Cytotoxic Triterpenoid Alkaloids from *Buxus microphylla*

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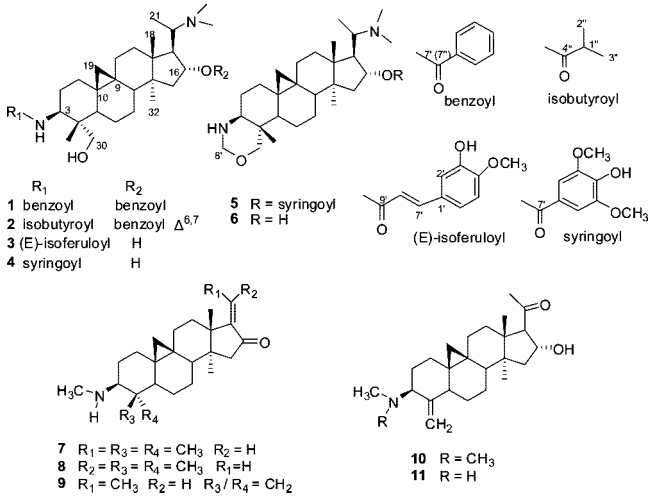
Five new triterpenoid alkaloids, buxmicrophyllines E−I (**1**−**5**), and six known ones (**6**−**11**) were isolated from the leaves and stems of *Buxus microphylla*. The structures of compounds **1**−**5** were elucidated by NMR and MS spectroscopic analysis, and the relative stereochemistry of **5** was determined by single-crystal X-ray crystallography. Compounds **3** and **9** were cytotoxic against HepG2 cells, with IC_{50} values of 0.89 and 0.78 μM, and compounds **2**, **3**, **7**, **8**, and **9** were cytotoxic against K562 cells, with IC_{50} values of 2.95, 4.44, 1.70, 5.61, and 0.37 μM, respectively.

More than 200 triterpenoid alkaloids have been isolated from plants of the genus *Buxus*.[1−3] Certain *Buxus* alkaloids have demonstrated antimalarial, antituberculosis, and anti-HIV effects.[4] *Buxus microphylla* Sieb. et Zucc. (Buxaceae) is an evergreen shrub distributed in southern China, which is usually planted to beautify the environment. The whole plant is also used as a folk medicine by natives for the treatment of acute myocardial ischemia.[5] In previous studies of plants from this genus, 10 new alkaloids were reported.[5−7] In our continuing studies on the secondary metabolites from leaves and stems of *B. microphylla*, five new triterpenoid alkaloids, buxmicrophyllines E−I (**1**−**5**), and six known ones, cyclobuxoxazine (**6**),[8] (*E*)-buxenone (**7**),[9] (*Z*)-buxenone (**8**),[9] buxmicrophylline B (**9**),[6] buxippine K (**10**),[10] and cyclomicrobuxinine (**11**),[6,11] were obtained. Compounds **1**−**3** and **7**−**11** were tested for cytotoxicity against human tumor cell lines HepG2 and K562. Herein we report the isolation and structural elucidation of the new compounds (**1**−**5**) and the results of cytotoxiciy tests of compounds **1**−**3** and **7**−**11**.



