JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

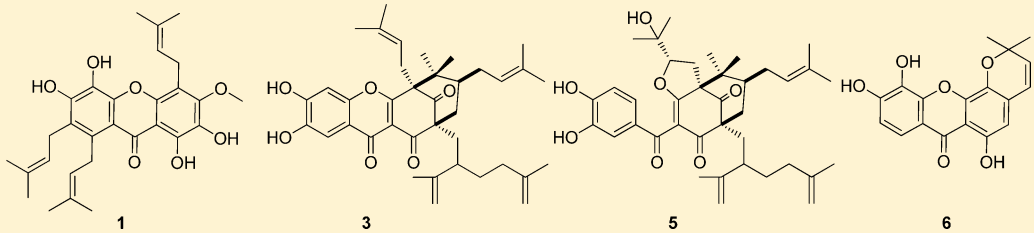
Bioassay-Guided Isolation of Prenylated Xanthones and Polycyclic Acylphloroglucinols from the Leaves of *Garcinia nujiangensis*

Zheng-Xiang Xia,[†] Dan-Dan Zhang,[†] Shuang Liang,[†] Yuan-Zhi Lao,[†] Hong Zhang,[†] Hong-Sheng Tan,[†] Shi-Lin Chen,[‡] Xin-Hong Wang,[†] and Hong-Xi Xu*[,†]

[†]Shanghai University of Traditional Chinese Medicine, Shanghai 201203, People's Republic of China

[‡]Chinese Academy of Medical Science and Peking Union Medical College, Beijing 100083, People's Republic of China

Supporting Information



ABSTRACT: Bioassay-guided fractionation of the acetone extract of the leaves of *Garcinia nujiangensis* resulted in the isolation of two new prenylated xanthones, nujiangexanthones A (**1**) and B (**2**), three new polycyclic polyprenylated acylphloroglucinols, nujiangefolins A–C (**3**–**5**), and 10 known related analogues. The structures of compounds **1**–**5** were elucidated by interpretation of their spectroscopic data. Compounds **3** and **4** are unusual polycyclic polyprenylated acylphloroglucinols in which the enol hydroxy group forms a six-membered ring with a benzene ring carbon. The compounds isolated were evaluated for their cytotoxic effects against 11 cancer cell lines and immortalized MIHA normal liver cells, and the test substances demonstrated selectivity toward the cancer cells. Isojacareubin (**6**) was found to be the most potent cytotoxic compound of those tested.

Cancer is a leading cause of mortality in all countries of the world. A standard treatment for cancer is surgical resection or irradiation with adjuvant chemotherapy.[1] In addition to rapid metastasis, problems such as a low response rate, a lack of selectivity toward cancer cells, and multidrug resistance have limited the success of chemotherapy.[2] However, there is a high demand for new antitumor agents that have high potency and selective toxicity toward cancer cells. Since some of the currently used anticancer drugs have originated from plants,[3,4] there is a growing interest in the use of traditional Chinese medicinal herbs and their isolated bioactive compounds as potential drug candidates for cancer treatment.

Garcinia species (Guttiferae) are rich in various oxidized and prenylated xanthones[5] and acylphloroglucinols,[6] of which some exhibit a wide range of biological and pharmacological effects including cytotoxic,[5b,h,6d,e] antimalarial,[5a] antioxidant,[5d,6b] anti-inflammatory,[5g,6c] and antiparasitic activities.[5c] In our ongoing search for novel plant-derived antitumor agents from plants in the genus *Garcinia*,[7] it was found that an acetone extract of the leaves of *Garcinia nujiangensis* showed cytotoxicity against a panel of human tumor cell lines. Bioassay-directed fractionation of *G. nujiangensis* resulted in the isolation of two new prenylated xanthones, nujiangexanthones A (**1**) and B (**2**), three new polycyclic polyprenylated acylphloroglucinols, nujiangefolins A–C (**3**–**5**), and 10 known related derivatives. Herein, we report the isolation, structure elucidation, and bioassay results of these compounds.

RESULTS AND DISCUSSION

The leaves of *G. nujiangensis* were pulverized and extracted with acetone at room temperature three times. The acetone-soluble extract was suspended in hot water and partitioned with CH_2Cl_2. The CH_2Cl_2 portion was chromatographed repeatedly over silica gel, reversed-phase C_{18} silica gel, and semipreparative HPLC, to afford 15 pure compounds.

