JOURNAL OF NATURAL PRODUCTS

Article

pubs.acs.org/jnp

Antiproliferative Prenylated Xanthones and Benzophenones from the Roots of *Cudrania tricuspidata* in HSC-T6 Cells

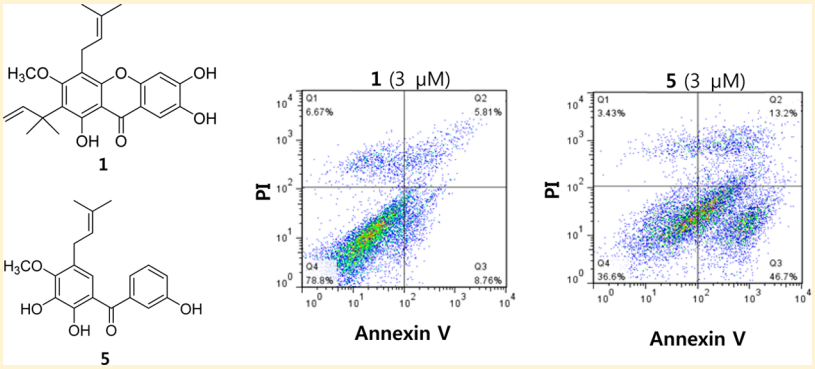
Yang Hee Jo,[†] Bora Shin,[‡] Qing Liu,[†] Ki Yong Lee,[§] Dong-Chan Oh,[‡] Bang Yeon Hwang,[†] and Mi Kyeong Lee*[,†]

[†]College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea

[‡]College of Pharmacy, Seoul National University, Seoul 151-742, Korea

[§]College of Pharmacy, Korea University, Sejong City 339-700, Korea

Supporting Information



ABSTRACT: Four new prenylated xanthones, cudracuspixanthones A–D (**1**–**4**), two new prenylated benzophenones, cudracuspiphenones A (**5**) and B (**6**), and 11 known xanthones (**7**–**17**) were isolated from the roots of *Cudrania tricuspidata*. The absolute configurations of compounds **2**–**4** were deduced by the comparison of the calculated optical rotation values with the measured data. Compounds **1**, **5**, and **6** showed moderate antiproliferative activity on HSC-T6 cells with IC_{50} values of 9.7, 3.3, and 7.1 μM, respectively. Compounds **2**–**4**, **10**, and **14**–**16** had weaker activity. Flow cytometric analysis suggested that compounds **1** and **5** inhibited HSC-T6 cell proliferation in part by inducing apoptosis.

Cudrania tricuspidata (Carr.) Bur. is a deciduous tree of the Moraceae family. The tree is widely distributed in Asia including Korea. In traditional medicine, the roots of *C. tricuspidata* are used for the treatment of hepatitis, rheumatism, and dysmenorrhea.[1] The roots are rich in xanthones and flavonoids,[2−4] and their anticancer, anti-inflammatory, and antiplatelet activities have been reported.[5−7]

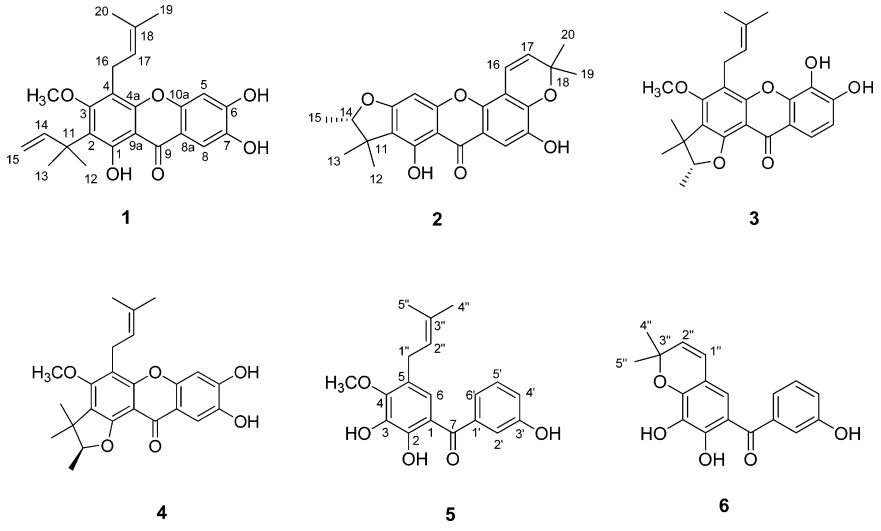
Chronic damage to the liver induces a wound-healing response, which can lead to liver fibrosis. This process is characterized by the abnormal accumulation of extracellular matrix components and an increased proliferation of hepatic stellate cells (HSCs). Although therapeutic options are limited, the reversibility of liver fibrosis has been suggested by experimental models, and the clearance of activated HSCs by apoptosis has been proposed as a therapeutic target.[8,9] In a continuation of our search for antiproliferative compounds from natural products, it was observed that the CH_2Cl_2- and EtOAc-soluble fractions of *C. tricuspidata* significantly reduced the viability of HSC-T6 cells, a rat hepatic stellate cell line. Further attempts to identify the active constituents of *C. tricuspidata* led to the isolation of four new xanthone derivatives (**1**–**4**), two new benzophenone derivatives (**5** and **6**), and 11 known xanthones.

RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow syrup, and its molecular formula of $C_{24}H_{26}O_6$ was determined by the ^{13}C NMR spectroscopic data and an HREIMS ion at m/z 433.1621 ($[M + Na]^+$, calcd 433.1627). Its UV absorption maxima at 256 and 323 nm suggested the presence of a xanthone derivative.[10] The ^1H and ^{13}C NMR spectra suggested the presence of a 3,3-dimethylallyl(prenyl) [δ_H 3.53 (2H, d, J = 6.8 Hz, H-16), 5.27 (1H, t, J = 6.8 Hz, H-17), 1.89 (3H, s, CH_3-19), and 1.69 (3H, s, CH_3-20); δ_C 22.6 (C-16), 123.0 (C-17), 131.1 (C-18), 17.3 (C-19), and 25.0 (C-20)] and a 1,1-dimethylallyl group [δ_H 1.62 (6H, s, H-12, 13), 6.46 (1H, dd, J = 17.2, 10.4 Hz, H-14), 4.96 (1H, dd, J = 17.2, 1.2 Hz, H-15a), and 4.78 (1H, dd, J = 10.8, 1.2 Hz, H-15b); δ_C 40.8 (C-11), 26.7 (C-12, 13), 149.8 (C-14), and 104.1 (C-15)], which were confirmed by HMBC correlations.[3] The presence of a methoxy group was deduced from the proton resonance at δ_H 3.61 (3H, s) connected to the carbon at δ_C 62.1 in the HSQC spectrum. Additionally, two isolated aromatic protons were observed at δ_H 7.00 (1H, s, H-

Received: March 28, 2014
Published: October 16, 2014

Chart 1



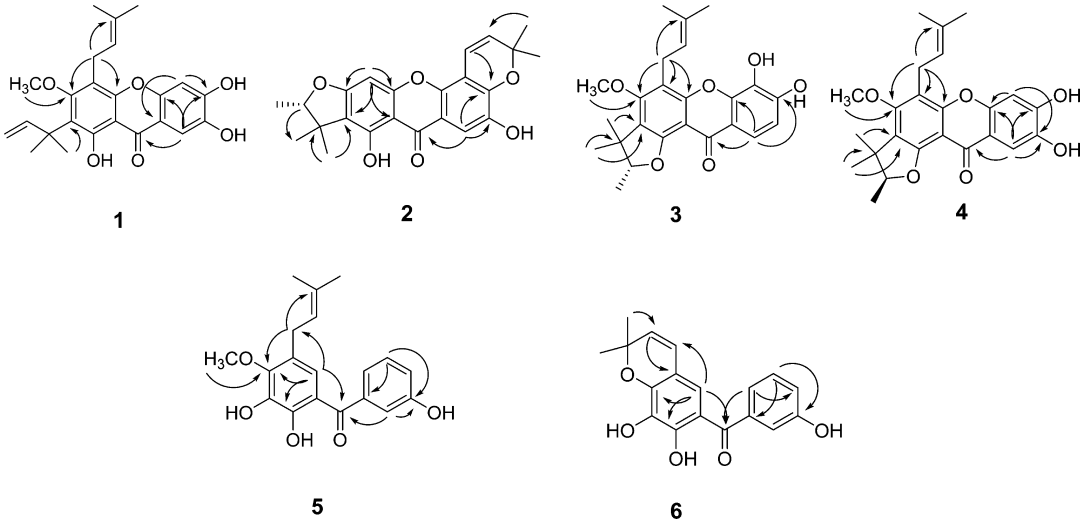


Figure 1. Key HMBC correlations of compounds **1**–**6**.

5) and 7.59 (1H, s, H-8) in the ^{1}H NMR spectrum, and 13 carbon resonances including one carbonyl carbon at δ_C 180.9 were observed in the ^{13}C NMR spectrum. These data suggested that compound **1** is a trihydroxyxanthone derivative with a prenyl group, a 1,1-dimethylallyl group, and a methoxy group. The prenyl, 1,1-dimethylallyl, and methoxy groups were placed at C-4, C-2, and C-3, respectively, by the correlations between H-16 (δ_H 3.53) and C-3 (δ_C 163.6), C-4 (δ_C 113.4), and C-4a (δ_C 153.4), between H-12 and -13 (δ_H 1.62) and C-2 (δ_C 122.1), and between OCH_3 (δ_H 3.61) and C-3 (δ_C 163.6) in the HMBC spectrum (Figure 1). On the basis of these data, the structure of compound **1** was defined as 1,6,7-trihydroxy-3-methoxy-4-(3-methylbut-2-en-1-yl)-2-(2-methylbut-3-en-2-yl)-xanthone, and the compound was named cudracuspixanthone A.

Compound **2** was purified as a yellow, amorphous powder, and its molecular formula of $C_{23}H_{22}O_6$ was determined by the ^{13}C NMR spectroscopic data and an HREIMS ion at m/z 417.1308 ($[M + Na]^+$, calcd 417.1314). The presence of two five-carbon moieties, i.e., a 2,3,3-trimethyl-2,3-dihydrofuran [δ_H 1.26 (3H, s, CH_3-12), 1.51 (3H, s, CH_3-13), 4.52 (1H, q, J = 6.8 Hz, H-14), and 1.40 (3H, d, J = 6.8 Hz, H-15); δ_C 43.1 (C-11), 19.5 (C-12), 24.2 (C-13), 90.8 (C-14), and 13.2 (C-15)][11] and a 2,2-dimethylpyran ring [δ_H 6.91 (1H, d, J = 10.0 Hz, H-16), 5.88 (1H, d, J = 10.0 Hz, H-17), and 1.55 (6H, s, H-19, 20); δ_C 114.7 (C-16), 130.3 (C-17), 78.2 (C-18), and 26.7 (C-19, 20)],[12] were deduced from the resonances in the ^{1}H and ^{13}C NMR spectra and confirmed by HMBC correlations. Compound **2** is suggested to be a derivative of a tetrahydroxyxanthone with a 2,3,3-trimethyl-2,3-dihydrofuran and a 2,2-dimethylpyran ring from additional isolated aromatic proton resonances at δ_H 6.35 (1H, s, H-4) and 7.40 (1H, s, H-8) in the ^{1}H NMR spectrum and ^{13}C carbon resonances including one carbonyl resonance at δ_C 180.1 in the ^{13}C NMR spectrum. The two aromatic protons were placed at C-4 and C-8, respectively, by correlations between H-4 (δ_H 6.35) and C-2 (δ_C 116.5), C-3 (δ_C 165.7), C-4a (δ_C 158.0), and C-9a (δ_C 103.1) and between H-8 (δ_H 7.40) and C-6 (δ_C 147.4), C-7 (δ_C 143.2), and C-9 (δ_C 180.1) in the HMBC spectrum. The positions of the 2,3,3-trimethyl-2,3-dihydrofuran and 2,2-dimethylpyran rings were also determined from the HMBC correlations between H-12 (δ_H 1.26) and C-2 (δ_C 116.5) and between H-16 (δ_H 6.91) and C-6 (δ_C 147.4) (Figure 1). On the basis of these data, the structure of compound **2** was elucidated