Buxmicrophylline E (**1**) was obtained as an amorphous powder. Its molecular formula was established to be $C_{40}H_{54}N_2O_4$ on the basis of HRESIMS and NMR spectra. IR absorptions at 3427, 1698, and 1714 cm^{-1} indicated the presence of OH, amide, and ester ketone groups. The ^1H and ^{13}C NMR spectra of **1** (Table 1) displayed 40 signals indicating six methyl, nine methylene, 16 methine, and nine quaternary carbons. The ^1H NMR spectrum featured three tertiary methyl singlets (δ_H 1.18, 0.69, and 1.05), and characteristic cyclopropyl methylene protons appeared as two doublets at δ_H 0.40 and 0.59 ($J_{19\alpha,19\beta}$ = 4.1 Hz). These observations favored a cycloartane-type triterpenoid skeleton typical of *Buxus* alkaloids.[5−7] Biogenetically, H-5 was assigned as α-oriented, while H-20 was β-oriented.[12,13] The ROESY correlations of H-5 (δ_H 2.09) with H-30 (δ_H 3.09 (m), 3.39 (m)) and of H-16 (δ_H 5.35) with H-20 (δ_H 2.52) indicated that H-30 was in α-orientation, while H-16 was in β-orientation, respectively. Furthermore, two downfield quaternary signals at δ_C 168.6 and 165.9 were assigned to carbonyl carbons, and two aromatic functionalities [10 methines at δ_C 126.8−132.3 and 10 protons appeared at δ_H 7.42−8.03] indicated there were two benzoyl units in **1**. The data indicated that compound **1** was similar in structure to semperviramidine[14] except for one more benzoyl group in **1**. The HMBC correlation from H-3 (δ_H 4.23) to C-7″ (δ_C 168.6) and from H-16 to C-7′ (δ_C 165.9) showed that the benzoyl groups were located at C-3 and C-16, respectively.

Buxmicrophylline F (**2**) was isolated as an amorphous powder. The HRESIMS showed a quasi-molecular ion at *m*/*z* 591.4178 [M + H]$^+$ indicating a molecular formula of $C_{37}H_{54}N_2O_4$. Comparion of the ^1H and ^{13}C NMR spectra of **2** with those of **1** (Table 1) revealed that a benzoyl group at C-3 in **1** was replaced by an isobutyroyl group in **2**, along with the appearance of a double bond between C-6 and C-7. This was confirmed by HMBC correlations of H-6 (δ_H 5.64) and H-7 (δ_H 5.47) with C-5 and C-8, and the upfield chemical shift of C-5 from δ_C 40.3 in **1** to δ_C 39.3 in **2** and C-8 from δ_C 47.7 in **1** to δ_C 43.2 in **2** also supported the above deduction. The additional HMBCs of H-3″ (δ_H 1.17) with C-1″ (55.7), C-2″ (δ_C 20.8), and C-4″ (178.3) and of H-1″ (δ_H 2.30) with C-2″ and C-4″ indicated the existence of an isobutyroyl functionality, which was attached to C-3 on the basis of the HMBC correlation between H-3 (δ_H 4.05) and the C-4″ carbonyl.

Buxmicrophylline G (**3**) was obtained as an amorphous powder and was assigned the molecular formula $C_{36}H_{54}N_2O_5$ on the basis of HRESIMS (at *m*/*z* 595.4108, calcd 595.4110). The NMR spectra of **3** (Table 2) were similar to those of **1** except for the absence of a benzoyl group at C-16 in **3** and an (*E*)-isoferuloyl group [δ_H 6.95 (d, J = 1.6 Hz, H-2′), 6.74 (d, J = 8.0, H-5′), 6.92 (dd, J = 1.6, 8.0, H-6′), δ_C 167.9 (C-9′), 116.9 (C-7′), 141.8 (C-8′), 126.8 (C-1′)] at C-3 in **3** instead of a benzoyl group in **1**. The two downfield olefinic protons of H-7′ (δ_H 6.24) and H-8′ (δ_H 7.42) resonated as doublets, and the coupling constant (J = 15.6 Hz) showed their *trans* relationship, respectively.[15] Furthermore, the HMBC cross-

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Table 1. ^{1}H and ^{13}C NMR Assignments of **1** and **2** ($CDCl_3$)