Compound **1** was shown to have the molecular formula $C_{29}H_{34}O_7$ by HRESIMS (*m*/*z* 493.2229 $[M - 1]^-$). The IR spectrum exhibited strong bands due to phenolic hydroxy (3423 cm^{-1}) and chelated carbonyl (1639 cm^{-1}) groups. The UV absorptions (MeOH) at λ_{max} 264 and 332 nm indicated **1** to be a hydroxylated xanthone derivative.[8] Analysis of the ^1H and ^{13}C NMR data (Table 1), aided by a HSQC experiment, disclosed the presence of a carbonyl, 15 sp^2 quaternary carbons (seven of which are oxygen bearing), three sp^2 methines, three sp^3 methylenes, a methoxy group, and six methyl groups. The initial analysis of the NMR spectroscopic data of **1** indicated that the molecule consists of a xanthone skeleton with a methoxy group and three isoprenyl moieties. The ^1H NMR spectrum of **1** revealed the proton signals of a methoxy signal at δ_H 3.88 (3H, s) and three isoprenyl moieties, of which the first showed a pair of *gem*-dimethyl signals at δ_H 1.78 (3H, s, H-15)

Received: May 23, 2012
Published: August 7, 2012

dx.doi.org/10.1021/np3003639 | *J. Nat. Prod.* 2012, 75, 1459–1464

Chart 1

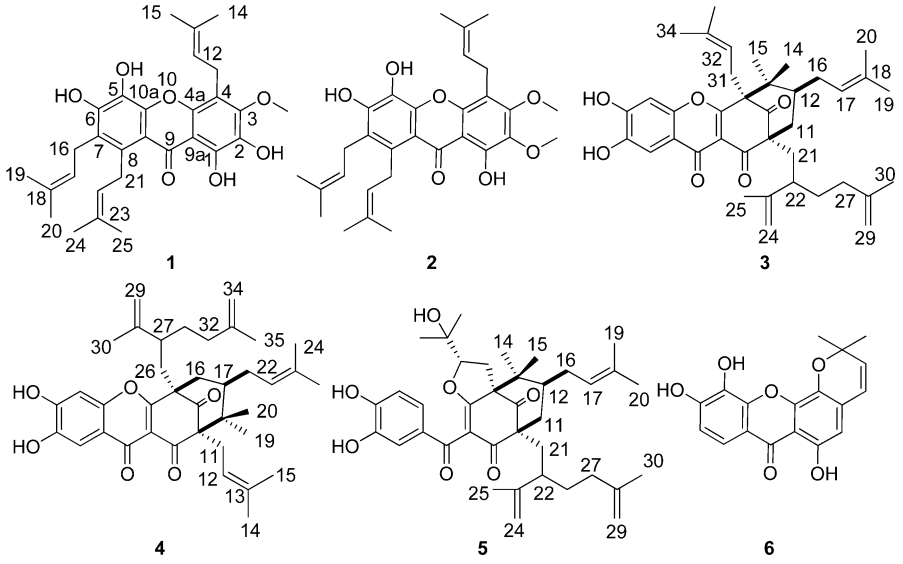


Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds 1[a] and 2[a] in DMSO-d_6

	1		2	
no.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		148.0		147.4
2		133.0		135.1
3		153.0		157.2
4		111.6		111.5
4a		145.2		148.1
10a		147.6		obscured
5		129.9		130.0
6		151.4		153.3
7		125.4		125.7
8		133.6		133.7
8a		110.2		111.5
9		183.1		183.1
9a		105.1		105.2
11	3.57, d (7.2)	21.9	3.56, m	21.8
12	5.20, d (7.2)	123.4	5.20, m	123.1
13		130.7		130.0
14	1.78, s	17.9	1.78, s	17.9
15	1.61, s	25.7	1.61, s	25.7
16	3.34, m	24.5	3.32[b]	24.5
17	4.98[b]	123.1	5.01[b]	123.1
18		131.0		130.1
19	1.71, s	18.1	1.72, s	18.2
20	1.63, s	25.7	1.62, s	25.8
21	3.98, d (4.8)	28.3	3.95, m	28.3
22	4.99[b]	125.4	4.98[b]	124.4
23		129.9		130.1
24	1.71, s	18.2	1.72, s	18.1
25	1.63, s	25.7	1.62, s	25.7
OH-1	13.40, brs		13.67, brs	
OCH_3-3	3.88, s	60.4	3.80, s	60.4
OCH_3-2			3.95, s	61.2

[a]Recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C). [b]Overlapping signals.

and δ_H 1.61 (3H, s, H-14), a methine signal at δ_H 5.30 (1H, t, J = 7.2 Hz, H-12), and a methylene signal at δ_H 3.57 (2H, d, J = 7.2 Hz, H-11). The second prenyl group exhibited a pair of *gem*-dimethyl signals at δ_H 1.71 (3H, s, H-20) and δ_H 1.63 (3H, s, H-19), a methine signal at δ_H 4.99 (1H, H-17), and a methylene signal at δ_H 3.41 (2H, d, J = 6.0 Hz, H-16). Finally, the third prenyl group showed a pair of *gem*-dimethyl signals at δ_H 1.63 (3H, s, H-25) and δ_H 1.71 (3H, s, H-24), a methine signal at δ_H 4.98 (1H, H-22), and a methylene signal at δ_H 3.98 (2H, d, J = 4.8 Hz, H-21). The locations of these three isoprenyl moieties were assigned at C-4 (δ_C 111.6), C-7 (δ_C 125.4), and C-8 (δ_C 133.6) based on correlations observed in the HMBC spectrum of **1** (Figure 1). Furthermore, a chelated

