Full Papers

Sesquiterpene Lactones from *Inula britannica* and Their Cytotoxic and Apoptotic Effects on Human Cancer Cell Lines

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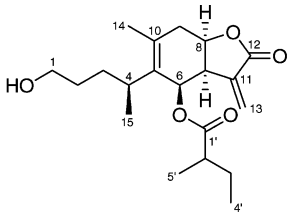
Three new sesquiterpenes (**1**−**3**), together with four known sesquiterpene lactones, were isolated from the flowers of *Inula britannica* var. *chinensis*. Structures were established on the basis of high-field 1D and 2D NMR methods supported by HRMS. All sesquiterpene lactones were tested for cytotoxicity as well as apoptotic ratio in human COLO 205, HT 29, HL-60, and AGS cancer cells. Compounds **3** and **4**, two α-methylene γ-lactone-bearing sesquiterpenes, were modestly active in these assays.

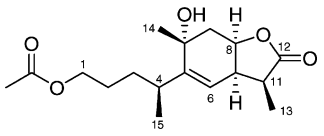
Inula, from Compositae, exists as more than one hundred species and is found mainly in Europe, Africa, and Asia. *Inula britannica* is a wild plant found in Eastern Asia, including China, Korea, and Japan. In traditional Chinese medicine, both *I. britannica* and *I. japonica* are called "Xuanfuhua". The flowers from these plants are used for the treatment of digestive disorders, bronchitis, and inflammation. Its extracts are reported to have anti-inflammatory, antibacterial, antihepatitic, antidiabetes, and antitumor activities.[1−5] Various sesquiterpene lactones have been isolated from Chinese *Inula* species such as *I. britannica*,[6−8] *I. salsoloides*,[9] *I. hupehensis*, and *I. helianthus-aquatica*.[10] Several isolated[11] and derivatized[12,13] sesquiterpene lactones have been shown to be cytotoxic. In addition, kaurane glycosides, as well as some flavonoids and steroids, are reported from *I. britannica*.[14] As part of an ongoing research program aimed at the discovery of antitumor constituents from plants, *Inula britannica* var. *chinensis* was further screened for anticarcinogenic activity. This investigation led to the isolation of three new and four known sesquiterpenes.

Results and Discussion

The air-dried, powered flower material was percolated with 95% EtOH at room temperature. A combination of column chromatography on silica gel and Sephadex LH-20 of the EtOAc-soluble portion of the EtOH extract from the flowers of *I. britannica* gave three new sesquiterpene lactones, 6*β*-*O*-(2-methylbutyryl)britannilactone (**1**), neobritannilactone A (**2**), and neobritannilactone B (**3**), together with the four known sesquiterpene lactones acetyl neobritannilactone B (**4**),[19,20] britannilactone (**5**),[6] 1-*O*-acetylbritannilactone (**6**),[6] and 1,6-*O*,*O*-diacetylbritannilactone (**7**).[6] The structures of the known compounds were identified by their physical and spectroscopic data with reported information. Compounds **1**, **2**, **5**, **6**, and **7** are 1,10-sec-eudesmanolide sesquiterpenoids with an uncommon carbon skeleton. Only a few of this class of sesquiterpene lactones have been previously reported.[15−18]



1



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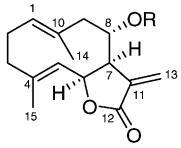

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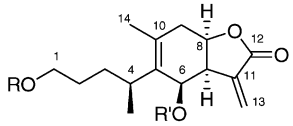
4 R = Ac



5 R = H R' = H
6 R = Ac R' = H
7 R = Ac R' = Ac

Compound **1** was obtained as an amorphous powder. The molecular formula $C_{20}H_{30}O_5$ was established by HRFABMS (obsd 351.2169, calcd 351.2171, $[M + H]^+$) and implied an unsaturation equivalence of six. The IR spectrum suggests the presence of hydroxyl (3382 cm^{-1}), ester (1749 cm^{-1}), and α-methylene γ-lactone (1654, 1762 cm^{-1}) groups. This was further confirmed by the ^1H and ^{13}C NMR signals appearing at δ_H 5.97 (H-13a) and 6.29 (each 1H, d, 2.0 Hz) (H-13b), δ_C 125.5 (C-13), 138.2 (C-11), and 171.9