	1		**2**	
position	δ_C	δ_H	δ_C	δ_H
1	32.6 t	1.32 (m), 1.81 (m)	32.3 t	1.44 (m), 1.75 (m)
2	27.8 t	1.22 (m), 1.83 (m)	27.4 t	1.66 (m)
3	51.7 d	4.23 (m)	50.1 d	4.05 (m)
4	44.6 s		43.8 s	
5	40.3 d	2.09 (s)	39.3 d	2.53 (s)
6	20.6 t	1.21 (m)	125.8 d	5.64 (d, $J = 13.5$)
7	26.3 t	1.32 (m)	128.2 d	5.47 (m)
8	47.7 d	1.55 (m)	43.2 d	2.63 (d, $J = 7.5$)
9	19.3 s		20.1 s	
10	25.5 s		27.6 s	
11	25.6 t	1.12 (m)	25.1 t	1.42 (m), 1.81 (m)
12	30.3 t	1.28 (m)	30.3 t	1.44 (m), 1.63 (m)
13	44.8 s		45.0 s	
14	47.7 s		49.8 s	
15	44.7 t	1.51 (m)	42.0 t	1.31 (d, $J = 17.5$)
16	80.4 d	5.35 (m)	80.1 d	5.39 (m)
17	56.6 d	2.35 (m)	59.8 d	2.55 (m)
18	19.6 q	1.18 (s)	15.9 q	1.00 (s)
19	29.6 t	0.40, 0.59 (AB, $J = 4.1$)	18.3 t	0.13, 0.75 (AB, $J = 4.5$)
20	59.7 d	2.52 (m)	55.6 d	2.28 (m)
21	9.5 q	0.85 (d, $J = 8.0$)	10.1 q	0.85 (d, $J = 8.0$)
30	64.2 t	3.09 (m), 3.39 (m)	63.7 t	2.95 (m), 3.45 (m)
31	11.4 q	0.69 (s)	11.6 q	0.61 (s)
32	19.2 q	1.05 (s)	17.7 q	1.10 (s)
1′	131.1 s		131.0 s	
2′	129.4 d	8.03 (d, $J = 6.8$)	129.3 d	8.01 (dd, $J = 8.5, 2.0$)
3′	128.1 d	7.42 (m)	132.4 d	7.43 (m)
4′	132.3 d	7.52 (m)	128.2 d	7.54 (m)
5′	128.1 d	7.42 (m)	132.4 d	7.43 (m)
6′	129.4 d	8.03 (d, $J = 6.8$)	129.3 d	8.01 (dd, $J = 8.5, 2.0$)
7′	165.9 s		166.0 s	
1″	133.9 s		55.7 d	2.30 (q, $J = 6.5$)
2″	126.8 d	7.75 (dd, $J = 7.2, 1.6$)	20.8 q	1.17 (d, $J = 6.5$)
3″	128.7 d	7.47 (m)	19.4 q	1.17 (d, $J = 6.5$)
4″	131.8 d	7.52 (m)	178.3 s	
5″	128.7 d	7.47 (m)		
6″	126.8 d	7.75 (dd, $J = 7.2, 1.6$)		
7″	168.6 s			
N $(CH_3)_2$	30.9 q	2.17 (br s)	40.3 q	2.07 (br s)

peak of H-3 (δ_H 3.95) with C-9′ indicated that the isoferuloyl group was located at C-3.

Buxmicrophylline H (**4**) was isolated as a white powder with molecular formula $C_{35}H_{54}N_2O_6$ as determined by HRESIMS. Comparison of NMR data of **4** with those of **3** (Table 2) indicated that the only difference was that the isoferuloyl group at C-3 in **3** was replaced by a syringoyl group in **4**. In addition, a singlet for two methoxy groups at δ_H 3.95 and a singlet for two aromatic protons at δ_H 6.99 indicated the presence of a syringoyl group, which was in accordance with the ^{13}C NMR spectrum [δ_C 125.0 (C-1′), 104.3 (C-2′, 6′), 146.9 (C-3′, C-5′), 138.4 (C-4′), and 56.6 (OCH_3-3′, 5′)].