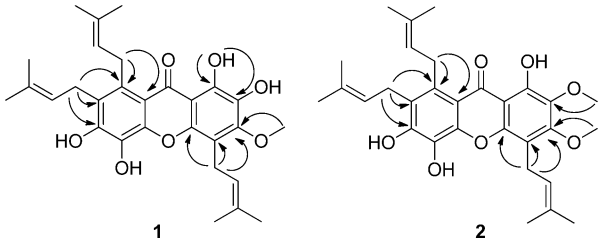


Figure 1. Key HMBC (H→C) correlations of **1** and **2**.

hydroxy proton signal at δ_H 13.40 (1H, s, OH-1) showed a cross-peak to the carbon signals at C-1 (δ_C 148.0) and C-2 (δ_C 133.0) (Figure 1). The location of the methoxy group at C-3 (δ_C 153.0) was confirmed in the HMBC spectrum from the correlation of the OCH_3 (δ_H 3.88) and C-3 (δ_C 153.0) signals (Figure 1). After comparing the ^{1}H and ^{13}C NMR data of **1** with those of known xanthones with similar structures,[5h,8,9] the substituted pattern of ring C was found to be close to that of 1,3,5,6-tetrahydroxy-4,7,8-tri(3-methyl-2-butenyl)xanthone.[8] Therefore, four hydroxy groups were located at C-1 (δ_C 148.0), C-3 (δ_C 153.0), C-5 (δ_C 129.9), and C-6 (δ_C 151.4) by analysis of the HSQC and HMBC spectra. Accordingly, **1** was determined as 1,2,5,6-tetrahydroxy-3-methoxy-4,7,8-tri(3-methylbut-2-enyl)xanthone and has been given the trivial name nujiangexanthone A.

Compound **2** was obtained as a yellow gum. It gave a molecular formula of $C_{30}H_{36}O_7$ according to its HRESIMS at m/z 507.2394 $[M - H]^-$ (calcd 507.2383). Comparison of the

dx.doi.org/10.1021/np3003639 | J. Nat. Prod. 2012, 75, 1459−1464

^{1}H and ^{13}C NMR data (Table 1) of **2** and **1** showed them to be closely related, with the exception of an additional methoxy group occurring in **2**. This methoxy group was located at C-2, as confirmed by the HMBC (Figure 1) correlation of the OCH_3-2 (δ_H 3.80) and C-2 (δ_C 135.1) signals. Correlations of two OCH_3 groups [δ_H 3.95 (3H, s), δ_H 3.80 (3H, s)] in the NOESY spectrum confirmed that the two methoxy groups are adjacent to one another. Thus, the structure of **2** (nujiangexanthone B) was determined to be 1,2,5,6-tetrahydroxy-2,3-dimethoxy-4,7,8-tri(3-methylbut-2-enyl)xanthone.

Compound **3** was isolated as a yellow gum. The molecular formula $C_{38}H_{48}O_6$ was deduced by HRESIMS at m/z 599.3387 $[M - 1]^-$. Two singlet aromatic protons (δ_H 7.43, 6.92) in the ^{1}H NMR spectrum indicated the presence of a 1,2,4,5-tetrasubstituted benzene ring. The ^{1}H NMR data (Table 2) suggested that **3** possesses six olefinic protons (with two characteristic signals ascribable to isoprenyl olefinic protons and four terminal double-bond protons), two methyl groups on sp^3 carbons, and six vinyl methyl groups. The analysis of the aromatic region of the ^{13}C NMR data (Table 2) revealed three oxygenated carbons at δ_C 147.0 (C-7), 154.7 (C-6), and 151.3 (C-10a). These data confirmed the presence of a 1,2,4,5-tetrasubstituted aromatic ring. Resonances for the functional groups of a six-membered ring, consisting of a nonconjugated ketone (δ_C 209.7) flanked by two quaternary carbons (δ_C 65.2, 63.3) and an enolized 1,3-diketone (δ_C 120.9, 173.8, 194.6), were observed in the ^{13}C NMR spectrum. The NMR data of **3** were closely comparable to those of symphonone H,[10] except for the signals due to a terminal double-bond group. This inference was also supported by the ^{13}C NMR data of C-28 (δ_C 147.0), C-29 (δ_C 110.4), and C-30 (δ_C 22.9) and the HMBC correlations between H-30/C-28, H-29/C-28, H-27/C-30, and H-30/C-27 (Figure 2).

The relative configuration in **3** was assigned on the basis of the analysis of the ^{13}C NMR and NOESY spectra. The relative configuration of C-12 was deduced by the ^{13}C NMR chemical shifts of C-12 (δ_C 47.7)[6a,7,10,11] and Me-14 (δ_C 27.5).[6a] The NOESY (Figure 2) correlations of H-31/H-14, H-31/H-12, and H-12/H-21 indicated that they are α-oriented, and H-15/H-16 were assigned as being oriented in the opposite direction. Consequently, the structure of **3** (nujiangefolin A) was deduced as shown.