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Table 1. ^{1}H and ^{13}C NMR and HMBC Data for Compounds **1** and **2** ($CDCl_3$)[a]

1				**2**			
no.	δ_H	δ_C	HMBC (H to C)	no.	δ_H	δ_C	HMBC (H to C)
1	3.37 m	62.8 t	3	1	3.97 t (6.4)	64.6 t	2,3,16
	3.43 m						
2	1.25 m	31.5 t		2	1.50 m	27.2 t	
3	0.98 m	32.2 t		3	1.41 m	34.6 t	
	1.42 m						
4	2.71 m	34.4 d		4	2.40 m	32.6 d	
5		133.6 s		5		151.8 s	
6	5.22 d (2.0)	70.2 d	4,5,7,8,10,11,1′	6	5.22 d (2.4)	115.3 d	8
7	3.53 m	44.1 d	6,11	7	3.01 m	38.8 d	
8	5.02 m	77.1 d		8	4.71 m	75.8 d	7,10
9	2.46 m 2.75 m	35.5 t	5,7,8,10,14	9	1.84 dd (3.6,14.8)	41.3 t	8,10
					2.46 dd (3.6,14.8)		
10		134.9 s		10		69.7 s	
11		138.2 s		11	2.84 m	40.5 d	6,12,13
12		171.9 s		12		178.9 s	
13	5.97 d (2.0)	125.5 t	7,12	13	1.16 d (7.2)	10.8 q	7,11,12
	6.29 d (2.0)		7,11,12				
14	1.80 s	20.6 q	5,9,10	14	1.30 s	29.4 q	5,9,10
15	0.88 d (7.6)	19.3 q	3,4,5	15	1.05 d (6.8)	23.8 q	3,4,5
1′		177.8 s		16 CH_3	1.98 s	21.1 q	17
2′	0.95 m	22.7 d		17 C=O		171.5 s	
3′	1.63 m	27.5 t					
	1.76 m						
4′	1.11 d (7.2)	16.9 q					
5′	1.15 d (8.0)	11.9 q					

[a] Carbon multiplicities were determined by DEPT experiments (s = C, d = CH, t = CH_2, q = CH_3). Figures in parentheses denote *J* values (Hz).

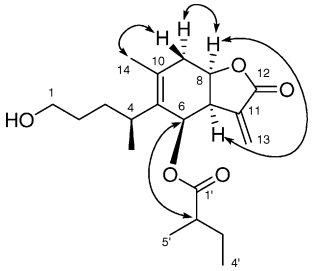

Figure 1. NOESY correlations of **1**.

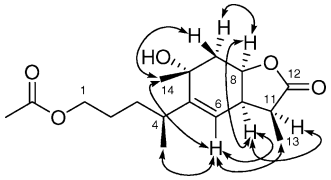

Figure 2. NOESY correlations of **2**.

(C-12). The ^{1}H and ^{13}C NMR spectra combined with a DEPT analysis show that **1** is composed of a total of 20 carbons, including four methyls, six methylenes (including one *exo*-methylene), five methines, and five quaternary carbons (see Table 1). All proton resonances were assigned with the aid of ^{1}H−^{1}H COSY and TOCSY spectra. The ^{13}C NMR signals were assigned by HMQC, HMBC, and DEPT spectra. Comparison of the ^{1}H and ^{13}C NMR data of **1** with those of britannilactone[6] (**5**) established the presence of the same backbone, except for a methylbutyryl group. This methylbutyryl group was established on the basis of the correlations observed between H-2′ at δ_H 0.95 (1H, m) and H-3′ at δ_H 1.63 (1H, m) and 1.76 (1H, m), as well as H-5′ at δ_H 1.15 (3H, d, 8.0 Hz). H-3′ was coupled with H-4′ at δ_H 1.11 (3H, d, 7.2 Hz). The group is located at C-6 (δ_C 70.2) due to the correlation between H-6 (δ_H 5.22, 1H, d, 2.0 Hz) and C-1′ (δ_C 177.8) in the HMBC. The relative stereochemistry of **1** was confirmed on the basis of the NOESY correlations. The strong cross-peak between H-7 at δ_H 3.53 (1H, m) and H-8 at δ_H 5.02 (1H, m) in the NOESY spectrum (Figure 1) suggests a *cis* configuration for the bicyclic ring of **1**. The presence of an NOE correlation between H-6 and H-7 indicated a β-orientation of the 6-hydroxyl group. Compound **1** was thus determined to be 6β-*O*-(2′-methylbutyryl)britannilactone, or 5-(4-hydroxy-1-methylbutyl)-6-methyl-4-(2-methylbutyryloxy)-3-methylene-3a,4,7,7a-tetrahydro-2(3*H*)-benzofuranone, a new compound from *I. britannica*. The complete ^{1}H and ^{13}C NMR signal assignments are listed in Table 1.