Buxmicrophylline I (**5**) had the molecular formula $C_{36}H_{54}N_2O_6$, as evidenced by the HRESIMS and NMR spectra. The ^{13}C NMR and DEPT spectra (Table 2) revealed 36 signals due to 10 quaternary, eight methine, 10 methylene (including two oxygenated ones), and eight methyl carbons. The HMBC correlations from H-30 (δ_H 3.21, 3.70) to C-3 (δ_C 63.2) and C-8′ (δ_C 79.5) and from H-8′ (δ_H 4.18, 4.55) to C-3 and C-30 (δ_C 77.2) indicated that **5** had a nonsubstituted tetrahydroxazine ring. All the data implied that the structure of **5** was very similar to that of **6**;[8] the only difference was an additional syringoyl group in **5**, which was located at C-16 by HMBC correlation of H-16 (δ_H 5.22) with C-7′. The single-crystal X-ray crystallographic results of **5** confirmed the proposed structure (Figure 1).

Compounds **1**−**3** and **7**−**11** were tested for cytotoxicity against human-tumor HepG2 and K562 cells lines (Table 3). Compared with the positive control cisplatin ($IC_{50} = 1.45$ μM), compounds **3** and **9** showed the most potent effect against HepG2 cells. Compounds **2**, **3**, **7**, **8**, and **9** were cytotoxic against K562 cells.

Experimental Section

General Experimental Procedures. Melting points were determined on a YU-HUA X-4 melting point apparatus. Optical rotations were obtained with a Horiba SEAP-300 polarimeter. NMR spectra were measured on Bruker AV-400 and DRX-500 instruments (Bruker, Zürich, Switzerland) with TMS as internal standard. HRESIMS data were recorded on a VG Autospec-3000 spectrometer. Infrared spectra were recorded on a Shimadzu IR-450 instrument by using KBr pellets. Thin-layer chromatography was performed on precoated TLC plates (200−250 μM thickness, F_{254} Si-gel 60 and F_{254} RP-18 Sigel-60, Qingdao Marine Chemical, Inc.). Silica gel (200−300 mesh, Qingdao Marine Chemical, Inc.), amino silica gel (75−100 μM, Fuji Silysia Chemical LTD, Japan), Lichroprep RP-18 (40−63 μM, Merk), and Sephadex LH-20 (Pharmacia) were used for column chromatography.

Plant Material. *Buxus microphylla* plant material was collected at Kunming (Yunnan), China, in August 2007. The sample was identified by Prof. Shu-Kun Chen of the Kunming Institute of Botany, and a voucher specimen (KIB 20070821) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The dried and powdered stems and leaves of *B. microphylla* (14.0 kg) were extracted with Me_2CO (3 × 25 L, each 2 days) at room temperature and filtered. The filtrate was evaporated to yield an extract, which was partitioned between EtOAc and 0.001 N HCl. The aqueous layer was adjusted to pH 10.0 with 2 N NaOH followed by exhaustive extraction with $CHCl_3$. The $CHCl_3$-soluble fraction (135 g) was chromatographed on a silica gel column, eluted with $CHCl_3$−MeOH (100:0, 50:1, 20:1, 10:1, 2:1), to give five

Table 2. ^{1}H and ^{13}C NMR Assignments of **3**−**5** ($CDCl_3/CD_3OD$ (4:1))