dx.doi.org/10.1021/np5002797 | J. Nat. Prod. 2014, 77, 2361−2366

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data for Compounds 1−4

	1[a]		2[b]		3[b]		4[b]	
	δ_C[c]	δ_H (J in Hz)[e]	δ_C[c]	δ_H (J in Hz)[e]	δ_C[d]	δ_H (J in Hz)[e]	δ_C[c]	δ_H (J in Hz)[e]
1	160.2		157.8		157.6		157.4	
2	122.1		116.5		124.4		124.0	
3	163.6		165.7		160.5		160.0	
4	113.4		88.8	6.35, s	115.8		113.6	
4a	153.4		158.0		155.3		155.4	
5	102.5	7.00, s	109.6		132.6		101.9	6.81, s
6	151.9		147.4		146.7		151.7	
7	143.3		143.2		112.1	6.88, d (8.8)	143.5	
8	108.3	7.59, s	108.1	7.40, s	116.6	7.62, d (8.8)	108.2	7.49, s
8a	112.7		113.1		115.2		115.1	
9	180.9		180.1		176.6		175.8	
9a	105.2		103.1		105.3		105.4	
10a	153.9		146.1		151.6		153.9	
11	40.8		43.1		43.6		43.6	
12	26.7	1.62, s	19.5	1.26, s	20.9	1.24, s	20.9	1.23, s
13	26.7	1.62, s	24.2	1.51, s	25.1	1.49, s	25.1	1.49, s
14	149.8	6.46, dd (17.2, 10.4)	90.8	4.52, q (6.8)	90.7	4.55, q (6.4)	90.6	4.54, q (6.4)
15	104.1	4.96, dd (17.2, 1.2) 4.78, dd (10.8, 1.2)	13.2	1.40, d (6.8)	12.9	1.46, d (6.4)	12.9	1.46, d (6.4)
16	22.6	3.53, d (6.8)	114.7	6.91, d (10.0)	22.3	3.66, d (6.0)	22.4	3.54, d (6.0)
17	123.0	5.27, t (6.8)	130.3	5.88, d (10.0)	122.7	5.39, t (6.8)	122.6	5.26, t (6.4)
18	131.1		78.2		131.5		131.5	
19	17.3	1.89, s	26.7	1.55, s	16.8	1.88, s	16.8	1.91, s
20	25.0	1.69, s	26.7	1.55, s	24.5	1.72, s	24.5	1.73, s
OCH_3	62.1	3.61, s			62.0	3.95, s	62.2	3.93, s

[a]Acetone-d_6. [b]Methanol-d_4. [c]100 MHz. [d]125 MHz. [e]400 MHz.

as 5,8-dihydroxy-3,3,9,9,10-pentamethyl-9,10-dihydrofuro[3,2-*b*]pyrano[3,2-*h*]xanthone, and the compound was named cudracuspixanthone B.

Compound **3** was purified as a light brown syrup, and its molecular formula of $C_{24}H_{26}O_6$ was determined by the ^{13}C NMR spectroscopic data and an HREIMS ion at m/z 411.1802 ([M + H]$^+$, calcd 411.1808). The ^{1}H and ^{13}C NMR spectra revealed the resonances for a 3,3-dimethylallyl group [δ_H 3.66 (2H, d, J = 6.0 Hz, H-16), 5.39 (1H, t, J = 6.8 Hz, H-17), 1.88 (3H, s, CH_3-19), and 1.72 (3H, s, CH_3-20); δ_C 22.3 (C-16), 122.7 (C-17), 131.5 (C-18), 16.8 (C-19), and 24.5 (C-20)] and resonances for a 2,3,3-trimethyl-2,3-dihydrofuran ring [δ_H 1.24 (3H, s, CH_3-12), 1.49 (3H, s, CH_3-13), 4.55 (1H, q, J = 6.4 Hz, H-14), and 1.46 (3H, d, J = 6.4 Hz, H-15); δ_C 43.6 (C-11), 20.9 (C-12), 25.1 (C-13), 90.7 (C-14), and 12.9 (C-15)], similar to those in **1** and **2**, respectively. In the ^{1}H NMR spectrum, resonances for *ortho*-coupled aromatic protons were observed at δ_H 6.88 (1H, d, J = 8.8 Hz, H-7) and 7.62 (1H, d, J = 8.8 Hz, H-8). The resonances for a methoxy group were observed at δ_H 3.95 (3H, s) and δ_C 62.0, in the ^{1}H and ^{13}C NMR spectra, respectively. The positions of *ortho*-coupled aromatic signals were confirmed to be at C-7 and C-8 from the HMBC correlations between H-8 (δ_H 7.62) and C-6 (δ_C 146.7), C-9 (δ_C 176.6), and C-10a (δ_C 151.6). The positions of the 3,3-dimethylallyl and 2,3,3-trimethyl-2,3-dihydrofuran groups were deduced from the HMBC correlations between H-16 (δ_H 3.66) and C-3 (δ_C 160.5), C-4 (δ_C 115.8), and C-4a (δ_C 155.3) and between CH_3-12 (δ_H 1.24), CH_3-13 (δ_H 1.49), and C-2 (δ_C 124.4). The HMBC correlation between OCH_3 (δ_H 3.95) and C-3 (δ_C 160.5) confirmed the position of the methoxy group as C-3. On the basis of these data, the structure of compound **3** was defined as 7,8-dihydroxy-4-methoxy-2,3,3-trimethyl-5-(3-methylbut-2-en-1-yl)-2,3-dihydrofuro[2,3-*a*]xanthone, and the compound was named cudracuspixanthone C.