Compound **2** has a molecular formula of $C_{20}H_{30}O_5$ as established by HRFABMS (obsd 311.1859, calcd 311.1858, $[M + H]^+$). According to the IR spectrum, **2** and **1** were similar in that they both contained a hydroxyl group (3450 cm^{-1}), an ester (1729 cm^{-1}), and a γ-lactone (1755 cm^{-1}), but not the α-methylene-γ-lactone due to the lack of an UV absorption at 210 nm. **2** exhibited a ^{1}H and ^{13}C NMR signal pattern similar to those of **1** and 1-*O*-acetylbritannilactone[6] (**6**), which indicates a similar skeleton for these structures. The ^{13}C NMR, aided by DEPT and HMQC, spectra showed signals due to a double bond, and an acetyl and a quaternary carbon connected with a hydroxyl group. The two spin systems from H-1 through H-15 and from H-6 through H-9 in **1** were also observed for **2** in the ^{1}H−^{1}H COSY and TOCSY spectra, and they were separated by a double bond. Unlike **1**, the only double bond in **2** was determined at position Δ^{5-6}, which was deduced by the direct observation of a correlation between H-6 at δ_H 5.22 (1H, d, 2.4 Hz) and C-8 at δ_C 75.8, between H-14 at δ_H 1.30 (3H, s) and C-5 at δ_C 151.8, and between H-15 at δ_H 1.05 (3H, d, 6.8 Hz) and C-5 in HMBC spectrum. The NOE signals of H-6 with H-15 and H-6 with H-7 also supported the double-bond position for **2**. By comparing the ^{1}H NMR data of **2** with those of **1**, it was found that two H_2-9 signals were shifted upfield due to the anisotropic effect of the double bond, which further confirms $\Delta^{5,6}$ as the position of the double bond. A methyl group, from a reduction of the double bond in the α-methylene-γ-lactone unit, appeared at C-11 and was confirmed by the correlation of H-13 (δ_H 1.16, 3H, d, 7.2 Hz) with C-7 (δ_C 38.8), C-11 (δ_C 40.525), and C-12 (δ_C 178.9) in the HMBC spectrum. Due to the similarity of the ^{1}H NMR pattern of H-1−H-9 in **1** and **2**, the only position left for a hydroxyl group is at C-10. The presence of the correlation between H-14 (δ_H 1.30, 3H, s) and C-10 (δ_C 69.7) in the HMBC spectrum suggested the location of the hydroxyl group at C-10. The H-1 resonance was deshielded in **2** (δ_H 3.97, 2H, t, 6.4 Hz) when compared to that of **1**, suggesting

Table 2. ^{1}H and ^{13}C NMR and HMBC Data for Compounds **3** and **4** (CD_3OD)[a]

	3			**4**		
no.	δ_H	δ_C	HMBC (H to C)	δ_H	δ_C	HMBC (H to C)
1	4.83 m	129.2 d		4.83 d (11.6)	130.6 d	
2	2.27 m 2.35 m	26.1 t	1,3,10,14	2.23 m 2.27 m	26.1 t	1,3,10,14
3	1.95 m 2.39 m	39.4 t	2,4,5,15	2.04 m 2.34 m	39.3 t	2,4,5,15
4		142.6 s			142.4 s	
5	4.78 d (10.2)	127.5 d	3,7,15	4.71 d (10.0)	127.2 d	3,7,15
6	5.23 t (8.6)	75.1 d	4	5.06 t (10.0)	75.5 d	4,7
7	2.78 m	53.6 d		2.85 m	52.4 d	
8	4.61 m	71.7 d	6,10	5.66 m	71.5 d	6,10,16
9	2.05 d (13.2) 2.76 d (13.2)	47. 8t		2.31 m 2.76 dd (4.8,14.4)	43.8 t	1,7,8,10
10		135.8 s			134.2 s	
11		138.3 s			136.7 s	
12		170.2 s			169.8 s	
13	5.59 d (3.6) 6.39 d (3.6)	120.4 t	7,12 7,11,12	5.54 d (2.4) 6.25 d (3.6)	120.9 t	7,12 7,11,12
14	1.63 s	19.5 q	1,9,10	1.44 s	18.8 q	1,9,10
15	1.74 d (1.6)	17.4 q	3,4,5	1.70 d (1.6)	17.4 q	3,4,5
16					169.6 s	
17				2.01 s	20.9 q	16