Compound **4** was isolated as a yellow gum. The molecular formula, $C_{38}H_{48}O_6$, was deduced by HRESIMS at m/z 599.3387 $[M - 1]^-$ and was found to be the same as that of **3**. The ^{1}H and ^{13}C NMR data (Table 2) were almost identical with those of **3**, suggesting that their bicyclic ring systems are the same. In the ^{13}C NMR spectrum, only the signals of carbons C-4 (δ_C 55.6) and C-2 (δ_C 71.4) in **4** differed from **3**. These differences could only be due to the modification of the side chain attached to C-4. This postulation was supported by the HMBC correlations shown in Figure 3. The relative configuration was assigned in a manner similar to **3**. Therefore, the structure of **4** (nujiangefolin B) was deduced as shown.

Compound **5** was obtained as a yellow gum. The HRESIMS showed a molecular ion peak at m/z 641.3441 $[M + Na]^+$ and 619.3621 $[M + 1]^+$, consistent with the molecular formula, $C_{38}H_{50}O_7$. Its ^{1}H and ^{13}C NMR spectra (Table 2), together with the HSQC spectrum, revealed the presence of eight methyls, eight methylenes, seven methines, and 15 quaternary carbons. The ^{1}H NMR spectrum of **5** revealed the occurrence of a 1,3,4-trisubstituted benzene ring [δ_H 6.74 (1H, d, J = 8.4 Hz), 7.18 (1H, dd, J = 2.0 and 8.4 Hz), and 7.38 (1H, d, J = 2.0 Hz)] and two terminal double bonds [δ_H 4.55 (1H, s), 4.51 (1H, s); 4.65 (1H, s), 4.69 (1H, s)]. The ^{13}C NMR spectrum was consistent with the presence of a bicyclo[3.3.1]nonane-2,4,9-trione moiety.[13] Several residues present were assigned as

Table 2. ^{1}H and ^{13}C NMR Spectroscopic Data of Compounds 3−5[a] in CD_3OD

	3		4		5	
no.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		194.6		193.7		196.6
2		63.3		71.4		61.8
3		209.7		207.3		208.6
4		65.2		55.6		69.1
4a		176.7		178.0		177.7
5	6.92, s	104.0	6.90, s	102.7	6.74, d (8.4)	115.5
6		154.7		153.4		152.8
7		147.0		146.7		147.2
8	7.43, s	109.6	7.44, s	108.2	7.38, d (2.0)	116.5
8a		118.2		118.7		130.6
9		173.8		173.8		192.8
9a		120.9		119.7		118.6
10a		151.3		149.6	7.18, d (8.4, 2.0)	126.2
11	2.20[b]	44.8	2.62[b]	25.6	2.11[b]	42.8
	2.04[b]					
12	1.55, d (7.6)	47.7	4.77, m	119.7	1.68, d (7.8)	47.0
13		50.6		133.9		49.2
14	1.14, s	27.5	1.54, s	24.8	1.14, s	27.5
15	1.29, s	24.1	1.73, s	17.0	1.25, s	24.1
16	2.01[b]	30.6	2.44[b]	41.2	2.45[b]	31.0
	1.77[b]		2.20[b]		2.53[b]	
17	4.79, m	125.2	1.62, d (7.6)	47.0	4.98, m	126.2
18		133.8		49.5		134.0
19	1.57, s	26.1	1.05, s	25.9	1.69, s	26.0
20	1.33, s	18.0	1.13, s	21.1	1.61, s	18.4
21	2.17[b]	38.7	1.99[b]	29.3	1.82[b]	38.0
	1.82[b]		1.72[b]		1.96[b]	
22	2.64[b]	44.1	4.73, m	123.4	2.45[b]	44.6
23		149.5		132.3		149.0
24	4.37, s	114.7	1.49, s	24.6	4.55, s	113.7
	4.46, s				4.51, s	
25	1.56, s	18.0	0.95, s	16.3	1.53, s	17.9
26	1.42[b]	32.5	2.30[b]	36.2	1.45, s	32.8
	1.35[b]		1.98[b]		1.47, s	
27	1.82[b]	36.8	2.28[b]	43.4	1.81[b]	36.7
					1.94[b]	
28		147.0		146.7		147.2
29	4.61, s	110.4	4.14, m	111.5	4.65[b]	110.1
	4.64, s		4.08, m		4.69[b]	
30	1.67, s	22.9	1.57, s	16.6	1.72[b]	22.9
31	2.89, d (6.8)	26.9	1.59[b]	31.5	2.63[b]	27.5
					2.28[b]	
32	4.68, m	120.3	1.85[b]	34.9	4.58, m	94.0
			1.79[b]			
33		135.9		145.1		71.9
34	1.76, s	18.6	4.60, s	109.3	1.09, s	25.6
			4.57, s			
35	1.44, s	25.9	1.66, s	21.1	1.02, s	25.3