Compound **4** was obtained as a brown syrup and had the same molecular formula, $C_{24}H_{26}O_6$ (m/z 411.1802 [M + H]$^+$), as compound **3**. The ^{1}H NMR spectrum of **4** differs from the spectrum of **3** in the aromatic resonances due to the replacement of the two *ortho*-coupled aromatic protons of **3** by two isolated aromatic resonances at δ_H 6.81 (1H, s, H-5) and 7.49 (1H, s, H-8). In the HMBC spectrum, H-5 (δ_H 6.81) showed correlations with C-7 (δ_C 143.5) and C-10a (δ_C 153.9), and H-8 (δ_H 7.49) showed correlations with C-6 (δ_C 151.7), C-7 (δ_C 143.5), C-9 (δ_C 175.8), and C-10a (δ_C 153.9). Therefore, the structure of compound **4** was defined as 8,9-dihydroxy-4-methoxy-2,3,3-trimethyl-5-(3-methylbut-2-en-1-yl)-2,3-dihydrofuro[2,3-*a*]xanthone, and the compound was named cudracuspixanthone D.

Compounds **2**−**4** commonly bear a stereogenic center at C-14. For the determination of the absolute configurations of C-14 in **2**−**4**, we initially calculated ECDs for both enantiomers of compounds **2**−**4** and compared the calculated and experimental ECD spectra. However, the ECD data did not provide unequivocal determination of the absolute configurations (see the Supporting Information). Therefore, we calculated optical rotation values of **2**−**4**. The specific rotation value of **2**, −83, enabled assignment of the (14*S*) configuration for **2** because the calculated value for the (14*S*) configuration was −78, whereas the (14*R*) configuration in **2** resulted in a +93 specific rotation. The absolute configurations of **3** and **4** were also deduced as (14*R*) and (14*S*) in the same manner by the comparison of their observed and calculated specific rotations (see the Supporting Information).

dx.doi.org/10.1021/np5002797 | J. Nat. Prod. 2014, 77, 2361−2366

Compound **5** was purified as a yellow, amorphous powder, and a molecular formula of $C_{19}H_{20}O_5$ was determined on the basis of the ^{13}C NMR spectroscopic data and an HREIMS ion at m/z 327.1231 ([M − H]$^-$, calcd 327.1232). The ^1H and ^{13}C NMR spectra of **5** showed resonances for a 3,3-dimethylallyl group at [δ_H 3.20 (2H, d, J = 7.2 Hz, H-1″), 5.18 (1H, t, J = 7.2 Hz, H-2″), 1.68 (3H, s, H-4″), and 1.65 (3H, s, H-5″); δ_C 27.5 (C-1″), 122.1 (C-2″), 132.6 (C-3″), 24.4 (C-4″), and 16.4 (C-5″)] and a methoxy group at δ_H 3.96 (3H, s, OCH_3); δ_C 59.3, similar to **1**, **3**, and **4**. In addition to the resonances for the 3,3-dimethylallyl and methoxy groups, five aromatic protons at δ_H 6.94 (1H, s, H-6), 7.03 (1H, d, J = 8.0 Hz, H-4′), 7.04 (1H, brs, H-2′), 7.07 (1H, d, J = 8.0 Hz, H-6′), and 7.33 (1H, t, J = 8.0 Hz, H-5′) were observed in the ^1H NMR spectrum, and a further 13 carbons including one carbonyl carbon were observed in the ^{13}C NMR spectrum. However, the carbonyl resonance was deshielded to δ_C 201.0 compared to compounds **1**−**4**, which suggested that compound **5** was a benzophenone.[13] In the HMBC spectrum, H-6 (δ_H 6.94) showed correlations with C-7 (δ_C 201.0), C-2 (δ_C 151.5), C-4 (δ_C 151.2), and C-1″ (δ_C 27.5), and C-4 (δ_C 151.2) showed correlations with H-1″ (δ_H 3.20) and OCH_3 (3.96), suggesting the positions of the 3,3-dimethylallyl and methoxy groups as C-5 and C-4, respectively. The HMBC correlations between H-2′ (δ_H 7.04) and C-3′ (δ_C 157.2) and C-7 (δ_C 201.0) and between H-5′ (δ_H 7.33) and C-1′ (δ_C 139.5) and C-3′ (δ_C 157.2) placed the hydroxy group at C-3′. Thus, the structure of compound **5** was elucidated as 2,3-dihydroxy-4-methoxy-5-(3-methylbut-2-en-1-yl)phenyl)(3-hydroxyphenyl)methanone, and the compound was named cudracuspiphenone A.