[a] Carbon multiplicities were determined by DEPT experiments (s = C, d = CH, t = CH_2, q = CH_3). Figures in parentheses denote *J* values (Hz).

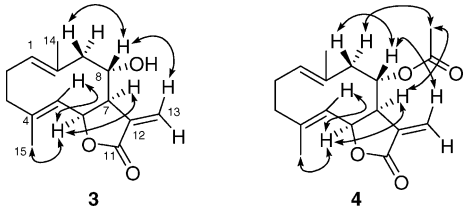

Figure 3. NOESY correlations of **3** and **4**.

the C-1 location of the acetyl group in **2**. This was confirmed by the obtained long-range correlation from H-1 to the carbonyl carbon of the acetyl group in the HMBC spectrum of **2**. Similar to **1**, the strong cross-peak between H-7 (δ_H 3.01, 1H, m) and H-8 (δ_H 4.71, 1H, m) in the NOESY spectrum (Figure 2) of **2** suggests a *cis* configuration of the bicyclic ring in **2**. The NOE correlations between H-7 and H-11 (δ_H 2.84, 1H, m) and between H-8 and H-9α (δ_H 1.84, 1H, dd, 3.6, 14.8 Hz), H-9*β* (δ_H 2.46, 1H, dd, 3.6, 14.8 Hz), and H-14 (CH_3) (δ_H 1.30, 3H, s) suggest the *β*-orientations of both CH_3-13 (δ_H 1.16, 3H, d, 7.2 Hz) and CH_3-14. Neobritannilactone A (**2**) is thus 5-(4-*O*-acetyl-1-methylbutyl)-3,6-dimethyl-6-hydroxy-3a,6,7,7a-tetrahydro-2(3*H*)-benzofuranone. The complete ^{1}H and ^{13}C NMR data of this new isolate were assigned as in Table 1.

Compound **3** was formulated as $C_{15}H_{20}O_3$ by HREIMS (obsd 248.1418, calcd 248.1413, $[M + H]^+$). The presence of the α-methylene *γ*-lactone group was evidenced from the ^{1}H NMR signals at δ_H 5.59 and 6.39 ppm and ^{13}C NMR signals at δ_C 120.4, 138.3, and 170.2 ppm. Further analysis of its ^{1}H and ^{13}C NMR data (Table 2) as well as the observed correlation in the ^{1}H−^{1}H COSY, TOCSY, HMQC, and HMBC spectra suggested that the structure of compound **3** is closely related to that of **4**. Comparison of the ^{1}H and ^{13}C NMR data of compounds **3** and **4** revealed that the difference between these two isolates is the absence of an acetyl group in compound **3**. This is consistent with its determined molecular formula. Compound **4** was previously reported by Galvez et al.[19] and Vickovic et al.[20] In the present study, the relative configurations of compounds **3** and **4** were established by interpretation of both 1D-NOE difference and 2D-NOESY (Figure 3) NMR data. Thus, the structure of **3** was determined to be 4-hydroxy-3a,4,5,8,9,11a-hexahydro-6,10-dimethyl-3-methylenecyclodeca[*b*]-furan-2(3*H*)-one. The ^{1}H and ^{13}C NMR data assignments are given in Table 2.

