 (usually around δ_C 125) and the methine signal for C-6 (usually around δ_C 42 or δ_C 46−48 according to the different configurations for C-6) in normal analogues were replaced by resonances for an olefinic methine (δ_C 120.3) and a methylene (δ_C 37.8), respectively. These observations indicated that the C-2 and C-6 carbons are both unsubstituted in **1**, which was confirmed by the HMBC correlations from H-2 to C-1, C-4, and C-8, as well as the HMBC correlations of Me-15 and H-16 with C-6 (Figure 1).

Only five signals (C-22 through C-26) remained in the ^{13}C NMR spectrum of **1**, excluding the core fragment (C-1 through C-9, a 2,2-dimethylbicyclo[3,3,1]nonane ring system), two prenyl groups, and one geranyl group. These signals were assigned for an isoamyl group connected to C-8, and an epoxy group between C-1 and C-24 was deduced by NMR and MS analysis with other 1,24-epoxy analogues.[10,11] In addition, HMBC correlations between H-7/C-8 and C-9; H-10/C-3, C-4, C-9, C-11, and C-12; Me-15/C-4, C-5, C-6, and C-16, H-17/C-5, C-16, C-18, and C-19; and H-27/C-8, C-28, and C-29 were observed, which were used to confirm the presence of the core fragment in **1** and the locations of the substituent prenyl and geranyl groups, respectively.

The relative configuration of **1** was elucidated by analysis of the NOESY spectrum. The NOE correlations of Me-15/H-27b and Me-15/H-10b suggested the α-orientation of both Me-15 and CH_2-10 together with the β-configuration of CH_2-27 (Figure 1). The Me-25 group was deduced to be β-oriented from the NOE correlations between Me-15/H-6a and H-6a/Me-25. In addition, correlations of Me-15/H-7, Me-25/H-2, and Me25/H-23 were evident in the NOESY spectrum. On the basis of all the above evidence, the structure and relative configuration of **1** were established, and this compound was named garcicowin A. This is the first compound of this type without any substitution at C-2 and C-6, although analogues have been reported with a lack of any substituent group at C-2.[15]

The HRESIMS indicated that **2** has a molecular formula of $C_{43}H_{58}O_5$, which was supported by the ^{13}C NMR spectrum (Table 2). In the ^{13}C and DEPT NMR spectra, the typical signals for a substituted acylphloroglucinol derivative with a 2,2-dimethylbicyclo[3,3,1]nonane ring system at δ_C 194.6 (s, C-1), 119.5 (s, C-2), 192.1 (s, C-3), 69.7 (s, C-4), 51.6 (s, C-5), 41.9 (d, C-6), 43.1 (t, C-7), 64.0 (s, C-8), 208.8 (s, C-9), 16.2 (q, C-22), and 37.4 (t, C-23) were observed. The ^1H and ^{13}C NMR data (Tables 2 and 3) also supported the presence of a 1,3-disubstituted benzene

* To whom correspondence should be addressed. Tel: +852-3406-2873. Fax: +852-3551-7333. E-mail: linge@cuhk.edu.hk; xuhongxi@hkjcicm.org.
[†] Hong Kong Jockey Club Institute of Chinese Medicine.
[‡] The Chinese University of Hong Kong.