	3		**4**		**5**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	32.5 t	1.22 (m), 1.64 (m)	32.7 t	1.30 (m), 1.72 (m)	32.8 t	1.26 (m)
2	26.0 t	1.35 (m), 1.55 (m)	25.8 t	1.23 (m), 1.33 (m)	25.0 t	1.09 (m), 1.23 (m)
3	51.4 d	3.95 (m)	51.8 d	4.21 (m)	63.2 d	2.53 (dd, J = 3.0, 12.5)
4	44.4 s		44.7 s		38.1 s	
5	40.4 d	1.86 (m)	40.5 d	2.02 (m)	44.9 d	1.96 (m)
6	20.7 t	1.47 (m), 1.88 (m)	20.8 t	1.77 (m)	19.6 t	1.22 (m)
7	27.2 t	1.53 (m), 2.03 (m)	27.9 t	1.80 (m)	27.0 t	1.40 (m), 1.54 (m)
8	47.9 d	1.37 (m)	48.0 d	1.45 (m)	46.7 d	1.37 (m)
9	19.3 s		19.4 s		19.5 s	
10	25.6 s		25.6 s		25.6 s	
11	25.7 t	1.97 (m)	26.1 t	2.11 (m)	26.0 t	1.16 (m), 1.98 (m)
12	31.3 t	1.56 (m)	31.7 t	1.48 (m), 1.68 (m)	32.2 t	1.58 (m), 1.72 (m)
13	44.6 s		45.2 s		44.9 s	
14	47.2 s		47.3 s		47.6 s	
15	44.1 t	1.84 (m)	45.2 t	1.42 (m), 1.89 (m)	44.5 t	1.40 (m), 1.98 (m)
16	79.0 d	3.97 (m)	77.2 d	4.39 (m)	80.4 d	5.22 (m)
17	62.4 d	2.55 (m)	56.6 d	2.27 (m)	56.3 d	2.27 (m)
18	18.9 q	0.87 (s)	19.1 q	0.98 (overlapped)	18.8q	0.98 (overlapped)
19	30.5 t	0.26, 0.44 (AB, J = 3.9)	30.5 t	0.37, 0.56 (AB, J = 3.6)	30.2 t	0.32, 0.58 (AB, J = 4.0)
20	56.2 d	1.84 (m)	56.9 d	1.93 (m)	56.3 d	1.84 (m)
21	9.5 q	0.79 (d, J = 6.4)	10.1 q	0.98 (overlapped)	11.1 q	0.98 (overlapped)
30	63.9 t	2.92, 3.25 (AB, J = 12.8)	64.2 t	3.08, 3.40 (AB, J = 11.6)	77.2 t	3.21, 3.70 (AB, J = 10.5)
31	11.2 q	0.55 (s)	11.6 q	0.69 (s)	11.1 q	0.85 (s)
32	21.0 q	1.02 (s)	20.8 q	1.14 (s)	19.5 q	1.09 (s)
1′	126.8 s		125.0 s		[a]121.6 s	
2′	122.0 d	6.95 (d, J = 1.6)	104.3 d	6.99 (s)	106.7 d	7.24 (s)
3′	147.3 s		146.9 s		146.8 s	
4′	147.9 s		138.4 s		[a]139.3 s	
5′	115.1 d	6.74 (d, J = 8.0)	146.9 s		146.8 s	
6′	110.2 d	6.92 (dd, J = 8.0, 1.6)	104.3 d	6.99 (s)	106.7 d	7.24 (s)
7′	116.9 d	6.24 (d, J = 15.6)	168.3 s		166.1 s	
8′	141.8 d	7.42 (d, J = 15.6)			79.5 t	4.55,4.18 (AB, J = 10.5)
9′	167.9 s					
OCH_3	55.7 q	3.79 (s)	56.6 q	3.95 (s)	56.3 q	3.86 (s)
$N(CH_3)_2$		2.14 (s)	40.3 q	2.36 (br.s)	40.2 q	2.10 (s)

[a] Observed in the HMBC spectrum (500 MHz).

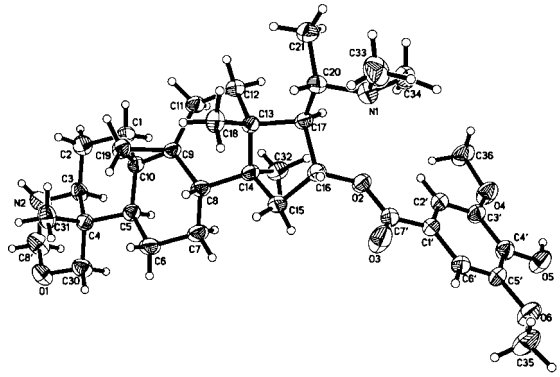

Figure 1. X-ray crystal structure of compound **5**.