Table 3. Effect of Compounds **1**−**7** on the Growth of Human Cancer Cells[a]

	IC_{50} (μM)			
compound	COLO 205	HT-29	HL-60	AGS
1	58.7 ± 1.32	48.1 ± 2.26	47.1 ± 1.55	31.3 ± 1.8
2	97.9 ± 1.16	>100	85.8 ± 4.97	>100
3	14.3 ± 0.84	56.1 ± 5.16	27.4 ± 4.41	21.4 ± 1.76
4	14.7 ± 1.18	57.0 ± 2.53	16.2 ± 2.93	5.4 ± 0.41
5	>100	>100	>100	>100
6	>100	96.0 ± 12.52	>100	>100
7	56.7 ± 3.66	76.6 ± 5.50	35.3 ± 0.44	66.4 ± 6.29

[a] Human cancer cells were treated with various concentrations of compounds **1**−**7** for 24 h. The numbers of viable cells were determined by counting the trypan blue-excluding cells in a hemocytometer. Three samples were analyzed in each group, and values represent the mean ± SE.

Seven compounds isolated from *I. britannica* were tested for their cytotoxicity in human cancer cells. Human cancer cells were treated with different concentrations (5−100 μM) of selected compounds for 24 h, and the viability of the cells was determined by trypan blue exclusion. As shown in Table 3, significant cytotoxicity was observed in all types of human cancer cells treated with compounds **3** and **4**. Compounds **3** and **4** have significant influence on the viability of COLO 205, HT-29, HL-60, and AGS cells, assuming an IC_{50} value of 14.3, 56.1, 27.4, and 21.4 μM, respectively, for **3** and 14.7, 57.0, 16.2, 5.4 μM, respectively, for **4**. Compound **3** strongly inhibited HL-60 and AGS cells. These results indicate that human cancer cells were more sensitive to compounds **3** and **4** than other compounds.

We also investigated the selected compounds from *Inula* for their effects on the apoptotic ratio in human cancer cells. A sub-G1 (sub-2N) DNA peak, which has been suggested to be apoptotic DNA,[21] was detected in cells that were treated with selected compounds (**1**−**7**), washed, and stained with propidium iodide. As shown in Table 4, compounds **3** and **4** appeared to be more potent apoptosis-inducing agents than others for COLO 205, HT-29, AGS, and HL-60 cells, and these apoptotic effects were found to be dose-dependent. The percentages of apoptotic COLO 205, HT-29, HL-60, and AGS cells were 41.62 and 76.87%; 66.54 and 69.70%; 77.57 and 95.17%; and 11.78 and 9.89% after 24 h of incubation with compounds **3** and **4** (25 μM), respectively. Compound **4** appears to be more potent and induced dose-dependent cell apoptosis in all types of human cancer cells.

Table 4. Induction of Apoptosis in Human Cancer Cells by Compounds **1**−**7**[a]