Compound **6** was obtained as a brown syrup with the molecular formula $C_{18}H_{16}O_5$, as determined by ^{13}C NMR and an HREIMS ion at m/z 311.0927 ([M − H]$^-$, calcd 311.0919). The ^1H and ^{13}C NMR spectra of **6** were similar to those of **5**, except for the difference of the nature of the five-carbon moiety and the absence of a methoxy group. In the ^1H NMR spectrum, the resonances at δ_H 6.26 (1H, d, J = 9.8 Hz, H-1″), 5.66 (1H, d, J = 9.8 Hz, H-2″), and 1.49 (6H, s, H-4″, 5″), which correspond to the carbon resonances at δ_C 121.0 (C-1″), 128.9 (C-2″), 77.8 (C-3″), and 27.2 (C-4″, C-5″) in the HSQC spectrum, were reminiscent of a 2,2-dimethylpyran ring. The HMBC correlations between H-2″ (δ_H 5.66) and C-5 (δ_C 112.8) and between H-6 (δ_H 6.88) and C-4 (δ_C 146.8), C-7 (δ_C 200.6), and C-1″ (δ_C 121.0) confirmed the structure of **6** as (7,8-dihydroxy-2,2-dimethyl-2*H*-chromen-6-yl)(3-hydroxyphenyl)methanone, and the compound was named cudracuspiphenone B.

The 11 known xanthones were identified as 2,6-dihydroxyxanthone (**7**),[14] laxanthone-I (**8**),[15] isocudraniaxanthone A (**9**),[16] isocudraniaxanthone B (**10**),[16] 1,3,5-trihydroxy-4-prenylxanthone (**11**),[17] cudraxanthone H (**12**),[3] cudratricusxanthone K (**13**),[18] macluraxanthone B (**14**),[19] 2-deprenylrheediaxanthone B (**15**),[20] cudraxanthone M (**16**),[21] and cudraxanthone A (**17**)[22] (Supporting Information) by the analysis of their spectroscopic data and comparison with literature values.

The inhibitory effects of the isolated compounds on the proliferation of HSC-T6 hepatic stellate cells were evaluated with an in vitro assay system. Among the compounds isolated, compounds **1**−**6**, **10**, and **14**−**16** showed antiproliferative activity toward HSC-T6 cells, with IC_{50} values of less than 20 μM (Table 2). The benzophenones **5** and **6** exhibited more potent inhibitory activities than the xanthones, with IC_{50} values of 3.3 and 7.1 μM, respectively, compared to IC_{50} values of >9.7 μM for the xanthones. The number and type of prenyl groups further divided the xanthones into several groups. Xanthones with no prenyl substituents (**7** and **8**) showed lower activity. The types of prenyl groups also affected the inhibitory activity, as was observed by comparing the stronger inhibitory activity of xanthone derivatives containing a 1,1-dimethylallyl group or 2,3,3-trimethyl-2,3-dihydrofuran moieties (**1**−**4**, **10**, and **14**−**16**) to xanthone derivatives containing the 3,3-dimethylallyl group or 2,2-dimethylpyran moieties. These results suggest that 1,1-dimethylallyl or 2,3,3-trimethyl-2,3-dihydrofuran moieties are important for the inhibitory activity of xanthones.

Table 2. Antiproliferative Activity of Compounds 1−17 on HSC-T6 Cells

compound	IC_{50} (μM)	compound	IC_{50} (μM)
1	9.7	**10**	17.3
2	15.9	**11**	>30
3	14.8	**12**	>30
4	15.2	**13**	>30
5	3.3	**14**	13.3
6	7.1	**15**	13.1
7	>30	**16**	10.4
8	>30	**17**	>30
9	NT	EGCG[a]	29.8

[a]Epigallocatechin-3-gallate was used as the positive control.

The apoptotic effects of compounds **1** and **5** were evaluated by flow cytometry using annexin-V/PI double staining. The percentage of apoptotic cells (annexin-V$^+$) was significantly increased by the treatment with 1 or 3 μM of compounds **1** and **5** (Figure 2). Interestingly, compounds **1** and **5** had differential dose−response effects on apoptosis. For compound **1**, concentrations of 1 and 3 μM induced similar total levels of apoptotic (annexin-V$^+$) cells (14.0% and 14.5%, respectively), but the late apoptotic cells (annexin-V$^+$/PI$^+$) accounted for 22.1% of the apoptotic cells at 1 μM and a significantly increase (66.3%) of the apoptotic cells at 3 μM. For compound **5**, the early apoptotic cells (annexin-V$^+$/PI$^-$) and late apoptotic cells, and thus, the total apoptotic cells, increased as the concentration increased. As shown in Figure 2, the total apoptotic cells increased from 14.1% (64.5% early, 35.5% late) to 59.9% (78% early, 22% late) from 1 to 3 μM.

Although the mechanism of action needs to be established by further studies, these results suggest that compounds **1** and **5** exert antiproliferative activity on HSC-T6 cells, in part, via inducing apoptosis.

EXPERIMENTAL SECTION

General Experimental Procedures. A JASCO DIP-1000 polarimeter was used for the measurement of optical rotations. A JASCO UV-550 and PerkinElmer model LE599 spectrometer were used respectively for the measurement of UV and IR spectra. NMR spectra were recorded on a Bruker DRX 400, 500, or 700 MHz spectrometer using methanol-d_4 or acetone-d_6 as solvents. EIMS data was obtained on VG Autospec Ultima mass spectrometers.

Plant Material. The roots of *C. tricuspidata* were obtained from an herbal market in Chungbuk, Korea, in July 2012. The identification of the roots was confirmed by the herbarium of the College of Pharmacy at Chungbuk National University, and a voucher specimen (CBNU2001207-CT) was deposited in a specimen room of the herbarium.