[a]Recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C). [b]Overlapping signals.

dx.doi.org/10.1021/np3003639 | J. Nat. Prod. 2012, 75, 1459−1464

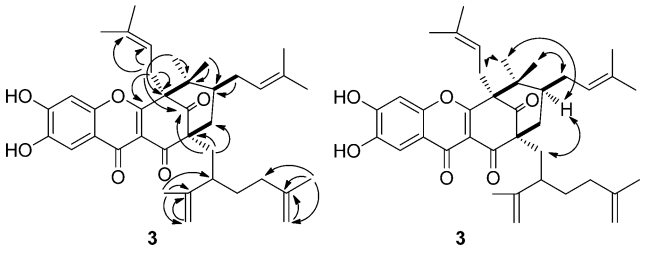


Figure 2. Key HMBC (H→C) and NOESY (H↔H) correlations of **3**.

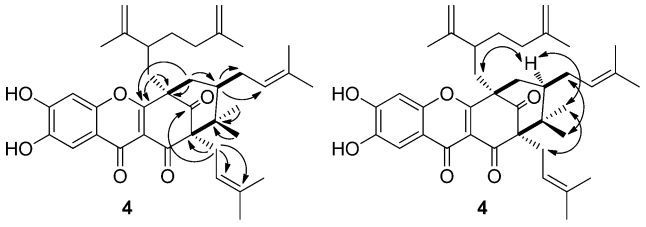


Figure 3. Key HMBC (H→C) and NOESY (H↔H) correlations of **4**.

gem-dimethyl groups (C-14 and C-15), a 2-isopropenyl-5-methylhex-5-enyl group (C-21 to C-30), an isoprenyl group (C-16 to C-20), and a (2-hydroxyisopropyl)dihydrofuran ring moiety (C-31 to C-35), on the basis of the 1D- and 2D-NMR spectroscopic data. The NMR spectra of **5** were similar to those of garcinielliptone FB[6b] and garcinialiptone C.[12] Inspection of the HMBC spectrum (Figure 4) showed long-range correla-

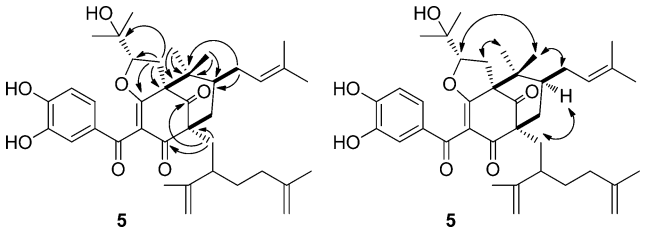


Figure 4. Key HMBC (H→C) and NOESY (H↔H) correlations of **5**.

tions between H-31/C-4a, C-4, and C-3 and between H-14, H-15/C-4, C-12, and C-13, so the (2-hydroxyisopropyl)-dihydrofuran ring was established at C-4a and C-4 and between H-21/C-2 and C-3, with the 2-isopropenyl-5-methylhex-5-enyl group connected at C-2. Therefore, the 3,4-dihydroxybenzoyl group must be located at C-9a. In order to determine the relative configuration of **5**, a NOESY experiment was performed. The NOESY correlations of H-31/H-14, H-31/H-12, and H-12/H-21 indicated they are α-oriented, with H-15/H-16 and H-15/H-32 oriented in the opposite direction. The α-orientation of H-12 was confirmed by comparing its NMR data with those of structurally related compounds. The ^{13}C NMR chemical shift of C-12 at δ_C 47.0 suggested that H-12 is α-oriented, since the expected signal of H-12 with a β-orientation would be between δ_C 41.0 and 44.0.[6a,b,7,10−12] The chemical shift of Me-14 (δ_C 27.5) 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-12 is β-oriented.[6a,b,7,10−12] Therefore, the structure of **5** (nujiangefolin C) was determined as shown.

The structures of the known compounds isojacareubin (**6**),[14] kaempferol,[15] 7-epi-garcinol,[16] xanthone V_{2a},[17] cycloxanthochymol,[16] 7-epi-isogarcinol,[16] (−)-cycloxanthochymol,[6e] garcinialiptone B,[6e] isogarcinol,[16] and (−)-garcinialiptone A[6e] were identified by comparing their spectroscopic data with reported values.

All isolates were evaluated for cytotoxic effects against 11 human tumor cell lines. Their selectivity was determined using the immortalized MIHA human normal liver cells, and the compounds tested were shown to be selectively cytotoxic for the cancer cells used. The anticancer drug paclitaxel was used as a positive control. However, only compound **6** exhibited significant activity, with IC_{50} values of 2.5, 3.8, 5.9, and 9.5 μM against the AGs, MCF7, MDAMB-231, and U87 tumor cell lines, respectively.