compound	conc (μM)	human cancer cells (apoptotic ratio) %			
		COLO 205	HT-29	HL-60	AGS
	control	4.38 ± 1.29	3.92 ± 0.47	7.62 ± 2.76	9.91 ± 0.11
1	5	5.33 ± 0.78	4.24 ± 0.40	6.41 ± 0.79	10.89 ± 1.69
	10	5.86 ± 0.32	3.68 ± 0.47	5.36 ± 0.61	11.38 ± 1.12
	25	15.20 ± 1.02	3.93 ± 0.16	10.53 ± 3.34	17.76 ± 0.76
	50	27.21 ± 5.51	5.16 ± 0.93	28.60 ± 2.67	28.48 ± 1.20
	100	36.24 ± 0.76	6.40 ± 0.28	27.05 ± 0.54	27.10 ± 0.54
2	5	4.08 ± 0.21	3.94 ± 1.05	6.68 ± 1.51	9.23 ± 0.22
	10	6.51 ± 2.28	4.24 ± 0.24	6.31 ± 0.98	9.83 ± 2.27
	25	4.28 ± .93	5.17 ± 1.70	6.19 ± 0.01	10.57 ± 2.11
	50	5.44 ± 0.47	4.09 ± 0.77	6.63 ± 2.21	9.41 ± 2.83
	100	25.50 ± 3.46	7.73 ± 3.22	16.31 ± 1.63	9.81 ± 2.30
3	5	10.73 ± 2.31	13.58 ± 0.87	9.82 ± 1.90	9.55 ± 3.05
	10	14.12 ± 1.06	24.46 ± .88	21.93 ± 2.81	8.66 ± 0.80
	25	41.62 ± 6.34	66.54 ± 1.58	77.57 ± 7.06	11.78 ± 1.40
	50	89.11 ± 1.47	82.27 ± 1.68	96.94 ± 0.57	15.95 ± 1.12
	100	96.81 ± 0.51	87.02 ± 0.30	98.29 ± 0.15	19.22 ± 0.57
4	5	15.47 ± 1.69	20.38 ± .17	22.93 ± 1.34	6.16 ± 1.17
	10	34.72 ± 2.72	47.56 ± 0.00	39.71 ± 2.85	7.87 ± 3.03
	25	76.87 ± 2.39	69.70 ± .22	95.17 ± 1.61	9.89 ± 4.44
	50	96.92 ± 0.20	81.43 ± 1.46	97.74 ± 0.44	13.64 ± 0.28
	100	98.66 ± 0.10	82.47 ± 2.06	98.05 ± .30	43.11 ± 0.99
5	5	11.90 ± 3.61	5.75 ± 1.77	7.22 ± 1.54	7.93 ± 1.71
	10	12.28 ± 5.24	6.16 ± 1.23	5.64 ± 2.00	10.37 ± 0.98
	25	12.79 ± 1.84	6.49 ± 1.07	5.24 ± 0.67	10.16 ± .28
	50	15.54 ± 2.14	6.59 ± 0.54	8.01 ± 1.34	8.97 ± 0.66
	100	28.31 ± 1.89	7.55 ± .08	12.83 ± .06	10.37 ± 0.66
6	5	13.44 ± .25	5.36 ± 1.80	4.05 ± 0.31	15.37 ± 6.22
	10	11.32 ± .421	6.81 ± 2.14	7.26 ± 2.15	17.28 ± 2.29
	25	16.48 ± 3.75	11.68 ± 3.02	4.37 ± 0.57	14.66 ± 1.38
	50	16.59 ± 0.71	9.26 ± 1.80	5.69 ± 0.60	14.84 ± .58
	100	35.79 ± 1.36	14.87 ± .90	22.19 ± 4.14	18.85 ± .68
7	5	6.94 ± .33	11.19 ± 3.98	6.14 ± .20	7.49 ± .88
	10	8.69 ± 1.65	11.15 ± 2.84	8.67 ± 2.06	5.79 ± 0.64
	25	26.95 ± 0.30	11.50 ± .92	21.13 ± 0.37	11.29 ± 2.16
	50	15.44 ± .08	13.08 ± 0.63	27.02 ± 2.69	10.79 ± 2.79
	100	17.82 ± 2.26	20.88 ± .09	22.24 ± 1.06	8.12 ± .26

[a] Cells were harvested 24 h after treatment, and apoptosis was quantified by flow cytometry. The method of flow cytometry used is described in the Experimental Section. Three samples were analyzed in each group, and the results are presented as means ± SE.

Experimental Section

General Experimental Procedures. Optical rotations were determined in MeOH solutions on a Perkin-Elmer 141 polarimeter. UV was measured on a Cary 300 Bio UV−visible spectrophotometer. FT-IR was performed on a Perkin-Elmer spectrum BX system. ^{1}H and ^{13}C NMR spectra were recorded on a U-400 instrument (Varian Inc., Palo Alto, CA). Chemical shifts are expressed in parts per million (δ) using TMS as internal standard. CD_3OD and $CDCl_3$ were purchased from Aldrich Chemical Co. (Allentown, PA). HRFABMS was run on a JEOL HX-110 double-focusing mass spectrometer. Silica gel (130−270 mesh) and Sephadex LH-20 and RP-18 (60 μm) (Sigma Chemical Co., St. Louis, MO) were used for column chromatography. All solvents used were purchased from Fisher Scientific (Springfield, NJ).

Plant Material. The dried flowers of *I. britannica* were purchased from Shanghai Drugs Company, cultivated in the Jiansu Province of China. It was identified by Professor Zhi Wei Wang, College of Pharmacy, Fudan University, Shanghai, China. Voucher specimens were deposited in the Laboratory of Phytochemistry, College of Pharmacy, Fudan University, Shanghai, China.