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Chart 1

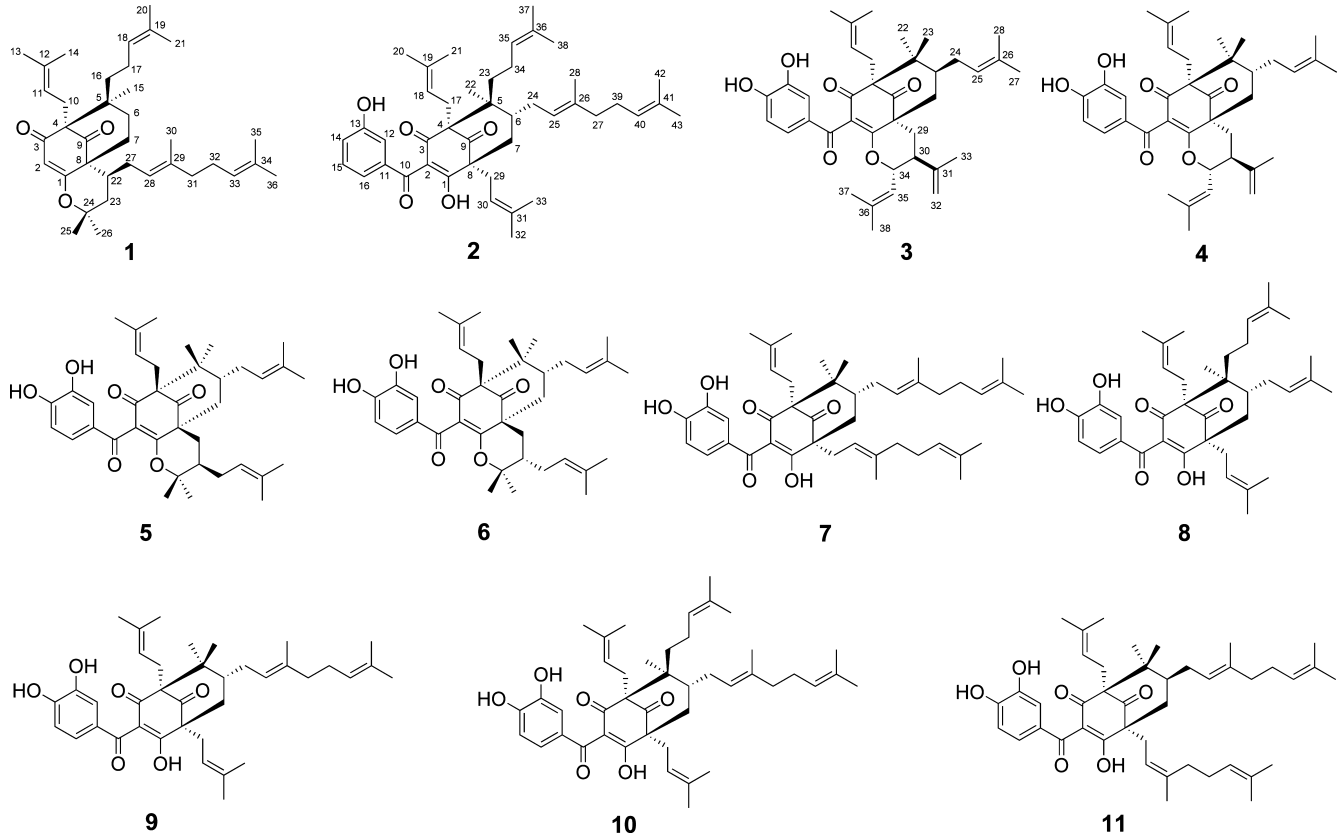

Table 1. ^{13}C and ^{1}H NMR Data of Garcicowin A (**1**) in CD_3OD (400 MHz, *J* in Hz)[a]

position	δ_C	δ_H	position	δ_C	δ_H
1	177.8		18	125.4	5.06, m
2	120.3	5.91, s	19	132.3	
3	200.9		20	18.1	1.59, s
4	64.2		21	26.1	1.66, s
5	49.8		22	37.7	1.99, m
6a	37.8	2.51, dt, 13.6, 3.6	23	34.4	1.81, m
6b		1.70, m	24	85.1	
7	19.0	1.78	25	29.8	1.24, s
8	63.9		26	26.3	1.45, s
9	208.6		27a	29.7	2.01, m
10a	31.0	2.38, d, 7.0	27b		1.75, m
10b		1.40, m	28	123.8	5.00, m
11	121.0	4.93, m	29	138.1	
12	134.5		30	17.8	1.57, s
13	18.1	1.64, s	31	40.6	2.01, m
14	25.9	1.60, s	32	27.5	2.07, m
15	16.5	0.71, s	33	125.2	5.00, m
16	40.8	1.87, dd, 13.1, 4.0	34	132.3	
17	23.8	2.06, m	35	17.4	1.69, s
			36	25.9	1.60, s

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

ring, a conjugated carbonyl carbon at δ_C 198.8 for C-10, and five prenyl groups in **2**. Detailed comparison of the NMR data of **2** with those of oblongifolin C (**10**) indicated that they are similar to one another except for the signal for the quaternary carbon for C-14 in **10** being replaced by an olefinic methine in **2**.[14] This observation indicated that **2** is the 14-dehydroxy derivative of oblongifolin C (**10**), which was confirmed by the HMBC correlations from H-14 to C-15 and C-16 as well as the HMBC correlations of H-12/C-14, H-15/C-14, and H-16/C-14.