Extraction and Isolation. The dried roots of *C. tricuspidata* (7.5 kg) were extracted twice with 80% MeOH, and the concentrated

dx.doi.org/10.1021/np5002797 | J. Nat. Prod. 2014, 77, 2361−2366

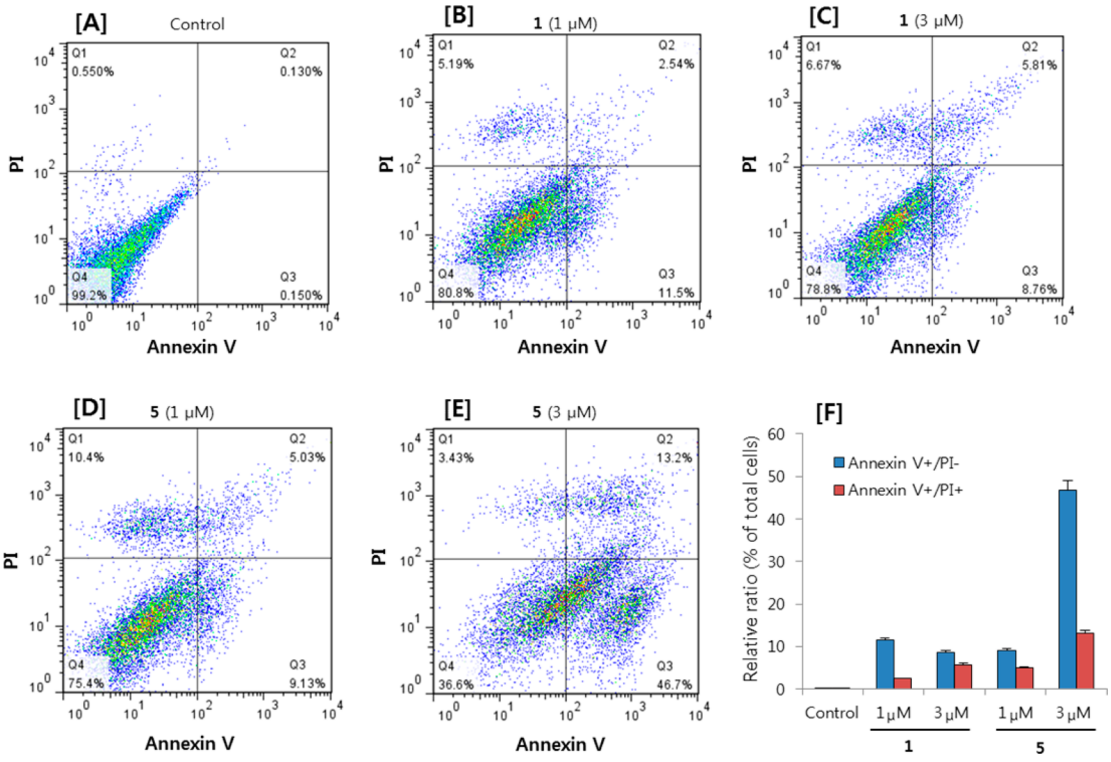


Figure 2. Analysis of the apoptosis stages of HSC-T6 cells: (A) control, (B) 1 μM compound **1**, (C) 3 μM compound **1**, (D) 1 μM compound **5**, and (E) 3 μM compound **5**. (F) Percentages of early apoptotic cells (annexin-V$^+$/PI$^-$) and late apoptotic cells (annexin-V$^+$/PI$^+$) are expressed as the mean values for apoptotic cells ± SD.

extract (461.4 g) was partitioned with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The CH_2Cl_2- and EtOAC-soluble fractions, which showed strong antiproliferative activity on HSC-T6 cells, were further separated.

The CH_2Cl_2 fraction (66.9 g) was chromatographed on silica gel with mixtures of *n*-hexane−CH_2Cl_2 to afford 17 fractions (C1−C17). Compound **7** (3.7 mg) was purified from C5 by recrystallization from MeOH. C11 was subjected to MPLC with mixtures of *n*-hexane−CH_2Cl_2 to yield four fractions (C11A−C11D). C11B was rechromatographed on Sephadex LH-20 eluted with *n*-hexane−CH_2Cl_2−MeOH (10:10:1) to afford seven subfractions (C11B1−C11B7). Compound **10** (3.8 mg) was obtained from C11B5 by semipreparative HPLC eluting with MeCN−H_2O (90:10). C14 was separated by MPLC with mixtures of *n*-hexane−CH_2Cl_2 to yield seven fractions (C14A−C14G). C14E was rechromatographed on Sephadex LH-20 using mixtures of *n*-hexane−CH_2Cl_2−MeOH (10:10:1) to yield 10 subfractions (C14E1−C14E10). Compound **2** (1.9 mg) was purified from C14E5 by semipreparative HPLC eluting with MeCN−H_2O (80:20). C16 was separated by MPLC with a mixture of CH_2Cl_2−MeOH to afford five fractions (C16A−C16E). C16B was subjected to RP-MPLC with a mixture of MeOH−H_2O to yield 17 fractions (C16B1−C16B17). Compounds **6** (4.6 mg), **8** (2.2 mg), and **9** (1.0 mg) were obtained from C16B10 by column chromatography over Sephadex LH-20 eluting with MeOH, followed by semipreparative HPLC using MeCN−H_2O (45:55). Compounds **5** (24.4 mg) and **15** (2.2 mg) were purified from C16B12 by column chromatography on Sephadex LH-20 using MeOH, followed by semipreparative HPLC eluting with MeCN−H_2O (45:55). C16B17 was rechromatographed on Sephadex LH-20 using MeOH to afford five fractions (C16B17A−C16B17E). C16B17E was separated by semipreparative HPLC column chromatography using MeCN−H_2O (80:20) to yield compounds **13** (3.0 mg), **14** (4.4 mg), **1** (6.2 mg), and **16** (7.5 mg). C17 was separated by RP-MPLC using MeOH−H_2O to afford 11 fractions (C17A−C17K). C17I was rechromatographed on Sephadex LH-20 using MeOH to give six subfractions (C17I1−C17I6). Compounds **3** (2.2 mg) and **4** (3.0 mg) were purified from C17I2 by semipreparative HPLC using MeCN−H_2O (45:55).