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 (200−300 mesh, Qingdao Haiyang Chemical Co., Ltd.) and reversed-phase C_{18} silica gel (250 mesh, Merck). Precoated TLC sheets of silica gel 60 GF_{254} (Qingdao Haiyang Chemical Co., Ltd.) were used. An Agilent 1200 Series machine equipped with a Zorbax SB-C_{18} column (4.6 × 250 mm, 5 μm) was used for HPLC analysis, and a semipreparative Zorbax SB-C_{18} column (9.4 × 250 mm, 5 μm) was used in sample preparation. Paclitaxel was purchased from Sigma-Aldrich Trading Co. Ltd. (Shanghai, People's Republic of China).

Plant Material. The leaves of *Garcinia nujiangensis* were collected in Nujiang, Yunnan Province, People's Republic of China, in August 2010. The plant material was identified by Prof. Yuanchuan Zhou, Yunnan University of Traditional Chinese Medicine. A voucher sample (G. N. 0001) was deposited in the Innovative Medicine Laboratory, Shanghai University of Traditional Chinese Medicine.

Extraction and Isolation. The leaves of *G. nujiangensis* (3.8 kg) were pulverized and extracted with acetone three times at room temperature. The acetone-soluble extract (160 g) was suspended in hot water and partitioned with CH_2Cl_2. The CH_2Cl_2-soluble extract (56 g) was then subjected to passage over a silica gel column (200−300 mesh, 1000 g) eluted with CH_2Cl_2−MeOH in a gradient (1:0 to 0:1), to afford eight fractions (A−H), monitored by TLC. Fractions A, B, and C showed cytotoxic activity against the U87, BXPC3, NCI2126, PANC1, A549, AGs, A375, MCF7, MDAMB-231, SMMC7721, and HepG2 tumor cell lines. Fraction A (6 g) was chromatographed over a column of silica gel and eluted with petroleum ether−ethyl acetate (3:1) to give four subfractions (A1−A4). Of these, subfraction A3 (32 mg) was subjected to passage over reversed-phase C_{18} silica gel, using methanol−water (9:1) as mobile phase, and further separated by semipreparative HPLC, using methanol−water (93:7, containing 0.1% trifluoroacetic acid, 2 mL/min) as mobile phase, to give compounds **1** (15 mg) and **2** (2 mg). Fraction B (12 g) was chromatographed over reversed-phase C_{18} silica gel and eluted with gradient mixtures of methanol−water (75:25 to 95:5) to give four subfractions (B1−B4). Subfraction B1 (8 mg) was separated by semipreparative HPLC, using methanol−water (85:15, containing 0.1% trifluoroacetic acid, 2 mL/min) as mobile phase, to yield **5** (5 mg) and isogarcinol (2 mg). Subfraction B2 (15 mg) was separated by semipreparative HPLC, using methanol−water (86:14, containing 0.1% trifluoroacetic acid, 2 mL/min) as mobile phase, to yield (−)-cycloxanthochymol (3 mg) and garcinialiptone B (2 mg). Subfraction B3 (206 mg) was separated by semipreparative HPLC, using methanol−water (85:15, containing 0.1% trifluoroacetic acid, 2 mL/min) as mobile phase, to give cycloxanthochymol (5 mg), 7-epi-isogarcinol (2 mg), and (−)-garcinialiptone A (6 mg). Subfraction B4 (69 mg) was separated by semipreparative HPLC, using methanol−water (90:10, containing

dx.doi.org/10.1021/np3003639 | J. Nat. Prod. 2012, 75, 1459−1464

0.1% trifluoroacetic acid, 2 mL/min) as mobile phase, to give **3** (5 mg), **4** (2 mg), and 7-epi-garcinol (6 mg). Fraction C (14 g) was chromatographed over reversed-phase C_{18} silica gel and eluted with gradient mixtures of methanol–water (65:35 to 90:10) to give four subfractions (C1–C4). Subfraction C2 (63 mg) was separated by semipreparative HPLC, using methanol–water (82:18, containing 0.1% trifluoroacetic acid, 2 mL/min) as mobile phase, to yield **6** (5 mg) and xanthone V_{2a} (10 mg). Subfraction C2 (34 mg) was separated by reversed-phase C_{18} silica gel, using methanol–water (5:1) as mobile phase, to give kaempferol (10 mg).

*Nujiangexanthone A (**1**):* yellow gum; UV (MeOH) λ_{max} (log ε) 332 (3.90), 264 (4.21), 245 (4.13) nm; IR (KBr) ν_{max} 3423, 2923, 2852, 1639, 1575, 1454, 1336 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; HRESIMS m/z 493.2229 $[M - 1]^-$ (calcd for $C_{29}H_{33}O_7$, 493.2226).

*Nujiangexanthone B (**2**):* yellow gum; UV (MeOH) λ_{max} (log ε) 330 (4.06), 269 (4.28), 246 (4.20) nm; IR (KBr) ν_{max} 3434, 2964, 2915, 2852, 1648, 1589, 1454, 1334, 970 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; HRESIMS m/z 507.2394 $[M - 1]^-$ (calcd for $C_{30}H_{35}O_7$, 507.2383).