Based on the analysis of the 1D NMR data and NOESY spectrum, the relative configuration of **2** was deduced to be the same as that of oblongifolin C (**10**). The large coupling constant between H-7b (δ_H 1.45, t, $J = 12.8$ Hz) and H-6 revealed the axial orientations for H-6 and H-7α together with the equatorial orientation of the isoprenyl group at C-6.[1,14] In addition, the ^{13}C NMR chemical shift of C-6 at δ_C 41.9 also suggested that H-6 is *β*-oriented, or otherwise its signal would be located at a lower field (δ_C 46.0−48.0).[4,10–14,16] The C-22 methyl group was determined to be α-oriented from the correlations of CH_2-23/H-6 and CH_2-23/H-24 found in the NOESY spectrum. Accordingly, the structure of **2** (garcicowin B) was established as shown.

The HRESIMS of compound **3** showed a protonated molecular ion at *m*/*z* 601.3524 $[M + H]^+$, which was used to establish its molecular formula as $C_{38}H_{48}O_6$. The IR spectrum exhibited bands for hydroxy (3445 cm^{-1}) and nonconjugated (1726 cm^{-1}) and conjugated (1638 cm^{-1}) carbonyl groups. The ^{1}H and ^{13}C NMR spectra of **3** (Tables 2 and 3) exhibited the typical signals for a substituted acylphloroglucinol derivative based on the observation of signals for a 2,2-dimethylbicyclo[3,3,1]nonane ring system, a conjugated carbonyl group, a 1,3,4-trisubstituted benzene ring, two prenyl groups, and another C_{10} unit (C-29 through C-38). Comparison of the NMR data with those of known analogues isolated in this study led to the conclusion that the structure of **3** is closely comparable to that of oblongifolin B (**9**), except for the signals for the geranyl group at C-6 and the prenyl group at C-8 in **9** being replaced in **3** by a prenyl group and the C_{10} unit mentioned above.[14] The HMBC correlations of H-24/C-6, H-24/C-26, H-6/C-24, and H-6/C-25 and H-25 observed in the HMBC spectrum were used to establish the connectivity of the prenyl group (C-24 through C-27) to C-6. In the same spectrum, correlations from H-30 to C-8, C-29, C-31, and C-34, from H-35 to C-34, C-37, and C-38, and from H-32 to C-30, C-31, and C-33 were observed. In this manner, the planar structure of compound **3** was determined.

The relative configuration of **3** was determined by analysis of the 1D NMR data and the NOESY spectrum. The NOE correlations between Me-22/H-6, Me-22/H-17b, and Me-22/H-7b indicated that H-6, CH_2-17, and Me-22 are all α-oriented. The α-orientation of

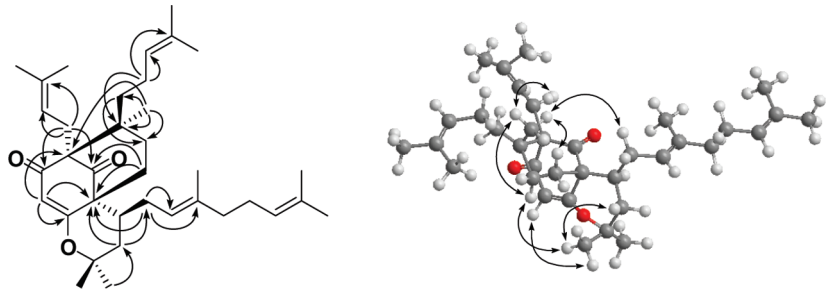

Figure 1. Key HMBC (→) and NOESY (↔) correlations of **1**.