Extraction and Isolation. The flowers (10 kg) of *I. britannica* were extracted three times with 95% EtOH at room temperature. The EtOAc-soluble part of the EtOH extract was chromatographed on a silica gel column, packed in $CHCl_3$, using a $CHCl_3$−MeOH gradient solvent system. The fractions from $CHCl_3$−MeOH (20:1 to 10:1) were evaporated under vacuum and repeatedly chromatographed on silica gel and Sephadex LH-20 columns, to give seven sesquiterpene lactones: 6β-*O*-(2-methylbutyryl)britannilactone, **1** (63 mg); neobritannilactone A, **2** (15 mg); neobritannilactone B, **3** (102 mg); acetyl neobritannilactone B, **4** (12 mg); britannilactone, **5** (21 mg); 1-*O*-acetylbritannilactone, **6** (1.1 g); and 1,6-*O*,*O*-diacetylbritannilactone, **7** (32 mg).

6β-*O*-(2-Methylbutyryl)britannilactone (1): amorphous powder; $[\alpha]^{20}_D$ +46.0 (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ϵ) 212.0 (4.11); IR (LF) ν_{max} 3382, 1762, 1749, 1654 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 351.2169 [M + H]$^+$ (calcd for $C_{20}H_{31}O_5$ 351.2171).

Neobritannilactone A (2): amorphous powder; $[\alpha]^{20}_D$ +14.0 (*c* 0.08, MeOH); IR (LF) ν_{max} 3450, 1755, 1729 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* 311.1859 [M + H]$^+$ (calcd for $C_{17}H_{27}O_5$ 311.1858).

Neobritannilactone B (3): amorphous powder; $[\alpha]^{20}_D$ −15.2 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ϵ) 215.0 (3.92); IR (LF) ν_{max} 3430, 3100, 1730, 1660, 820 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 2; DI EI HRMS *m*/*z* 248.1418 [M + H]$^+$ (calcd for $C_{15}H_{21}O_3$ 248.1413).

Acetyl neobritannilactone B (4): amorphous powder; $[\alpha]^{20}_D$ −17.3 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 217.0 (4.32); ^{1}H and ^{13}C NMR data, see Table 2; DI EI HRMS *m*/*z* 290.1516 [M + H]$^+$ (calcd for $C_{17}H_{23}O_4$ 291.1518).

Cell Culture and Chemicals. The COLO 205 and HT 29 cell lines were isolated from human colon adenocarcinoma (ATCC CCL-222 and HTB-38); human promyelocytic leukemia (HL-60) cells were obtained from American Type Culture Collection (Rockville, MD). The human AGS gastric carcinoma cell lines (CCRC 60102) were obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). Cell lines were grown at 37 °C in 5% CO_2 dioxide atmosphere in RPMI for COLO 205, HT-29, and HL-60 cells and DMEM/F12 for AGS cells, all supplemented with 10% heat-inactivated fetal bovine serum (Gibco BRL, Grand Island, NY) (100 units/mL of penicillin, 100 μg/mL of streptomycin) and 2 mM L-glutamine (Gibco BRL). Selected compounds were dissolved in dimethyl sulfoxide (DMSO). Propidium iodide was obtained from Sigma Chemical Co. (St. Louis, MO).

Determination of Cell Viability. Human cancer cells were treated with either DMSO (0.01%) or the selected compounds (5−100 μM). Cell viability was determined at 24 h based on trypan blue exclusion assay. The viability percentage was calculated on the basis of the

percentage of unstained cells. Suspensions of cells were diluted 1:1 with 0.5% trypan blue solution. Stained and unstained cells were counted in a hemocytometer.

Flow Cytometry. Human cancer cells (2×10^5) were cultured in 60 mm Petri dishes and incubated for 24 h. The apoptotic cells (sub-G1) in the selected compounds and treated cells were measured by flow cytometry analysis. Then cells were harvested, washed with PBS, resuspended in 200 μL of PBS, and fixed in 800 μL of iced 100% ethanol at −20 °C. After being left to stand overnight, the cell pellets were collected by centrifugation, resuspended in 1 mL of hypotonic buffer (0.5% Triton X-100 in PBS and 0.5 μg/mL RNase), and incubated at 37 °C for 30 min. Then 1 mL of propidium iodide solution (50 μg/mL) was added, and the mixture was allowed to stand on ice for 30 min. Fluorescence emitted from the propidium iodide−DNA complex was quantitated after excitation of the fluorescent dye by FACScan cytometry (Becton Dickinson, San Jose, CA).

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