Table 3. Cytotoxicity Data of Compounds **1**−**3** and **7**−**11** with IC_{50} Values (μM)

	cell line	
compound	HepG2	K562
1	39.66	>100
2	20.13	2.95
3	0.89	4.44
7	>100	1.70
8	14.92	5.61
9	0.78	0.37
10	57.13	35.29
11	>100	>100
cisplatin	1.45	8.54

fractions (A−E). Fraction A (7 g) was chromatographed on silica gel eluted with petroleum ether (PE)−EtOAc (80:1, 20:1, 3:1) to afford fractions A1, A2, and A3. Fraction A2 (500 mg) was subjected to silica gel column chromatography, eluted with PE−EtOAc−Et_2NH (1000:10:3), to yield **7** (23 mg) and **8** (8 mg). Fraction B (18 g) was chromatographed on silica gel using PE−Me_2CO (20:1, 5:1) as solvent to afford fractions B1 and B2. Fraction B1 (4 g) was further separated using a Sephadex LH-20 column eluted with MeOH and then an amino silica gel column eluted with PE−EtOAc (10:1) to afford **9** (13 mg), **10** (19 mg), and **11** (38 mg). Fraction B2 (3 g) was further separated by RP_{18}-gel column chromatography using aqueous MeOH (60%−90%) to afford fractions B2-(1−4). Fraction B2-3 (1 g) was initially subjected to a silica gel column using $CHCl_3$−Me_2CO (20:1) as eluent to yield **1** (25 mg) and **2** (13 mg). Fraction C (14 g) was chromatographed on silica gel using $CHCl_3$−MeOH (20:1, 5:1) as solvent to afford C1 and C2. Fraction C1 (2 g) was repeatedly separated by amino silica gel column chromatography, eluted with $CHCl_3$−MeOH (50:1, 10:1), to give **3** (42 mg), **4** (7 mg), **5** (28 mg), and **6** (7 mg).

Buxmicrophylline E (1): amorphous powder; mp 259 °C; $[\alpha]^{24}_D$ +12.5 (*c* 0.96, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 228 (6.89), 271 (0.71) nm; IR (KBr) ν_{max} 3427, 3245, 1714, 1698, 1628 cm^{-1}; ^{1}H and ^{13}C NMR spectra, see Table 1; ESIMS *m/z* 627 $[M + H]^+$; HRESIMS *m/z* 627.4167 (calcd for $C_{40}H_{54}N_2O_4$, 627.4161).

Buxmicrophylline F (2): amorphous powder; mp 248 °C; $[\alpha]^{24}_D$ +16.9 (*c* 1.03, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 228 (2.07), 272 (0.05) nm; IR (KBr) ν_{max} 3420, 3306, 1716, 1642 cm^{-1}; ^{1}H and ^{13}C NMR spectra, see Table 1; ESIMS *m/z* 591 $[M + H]^+$; HRESIMS *m/z* 591.4178 (calcd for $C_{37}H_{54}N_2O_4$, 591.4161).

Buxmicrophylline G (3): amorphous powder; mp 286 °C; $[\alpha]^{24}_D$ +8.6 (*c* 1.52, MeOH); UV (MeOH) λ_{max} (log ε) 218 (2.76), 235 (2.51), 295 (2.79), 321 (3.54) nm; IR (KBr) ν_{max} 3410, 3364, 1629 cm^{-1}; ^{1}H and ^{13}C NMR spectra, see Table 2; ESIMS *m/z* 595 $[M + H]^+$; HRESIMS *m/z* 595.4108 (calcd for $C_{36}H_{54}N_2O_5$, 595.4110).

Buxmicrophylline H (4): white powder; mp 289 °C; $[\alpha]^{24}_D$ +30.77 (*c* 1.90, MeOH); UV (MeOH) λ_{max} (log ε) 219 (1.73), 232 (1.87), 272 (2.97) nm; IR (KBr) ν_{max} 3379, 1636, 1724 cm^{-1}; ^{1}H and ^{13}C NMR

spectra, see Table 2; ESIMS *m/z* 599 $[M + H]^+$; HRESIMS *m/z* 599.4062 (calcd for $C_{35}H_{54}N_2O_6$, 599.4060).