Table 2. ^{13}C NMR Data of Garcicowins B−D (**2**−**4**) in CD_3OD (100 MHz)[a]

carbon	2	3	4	carbon	2	3	4
1	194.6	173.1	172.2	23	37.4	22.7	22.8
2	119.5	124.3	126.3	24	29.9	30.6	28.8
3	192.1	196.1	195.9	25	123.7	126.4	123.9
4	69.7	70.6	72.6	26	138.1	134.0	134.3
5	51.6	47.6	47.3	27	40.7	18.0	18.2
6	41.9	47.6	43.4	28	16.5	26.1	25.7
7	43.1	38.7	41.3	29	31.4	34.5	33.7
8	64.0	49.3	50.9	30	120.8	44.1	44.1
9	208.8	209.7	208.9	31	135.5	145.4	145.4
10	198.8	193.6	194.6	32	18.2	114.2	114.3
11	140.5	131.3	130.9	33	26.2	20.5	20.5
12	116.2	116.2	116.4	34	25.2	81.3	81.3
13	158.5	146.5	146.7	35	125.2	122.9	122.8
14	120.6	152.6	152.9	36	132.5	143.1	143.1
15	129.8	115.8	115.7	37	17.9	18.6	18.2
16	121.2	124.1	124.6	38	25.9	25.7	26.0
17	26.6	26.3	25.5	39	27.5		
18	121.3	121.2	121.5	40	125.4		
19	135.0	135.3	134.9	41	132.3		
20	18.3	26.4	26.3	42	17.8		
21	26.2	18.2	18.1	43	26.0		
22	16.2	27. 1	16.2				

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

Table 3. ^{1}H NMR Data of Garcicowins B−D (**2**−**4**) in CD_3OD (400 MHz, *J* in Hz)[a]

proton	2	3	4
6	1.80, m	1.57, m	1.91, m
7a	2.06, m, eq	2.62, m, eq	2.48, m, eq
7b	1.45, t, 12.8, ax	1.93, m, ax	1.44, t, 13.2, ax
12	7.01, m	7.27, d, 2.0	7.30, d, 2.0
14	6.96, dd, 1.7, 7.8		
15	7.15, t, 7.8	6.76, d, 8.0	6.76, d, 8.0
16	6.91, dd, 1.8, 7.8	7.15, dd, 2.0, 8.0	7.15, dd, 2.0, 8.0
17a	2.72, dd, 7.8, 13.7	2.60, dd, 7.2, 13.2	2.60, dd, 7.2, 13.2
17b	2.66, m	2.47, m	2.45, m
18	4.86, m	4.87, m	4.82, m
19			
20	1.69, s	1.56, s	1.57, s
21	1.63, s	1.59, s	1.61, s
22	0.82, s	1.00, s	0.78, s
23	1.66, m	1.15, s	1.10, s
24a	2.08, m	2.54, m	2.23, m
24b	1.78, m	2.20, m	1.80, m
25	5.00, m	4.94, m	5.19, m
27	1.99, m	1.62, s	1.63, s
28	1.56, s	1.70, s	1.75, s
29a	2.51, m	2.32, t, 13.8	2.38, t, 13.6
29b	2.46, m	1.75, overlap	1.75, overlap
30	5.10, m	2.51, m	2.48, m
32	1.63, s	4.86, m	4.87, m
33	1.70, s	1.67, s	1.67, s
34	1.98, m	4.33, t, 9.6	4.40, t, 9.5
35	5.05, m	5.05, m	5.06, m
37	1.59	1.18, s	1.25, s
38	1.69	1.62, s	1.60, s
39	2.06, m		
40	5.04, m		
42	1.56, s		
43	1.66, s		

[a] Assignments are based on HMQC, HMBC, and NOESY experiments. Chemical shifts are given in ppm.

H-6 could be confirmed by comparison of its NMR data with those of structurally related compounds. As discussed above, the ^{13}C NMR chemical shift of C-6 at δ_C 47.6 suggested that H-6 was α-oriented, since the signal of H-6 with *β*-orientation would be located between δ_C 41.0 and 44.0.[4,10–14] In addition, the chemical shift of Me-22 (δ_C 27.1) also suggested the α-orientation of H-6 since the chemical shift of this methyl group is usually between δ_C 16.0 and 18.0 when H-6 is *β*-oriented.[4,10–14] The configuration of H-34 was deduced as being *β*-oriented by the NOE correlations of H-29a/H-7a and H-29a/H-34. Similarly, H-30 was suggested to be in the α-orientation by the NOE correlations of H-30/H-29b, Me-33/H-34, and Me-33/H-29a. Therefore, the structure of **3** (garcicowin C) was established as shown.