*Nujiangefolin A (**3**):* yellow gum; $[\alpha]^{20}_D$ −2 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 331 (3.64), 263 (3.96), 245 (3.95) nm; IR (KBr) ν_{max} 3425, 3072, 2966, 2923, 2852, 1731, 1685, 1620, 1465, 1390, 1143, 889, 798 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS m/z 599.3387 $[M - 1]^-$ (calcd for $C_{38}H_{47}O_6$, 599.3373).

*Nujiangefolin B (**4**):* yellow gum; $[\alpha]^{20}_D$ +5 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 340 (3.47), 326 (3.52), 264 (3.83) nm; IR (KBr) ν_{max} 3429, 2923, 2852, 1730, 1674, 1624, 1464, 1398, 1143, 891 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 2; HRESIMS m/z 599.3387 $[M - 1]^-$ (calcd for $C_{38}H_{47}O_6$, 599.3373).

*Nujiangefolin C (**5**):* yellow gum; $[\alpha]^{20}_D$ +20 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 313 (4.56), 275 (4.78), 234 (4.81) nm; IR (KBr) ν_{max} 3429, 2970, 2925, 2856, 1730, 1672, 1608, 1442, 1373, 1292, 1203, 974, 891 cm^{-1}; ^1H and ^{13}C NMR, see Table 2; HRESIMS m/z 641.3441 $[M + Na]^+$, 619.3621 $[M + 1]^+$ (calcd for $C_{38}H_{50}O_7Na$, 641.3454, $C_{38}H_{51}O_7$, 619.3635).

Cytotoxicity Assay. All test samples were dissolved in dimethyl sulfoxide (DMSO) to make stock solutions and further diluted in culture medium upon assay. The U87 (brain), BXPC-3 (pancreas), NCI-2126 (lung), PANC-1 (pancreas), A549 (lung), AGs (stomach), A375 (melanoma), MCF-7 (breast), MDA-MB-231 (breast), SMMC-7721 (liver), and HepG2 (hepatic) human cancer cell lines and the immortalized MIHA liver cell line were cultured in RPMI 1640, DMEM, or DMEM/F12 medium, containing 10% fetal bovine serum. The 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, the cell number was quantified using a standard colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were plated in a 96-well plate (5×10^3 cells/well) and allowed to attach overnight. Cells were treated with 5, 10, 20, and 40 μM of each compound in culture medium for 72 h. Then, new culture medium was added with 20 μL of MTT (5 mg/mL stock in PBS) per well and incubated for 4 h at 37 °C. Finally, the culture medium was discarded, and 150 μL of DMSO was added per well to dissolve the purple formazan crystals. Absorbance of the solution was measured using a microplate reader spectrophotometer (Bio-Rad Laboratories, Inc., Hercules, CA, USA), at a wavelength of 490 nm. The absorbance of untreated cells in the medium (negative control) was 100%. Paclitaxel was used as a positive control and showed cytotoxic activity with IC_{50} values of 0.09, 3.0×10^{-3}, 5.0, 4.4×10^{-4}, 0.72, 8.1, 0.15, 9.2×10^{-3}, 2.5, 0.26, and 1.6 μM against the BXPC-3, MCF-7, MDA-MB-231, HepG2, A549, U87, SMMC-7721, A375, NCI-2126, PANC-1, and AGs tumor cell lines, respectively.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HRESIMS, ^1H NMR, ^{13}C NMR, HMBC, HSQC, and NOESY spectra of compounds **1**–**5** are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +86-21-51323089. E-mail: xuhongxi88@gmail.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the National Natural Science Foundation of China (No. 81173485) and the Key National Natural Science Foundation of China (No. 81130069).