Buxmicrophylline I (5): colorless crystals (MeOH−EtOAc); mp 257 °C; $[\alpha]^{24}_D$ +15.6 (*c* 1.52, MeOH); UV (MeOH) λ_{max} (log ε) 216 (6.17), 275 (2.60) nm; IR (KBr) ν_{max} 3392, 3271, 1705 cm^{-1}; ^1H and ^{13}C NMR spectra, see Table 2; ESIMS *m/z* 611 $[M + H]^+$; HRESIMS *m/z* 611.4058 (calcd for $C_{36}H_{54}N_2O_6$, 611.4010).

Crystal Data for 5. Crystals of **5** suitable for X-ray analysis were recrystallized from MeOH−EtOAc (8:1). Crystal data: $C_{36}H_{54}N_2O_6$, M = 610.82, space group triclinic, P_1, a = 6.7780(12) Å´, b = 7.8277(14) Å´, c = 16.835(3) Å´, V = 843.2(3) Å´^{3}, Z = 1, d = 1.205 Mg/m^3. The data were collected on a MAC DIP-2030K diffractometer, with graphite-monochromated Mo Kα radiation using a colorless crystal of dimensions 0.18 × 0.17 × 0.08 mm^3, maximum 2θ value of 56.34°. The crystal structure of **5** was solved by direct methods with SHELXS-8610 and expanded using difference Fourier techniques, refined by the program and method NOMCSDP11 and full-matrix, least-squares calculations. The final indices were R_1 = 0.0553, wR_2 = 0.1158. Crystallographic data for the structure of **5** have been deposited in the Cambridge Crystallographic Data Centre (deposition number 702205).

Cell Lines and Culture. The human hepatoma HepG2 and human chronic myelogenous leukemia K562 cell lines were obtained from the American Type Culture Collection (ATCC). The cells were grown in RPMI-1640 medium (GIBCO) supplemented with 10% heat-inactivated bovine serum, 2 nM L-glutamine, 10^5 IU/L penicillin, 100 mg/L streptomycin, and 10 mM HEPES pH 7.4. Cells were kept at 37 °C in a humidified 5% CO_2 incubator.

Cytotoxicity Assay. Growth inhibition of **1**−**3** and **7**−**11** on human tumor cells HepG2 and K562 was measured using the microculture tetrazolium (MTT) assay[16,17] with minor modification.[18] Briefly, tumor cells were seeded into 96-well microculture plates and allowed to adhere for 24 h before drug addition. The cell densities were selected on the basis of the results of preliminary tests to maintain the control cells in an exponential phase of growth during the period of the experiment and to obtain a linear relationship between the optical density and the number of viable cells. Each tumor cell line was exposed to **1**−**3** and **7**−**11** at 0.01, 0.1, 1, 10, and 100 μg/mL concentrations for 72 h, and each concentration was tested in triplicate. At the end of exposure, 20 μL of 5 g/L MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma Chemical Co.] was added to each well, and the plates were incubated for 4 h at 37 °C. Then "triplex solution (10% SDS−5% isobutanol−0.012 M HCl)" was added, and the plates were incubated for 12−20 h at 37 °C. The optical density (OD) was read on a plate reader at a wavelength of 570 nm. Media and DMSO control wells were included in all the experiments in order to eliminate the influence of DMSO. The inhibitory rate of cell proliferation was calculated by the following formula: Growth inhibition (%) = $OD_{control} - OD_{treated}/OD_{control} \times 100\%$. The cytotoxicity of **1**−**3** and **7**−**11** on tumor cells was expressed as IC_{50} values (the drug concentration reducing by 50% the absorbance in treated cells, with respect to untreated cells) that was calculated by the LOGIT method.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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