The molecular formula of **4** was deduced to be $C_{38}H_{48}O_6$, which was the same as that of **3** from the positive HRESIMS. Comparison of the 1D (Tables 2 and 3) and 2D NMR data of these two compounds indicated that they are stereoisomers. The key differences between the two NMR spectra were that the chemical shifts of C-6 and Me-22 were both shifted upfield from δ_C 47.6 and 27.1 in **3** to δ_C 43.4 and 16.2 in **4**, respectively. These observations indicated that H-6 is *β*-oriented, as discussed above,[4,10–14] which was confirmed by the NOE correlation between Me-23/H-6. In addition, the NOE correlation between Me-22/CH_2-17 suggested that Me-22 and CH_2-17 are both α-oriented. Similar to those of **3**, the NOE correlations of H-34/H-29a and H-34/Me-33 suggested that H-34 and H-30 are *β*- and α-oriented, respectively. Furthermore, the ^{1}H NMR chemical shifts of H-34 and H-29a in ^{1}H NMR spectra of **4** (H-34: δ_H 4.33, t, J = 9.6; H-29a: δ_H 2.32, t, 13.8) were almost identical to those of **3** (H-34: δ_H 4.40, t, J = 9.5; H-29a: δ_H 2.38, t, 13.6), which suggested the same relative configuration at H-30 and H-34 in these isolates. Therefore, the structure of **4** (garcicowin D) was determined as shown.

The four new isolates (**1**−**4**), along with seven known compounds (**5**−**11**), were tested for their cytotoxic effects against two human colon cancer cell lines, HT-29 and HCT116, and their selectivity using the human normal colon cell line, CCD-18Co. Due to the limited amount of isolated pure compounds available, only one concentration of each compound was examined in both 24 and 48 h treatments. After 24 h treatment with 5 *μ*M (Figure 2), compounds **3** and **4** significantly ($p < 0.01$) reduced the viability of HT-29 cells to 73.8 ± 2.8% and 57.7 ± 4.6% when compared with the untreated control, respectively. All the known isolates except for oblongifolin D (**11**) significantly reduced the viability of HT-29 cells ($p < 0.01$). Similar results were observed using the second human colon cancer cell line, HCT116. Interestingly, none of the compounds tested showed significant toxicity to the normal human CCD-18Co colon cell line, demonstrating a selective toxicity of isolates toward the

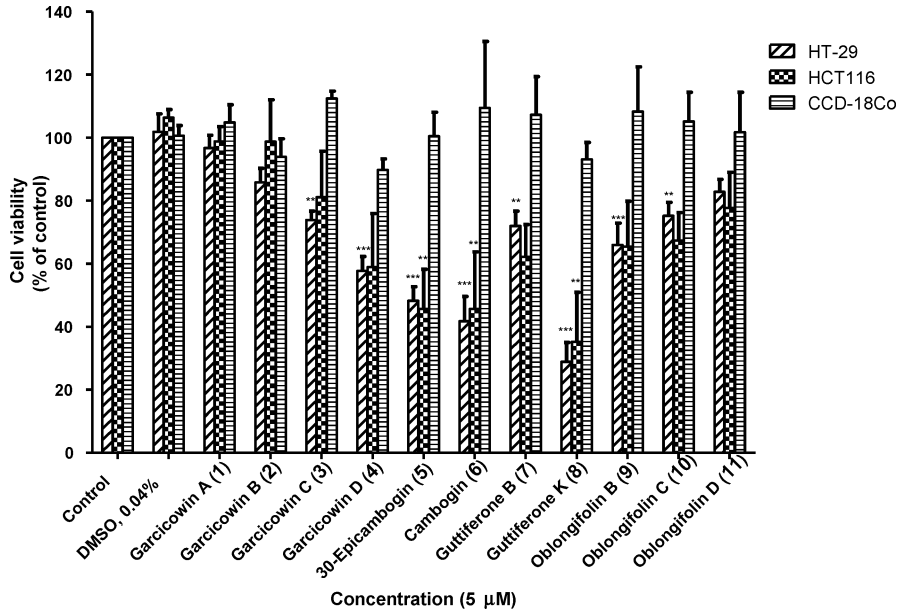

Figure 2. Cytotoxic effects of 11 isolated acylphloroglucinol derivatives (**1**−**11**) for HT-29, HCT116, and CCD-18Co colon cells after 24 h treatment.