The EtOAc fraction (69.4 g) was chromatographed on silica gel using mixtures of CH_2Cl_2−MeOH to yield seven fractions (E1−E7). E2 was separated by RP-MPLC with mixtures of MeOH−H_2O to afford five fractions (E2A−E2E). E2E was rechromatographed on Sephadex LH-20 using MeOH to yield four fractions (E2E1−E2E4). Compounds **10** (1.9 mg), **11** (2.1 mg), and **12** (1.3 mg) were purified from E2E4 by semipreparative HPLC using MeCN−H_2O (60:40).

Cudracuspixanthone A (***1***)*:* yellow syrup; UV (MeOH) λ_{max} 256 (4.56), 323 (4.21); IR ν_{max} 3254, 1647 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS (negative mode) *m*/*z* 409 [M − H]$^-$; HRESIMS (positive mode) *m*/*z* 433.1621 (calcd for $C_{24}H_{26}O_6Na$, 433.1627).

Cudracuspixanthone B (***2***)*:* yellow, amorphous powder; $[\alpha]_D^{25}$ −83 (*c* 0.5, MeOH); UV (MeOH) λ_{max} 256 (4.46), 334 (4.20); IR ν_{max} 3317, 1647 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS (negative mode) *m*/*z* 393 [M − H]$^-$; HRESIMS (positive mode) *m*/*z* 417.1308 (calcd for $C_{23}H_{22}O_6Na$, 417.1314).

Cudracuspixanthone C (***3***)*:* light brown syrup; $[\alpha]_D^{25}$ −206 (*c* 0.3, MeOH); UV (MeOH) λ_{max} 250 (4.41), 317 (3.77); IR ν_{max} 3319, 1646 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS (positive mode) *m*/*z* 411 [M + H]$^+$; HRESIMS (positive mode) *m*/*z* 411.1802 (calcd for $C_{24}H_{27}O_6$, 411.1808).

Cudracuspixanthone D (***4***)*:* brown syrup; $[\alpha]_D^{25}$ +96 (*c* 0.2, MeOH); UV (MeOH) λ_{max} 256 (4.46), 366 (3.91); IR ν_{max} 3272, 1646 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS (negative mode) *m*/*z* 409 [M − H]$^-$; HRESIMS (positive mode) *m*/*z* 411.1802 (calcd for $C_{24}H_{27}O_6$, 411.1808).

Cudracuspiphenone A (***5***)*:* yellow, amorphous powder; UV (MeOH) λ_{max} (log ε) 211 (4.37), 294 (3.96); IR ν_{max} 3336, 1623 cm^{-1}; ^{1}H NMR (400 MHz, methanol-d_4) δ 7.33 (1H, t, *J* = 8.0 Hz, H-5′), 7.07 (1H, d, *J* = 8.0 Hz, H-6′), 7.04 (1H, brs, H-2′), 7.03 (1H, d, *J* = 8.0 Hz, H-4′), 6.94 (1H, s, H-6), 5.18 (1H, t, *J* = 7.2 Hz, H-2″), 3.96 (3H, s, OCH_3), 3.20 (2H, d, *J* = 7.2 Hz, H-1″), 1.68 (3H, s, H-4″), 1.65 (3H, s, H-5″); ^{13}C NMR (100 MHz, methanol-d_4) δ 201.0 (C-7), 157.2 (C-3′), 151.5 (C-2), 151.2 (C-4), 139.5 (C-1′), 138.0 (C-3),

dx.doi.org/10.1021/np5002797 | *J. Nat. Prod.* 2014, 77, 2361−2366

132.6 (C-3″), 129.0 (C-5′), 125.1 (C-5), 123.8 (C-6), 122.1 (C-2″), 119.8 (C-6′), 118.4 (C-4′), 115.2 (C-2′), 114.8 (C-1), 59.3 (OCH_3), 27.5 (C-1″), 24.4 (C-4″), 16.4 (C-5″); ESIMS (negative mode) m/z 327 $[M - H]^-$; HRESIMS (negative mode) m/z 327.1231 (calcd for $C_{19}H_{19}O_5$, 327.1232).

*Cudracuspiphenone B (**6**):* brown syrup; UV (MeOH) λ_{max} (log ε) 263 (3.96), 327 (3.81); IR ν_{max} 3326, 1640 cm^{-1}; ^1H NMR (700 MHz, methanol-d_4) δ 7.35 (1H, t, J = 7.7 Hz, H-5′), 7.09 (1H, d, J = 7.7 Hz, H-6′), 7.05 (1H, brs, H-2′), 7.03 (1H, d, J = 7.7 Hz, H-4′), 6.88 (1H, s, H-6), 6.26 (1H, d, J = 9.8 Hz, H-1″), 5.66 (1H, d, J = 9.8 Hz, H-2″), 1.49 (6H, s, H-4″, 5″); ^{13}C NMR (175 MHz, methanol-d_4) δ 200.6 (C-7), 157.2 (C-3′), 153.0 (C-2), 146.8 (C-4), 139.5 (C-1′), 133.1 (C-3), 129.1 (C-5′), 128.9 (C-2″), 121.7 (C-6), 121.0 (C-1″), 119.6 (C-6′), 118.3 (C-4′), 115.0 (C-2′), 113.5 (C-1), 112.8 (C-5), 77.8 (C-3″), 27.2 (C-4″, C-5″); ESIMS (negative mode) m/z 311 $[M - H]^-$; HRESIMS (negative mode) m/z 311.0927 (calcd for $C_{18}H_{15}O_5$, 311.0919).

Measurement of HSC-T6 Cell Proliferation. The viability of HSC-T6 cells, an immortalized rat hepatic stellate cell line, was measured as previously reported.[23]

Flow Cytometry for Apoptosis. For measuring apoptosis, HSC-T6 cells were treated with compounds **1** and **5** at 1 and 3 μM. After 48 h of incubation, cells were collected, washed with PBS and annexin V binding buffer, and centrifuged at room temperature. The measurement of apoptosis was performed by flow cytometry using the FITC-annexin V apoptosis detection kit (BD Biosciences) according to the manufacturer's protocol. Flow cytometric analysis was performed on a BD FACSCanto flow cytometer (BD Biosciences Immunocytometry Systems, USA).