■ REFERENCES

(1) Chau, I.; Cunningham, D. *Ann. Oncol.* **2006**, *17*, 1347–1359.
(2) Kosmider, S.; Lipton, L. *J. Gastroenterol.* **2007**, *13*, 3799–3805.
(3) Newman, D. J. *J. Med. Chem.* **2008**, *51*, 2589–2599.
(4) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2012**, *75*, 311–335.
(5) (a) Likhitayawuid, K.; Padungcharoen, T.; Krungkrai, J. *Planta Med.* **1998**, *64*, 70–72. (b) Matsumoto, K.; Akao, Y.; Kobayashi, E.; Ohguchi, K.; Ito, T.; Tanaka, T.; Inuma, M.; Nojawa, Y. *J. Nat. Prod.* **2003**, *66*, 1124–1127. (c) Cos, P.; Ferreira, D.; Marais, J. P. J.; Berghe, D. V.; Maes, L.; Vlietinck, A.; Pieters, L. *J. Nat. Prod.* **2006**, *69*, 369–372. (d) Jung, H. A.; Su, B. N.; Keller, W. J.; Mehta, R. G.; Kinghorn, A. D. *J. Agric. Food Chem.* **2006**, *54*, 2077–2082. (e) Balunas, M. J.; Su, B.; Brueggemeier, R. W.; Kinghorn, A. D. *J. Nat. Prod.* **2008**, *71*, 1161–1166. (f) Chin, Y. W.; Jung, H. A.; Chai, H.; Keller, W. J.; Kinghorn, A. D. *Phytochemistry* **2008**, *69*, 754–758. (g) Ngoupayo, J.; Tabopda, T. K.; Ali, M. S. *Bioorg. Med. Chem.* **2009**, *17*, 5688–5695. (h) Han, A. R.; Kim, J. A.; Lantvit, D. D.; Kardono, L. B. S.; Riswan, S.; Chai, H.; Carcache de Blanco, E. J.; Farnsworth, N. R.; Swanson, S. M.; Kinghorn, A. D. *J. Nat. Prod.* **2009**, *72*, 2028–2031.
(6) (a) Hamed, W.; Brajeul, S.; Mahuteau-Betzer, F.; Thoison, O.; Mons, S.; Delpech, B.; Hung, N. V.; Sévenet, T.; Marazano, C. *J. Nat. Prod.* **2006**, *69*, 774–777. (b) Wu, C. C.; Lu, Y. H.; Wei, B. L.; Yang, S. C.; Won, S. J.; Lin, C. N. *J. Nat. Prod.* **2008**, *71*, 246–250. (c) Chen, J. J.; Ting, C. W.; Hwang, T..; Chen, I. S. *J. Nat. Prod.* **2009**, *72*, 253–258. (d) Carroll, A. R.; Suraweera, L.; King, G.; Rali, T.; Quinn, R. J. *J. Nat. Prod.* **2009**, *72*, 1699–1701. (e) Zhang, L. J.; Chiou, C. T.; Cheng, J. J.; Huang, H. C.; Yang Kuo, L. M.; Liao, C. C.; Bastow, K. F.; Lee, K. H.; Kuo, Y. H. *J. Nat. Prod.* **2010**, *73*, 557–563.
(7) (a) Huang, S. X.; Feng, C.; Zhou, Y.; Xu, G.; Han, Q. B.; Chang, D. C.; Luo, K. Q.; Xu, H. X. *J. Nat. Prod.* **2009**, *72*, 130–135. (b) Xu, G.; Winnie, Y. K.; Zhou, Y.; Song, J. Z.; Han, Q. B.; Qiao, C. F.; Lin, G.; Xu, H. X. *J. Nat. Prod.* **2010**, *73*, 104–108. (c) Liu, X.; Yu, T.; Gao, X. M.; Zhou, Y.; Qiao, C. F.; Peng, Y.; Chen, S. L.; Luo, K. Q.; Xu, H. X. *J. Nat. Prod.* **2010**, *73*, 1355–1359.
(8) Chanmahasathien, W.; Li, Y. S.; Satake, M.; Oshima, Y.; Ishibashi, M.; Ruangrungsi, N.; Ohizumi, Y. *Chem. Pharm. Bull.* **2003**, *51*, 1332–1334.
(9) Ren, Y. L.; Yuan, C. H.; Chai, H. B.; Ding, Y. Q.; Li, X. C.; Ferreira, D.; Kinghorn, A. D. *J. Nat. Prod.* **2011**, *74*, 460–463.
(10) Fuller, R. W.; Blunt, J. W.; Boswell, J. L.; Cardellina, J. H., II; Boyd, M. R. *J. Nat. Prod.* **1999**, *62*, 130–132.
(11) Piccinelli, A. L.; Cuesta-Rubio, O.; Chica, M. B.; Mahmood, N.; Pagano, B.; Pavone, M.; Barone, V.; Rastrelli, L. *Tetrahedron* **2005**, *61*, 8206–8211.
(12) Matsuhisa, M.; Shikishima, Y.; Takaishi, Y.; Honda, G.; Ito, M.; Takeda, Y.; Shibata, H.; Higuti, T.; Kodzhimatov, O. K.; Ashurmetov, O. *J. Nat. Prod.* **2002**, *65*, 290–294.
(13) Ciochina, R.; Grossman, R. B. *Chem. Rev.* **2006**, *106*, 3963–3986.
(14) Wu, Q. L.; Wang, Q. W.; Du, L. J.; Yang, J. S.; Xiao, P. G. *Phytochemistry* **1998**, *49*, 1395–1402.
(15) Lu, H. X.; Lin, N.; Li, S. H.; Zhang, L. Y.; Gao, Y. H. *J. Guangzhou Univ. Trad. Chin. Med.* **2012**, *29*, 56–58.
(16) Marti, G.; Eparvier, V.; Moretti, C.; Susplugas, S.; Prado, S.; Grellier, P.; Retailleau, P.; Guéritte, F.; Litaudon, M. *Phytochemistry* **2009**, *70*, 75–85.

(17) Botta, B.; Delle Monache, G.; Delle Monache, F.; Marini Bettolo, G. B.; Menichini, F. *Phytochemistry* **1986**, *25*, 1217−1219.