colon cancer cell lines. Furthermore, similar results were also obtained for the 48 h treatment (data not shown). The results demonstrated that among the 11 compounds tested 30-epicambogin (**5**), cambogin (**6**), and guttiferone K (**8**) exhibited cytotoxicity toward both the HT-29 and HCT116 cell lines. Constricted by the availability of the isolated samples, only **5** and **8** were then selected for a further dose−response relationship study. The results demonstrated that after 24 h treatment both compounds reduced the viability of HT-29 cells in a concentration-dependent manner, and their potencies based on their IC_{50} values (**5**, 5.1 ± 0.1 μM, **8**, 5.4 ± 0.2 μM) were significantly greater than that of the positive control, cisplatin (26.6 ± 4.2 μM), as determined in a parallel study.

Among the compounds tested, guttiferone K (**8**) has been reported previously to have antiproliferative effects for a human ovarian cancer cell line, and oblongifolin C (**10**) has been shown to be a potent inducer of apoptosis for the HeLa-C3 cell line.[4,13] On the basis of their potency and selectivity for the cancer cell lines used, both compounds **5** and **8** are potential cancer chemotherapeutic lead compounds and are worthy of investigation for their in vivo activity and mechanistic effects.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEPA-300 polarimeter. Ultraviolet absorption spectra were recorded on a UV-2401 PC spectrophotometer. IR spectra were obtained from a Bio-Rad FtS-135 spectrometer. NMR spectra were measured on a Bruker AV-400 spectrometer with TMS as the internal standard. Mass spectrometry was performed on a Waters Q-TOF Premier instrument (Micromass MS Technologies, Manchester, UK) equipped with an ESI source in the positive-ion mode. Column chromatography was performed with silica gel 60 (200−300 mesh, Merck), Sephadex LH-20, and reversed-phase C_{18} silica gel (250 mesh, Merck). Precoated TLC sheets of silica gel 60 GF254 were used. An Agilent 1100 series instrument equipped with an Alltima C_{18} column (4.6 × 250 mm) was used for HPLC analysis, and a semipreparative Alltima C_{18} column (22 × 250 mm) was used in the sample preparation.

Plant Material. The twigs of *Garcinia cowa* were collected in Xishuangbanna, Yunnan Province, People's Republic of China, in August 2006. The plant material was identified by Prof. Wang Hong, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher sample (CMED-0472) was deposited in the Hong Kong Jockey Club Institute of Chinese Medicine.

Extraction and Isolation. The air-dried and powdered twigs of *G. cowa* (8.5 kg) were extracted with acetone (3 × 20 L, each two days) at room temperature. The solution obtained was evaporated under reduced pressure to yield a dark green residue, which was then partitioned further between H_2O and $CHCl_3$. The $CHCl_3$ portion (113 g) was subjected to column chromatography over MCI gel, eluting with a gradient H_2O/MeOH system (from 2:8 to 0:1). In total, six fractions (A−F) were obtained based on TLC analysis. Fraction C was subjected to column chromatography over RP-18 gel eluted in a step gradient manner with methanol−water (from 80:80 to 100:0), to afford **6** (210 mg) and **5** (200 mg) as yellow gums, along with several subfractions. These subfractions were finally purified by semipreparative HPLC, to afford **3** (8 mg), **4** (5 mg), and guttiferone F (8 mg). Fraction F was also subjected to column chromatography over RP-18 gel, eluted in a step gradient manner with methanol−water (from 80:20 to 100:0), to afford **8** (30 mg), **10** (100 mg), and **11** (12 mg), together with five subfractions (I−V). Compounds **1** (2.8 mg) and **2** (10 mg) were obtained from subfractions III and II, respectively, by semipreparative HPLC (3 mL/min), eluting with MeCN−H_2O (95:5) containing 0.3% formic acid. Subfraction IV was subjected to preparative HPLC and then purified by Sephadex LH-20 column chromatography (methanol) to afford **7** (6 mg). Oblongifolins A (4 mg) and B (**9**, 8 mg) were obtained from subfraction V by preparative HPLC (MeOH−H_2O, 95:5) and then semipreparative HPLC purification (3 mL/min, eluting with MeCN−H_2O (92.5:7.5 containing 0.3% formic acid).