Calculation of ECD Spectra and Optical Rotations. The ground-state geometries were optimized using density functional theory (DFT) calculations. All atoms were estimated with the basis set def-SV(P). The calculated ECD and optical rotation data were acquired with TDDFT at the functional B3LYP/DFT level with the basis set def2-TZVPP for all atoms. Optical rotations were estimated at 540 nm by dipole electric field polarizability calculations. These calculations were performed by Turbomole 6.5.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

1D and 2D NMR spectra of compounds **1**–**6**, structures of known compounds, calculated optical rotations of compounds **2**–**4**, and calculated ECDs and CD spectra of compounds **2**–**4** are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 82-43-261-2818. E-mail: mklee@chungbuk.ac.kr.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Financial support from the Medical Research Center Program (2008-0062275) and the Basic Science Research Program (2010-0025054) through the National Research Foundation (NRF) of Korea is greatly appreciated. The authors thank the Center for Research Instruments and Experimental Facilities, Chungbuk National University, for providing the NMR spectroscopic measurements.

■ REFERENCES

(1) Bae, K. H. *The Medicinal Plants of Korea*; Kyo-Hak Publishing Co.: Seoul, 2000; pp 68–69.
(2) Lee, B. W.; Gal, S. W.; Park, K. M.; Park, K. H. *J. Nat. Prod.* **2005**, *68*, 456–458.
(3) Hwang, J. H.; Hong, S. S.; Han, X. H.; Hwang, J. S.; Lee, D.; Lee, H.; Yun, Y. P.; Kim, Y.; Ro, J. S.; Hwang, B. Y. *J. Nat. Prod.* **2007**, *70*, 1207–1209.
(4) Chen, L.; Duan, Y.; Li, C.; Wang, Y.; Tong, X.; Dai, Y.; Yao, X. *Magn. Reson. Chem.* **2013**, *51*, 842–846.
(5) Kuang, L.; Wang, L.; Wang, Q.; Zhao, Q.; Du, B.; Li, D.; Luo, J.; Liu, M.; Hou, A.; Qian, M. *Biochem. Pharmacol.* **2011**, *81*, 1192–1200.
(6) Lim, J. Y.; Hwang, B. Y.; Hwang, K. W.; Park, S. Y. *Phytother. Res.* **2012**, *26*, 1948–1956.
(7) Zheng, Z. P.; Tan, H. Y.; Chen, J.; Wang, M. *Fitoterapia* **2013**, *84*, 242–247.
(8) Pellicoro, A.; Ramachndran, P.; Iredale, J. P. *Fibrogenesis Tissue Repair* **2012**, *5* (Suppl 1), S26–29.
(9) Ramachandran, P.; Iredale, J. P. *Q. J. Med.* **2012**, *105*, 813–817.
(10) Tanaka, N.; Takaishi, Y.; Shikishima, Y.; Nakahishi, Y.; Bastow, K.; Lee, K. H.; Honda, G.; Ito, M.; Takeda, Y.; Kodzhimatov, O. K.; Ashurmetov, O. *J. Nat. Prod.* **2004**, *67*, 1870–1875.
(11) Zou, Y. S.; Hou, A. J.; Zhu, G. F.; Chen, Y. F.; Sun, H. D.; Zhao, Q. S. *Bioorg. Med. Chem.* **2004**, *12*, 1947–1953.
(12) Nguyen, L. D.; Vo, H. T.; Phan, H. D.; Connolly, J. D.; Harrison, L. J. *Phytochemistry* **2003**, *63*, 467–470.
(13) Huang, Y. L.; Chen, C. C.; Chen, Y. J.; Huang, R. L.; Shieh, B. J. *J. Nat. Prod.* **2001**, *64*, 903–906.
(14) Bhardwaj, D. K.; Seshadri, T. R.; Singh, R. *Phytochemistry* **1977**, *16*, 1616–1617.
(15) Kobayashi, M.; Mahmud, T.; Yoshioka, N.; Shibuya, H.; Kitagawa, I. *Chem. Pharm. Bull.* **1997**, *45*, 1615–1619.
(16) Sabphon, C.; Sermboonpaisarn, T.; Sawasdee, P. *J. Med. Plants Res.* **2012**, *6*, 3781–3785.
(17) Hano, Y.; Matsumoto, Y.; Sun, J. Y.; Nomura, T. *Planta Med.* **1990**, *56*, 478–481.
(18) Ryu, Y. B.; Curtis-Long, M. J.; Lee, J. W.; Kim, J. H.; Kim, J. Y.; Kang, K. Y.; Lee, W. S.; Park, K. H. *Bioorg. Med. Chem.* **2009**, *17*, 2744–2750.
(19) Rath, G.; Potterat, O.; Mavi, S.; Hostettmann, K. *Phytochemistry* **1996**, *43*, 513–520.
(20) Hano, Y.; Matsumoto, Y.; Shinohara, K.; Sun, J. Y.; Nomura, T. *Planta Med.* **1991**, *57*, 172–175.
(21) Chang, C. H.; Lin, C. C.; Hattori, M.; Namba, T. *Phytochemistry* **1989**, *28*, 595–598.
(22) Kinjo, J.; Kurusawa, J.; Baba, J.; Takeshita, T.; Yamasaki, M.; Nohara, T. *Chem. Pharm. Bull.* **1987**, *35*, 4846–4850.
(23) Liu, Q.; Ahn, J. H.; Kim, S. B.; Lee, C.; Hwang, B. Y.; Lee, M. K. *Phytochemistry* **2013**, *87*, 112–118.