Garcicowin A (1): yellow gum; $[\alpha]_D^{15}$ −219.0 (*c* 0.09, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 263 (4.04), 235 (3.88), 233 (3.88) nm; IR (KBr) ν_{max} 2973, 2926, 1725, 1657, 1646, 1450, 1385, 1318, 1202, 1109 cm^{-1}; ^1H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data, see Table 1; positive HRESIMS *m*/*z* 535.4148 (calcd for $C_{36}H_{54}O_3$, $[M + H]^+$, 535.4151).

Garcicowin B (2): yellow gum; $[\alpha]_D^{15}$ −16.0 (*c* 0.21, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 301 (3.96), 244 (4.05), 227 (3.89) nm; IR (KBr) ν_{max} 3443, 2967, 2923, 2855, 1729, 1653, 1561, 1549, 1447, 1378, 1305, 1288, 1212, 1111, 1059, 998 cm^{-1}; ^1H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data, see Tables 2 and 3; positive HRESIMS *m*/*z* 655.4362 (calcd for $C_{43}H_{59}O_5$, $[M + H]^+$, 655.4363).

Garcicowin C (3): yellow gum; $[\alpha]_D^{14}$ −72.1 (*c* 0.10, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 308 (3.77), 274 (4.09), 238 (3.91) nm; IR (KBr) ν_{max} 3445, 2925, 2856, 1726, 1638, 1528, 1443, 1374, 1294, 1178, 1114, 1064, 966 cm^{-1}; ^1H (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) data, see Tables 2 and 3; positive HRESIMS *m*/*z* 601.3524 (calcd for $C_{38}H_{49}O_6$, $[M + H]^+$, 601.5329).

Garcicowin D (4): yellow gum; $[\alpha]_D^{14.3}$ 336.0 (*c* 0.12, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 308 (3.82), 273 (4.15), 238 (4.03) nm; IR (KBr) ν_{max} 3443, 2970, 2924, 1726, 1640, 1604, 1493, 1450, 1375, 1293, 1180, 1107, 1030, 955 cm^{-1}; ^1H (CD_3OD, 400 MHz) and ^{13}C NMR

(CD_3OD, 100 MHz) data, see Tables 2 and 3; positive HRESIMS *m/z* 601.3526 (calcd for $C_{38}H_{49}O_6$, $[M + H]^+$, 601.5329).

Cytotoxicity Bioassay. All test samples were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions and further diluted in culture medium upon assay. Human colorectal cancer HT-29 and HCT116 cell lines were cultured in RPMI 1640 medium, containing 10% fetal bovine serum, 2.0 g/L sodium bicarbonate, 0.1 g/L streptomycin sulfate, and 0.06 g/L penicillin G. The human colon fibroblast CCD-18Co cell line was cultured in minimum essential medium (MEM), containing 10% fetal bovine serum, 2.2 g/L sodium bicarbonate, 0.1 g/L streptomycin sulfate, 0.06 g/L penicillin G, and 5.958 g/L HEPES. All cell lines were maintained at 37 °C in a humidified environment containing 5% CO_2. To determine the effects of the compounds on cell viability, cell number was quantified using a standard colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded overnight in 96-well culture plates at a density of 7×10^3 cells/well. Cells were treated with 4.63 μM of each compound in culture medium for 24 and 48 h, respectively. Then, culture media were aspirated and replaced with 100 μL of fresh medium containing 10 μL of MTT (5 mg/mL stock in PBS) per well and incubated for 3 h at 37 °C. Next, culture media were aspirated and 200 μL of DMSO was added per well to dissolve the purple formazan crystals on an orbital shaker at 150 rpm for 15 min. Absorbance of the solution was measured using a Bio-Rad Benchmark Plus microplate reader spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA), at a wavelength of 570 nm, with background subtraction at 620 nm. Absorbance of untreated cells in medium (negative control) was 100%. Cisplatin (Sigma-Aldrich, St. Louis, MO), a commonly used anticancer drug, was dissolved in saline and used as a positive control. All data are presented as mean values + SEM. Results are in triplicate from at least three independent experiments. Data were analyzed by one-way ANOVA followed by Bonferroni's multiple comparison posthoc test using GraphPad Prism 4.0. In all statistical comparisons between experimental and vehicle control groups, a p value < 0.05 was considered to be statistically significant.

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Supporting Information Available: HRESIMS and NMR spectra of **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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