New Triterpenoid Saponins from *Glochidion eriocarpum* and Their Cytotoxic Activity

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Combined chromatographic methods led to the isolation of two new triterpenoid saponins, glochieriosides A and B (1, 2), from the aerial parts of *Glochidion eriocarpum*, along with three known triterpenes, glochidone (3), lup-20(29)-en-3β,23-diol (4), and lup-20(29)-en-1β,3β-diol (5). The structures of the new saponins were determined to be 22β-benzoyloxy-3β,16β,28-trihydroxyolean-12-ene 3-*O*-[β-D-glucopyranosyl-(1→3)-α-L-arabinopyranoside] (1) and 22β-benzoyloxy-3β,16β,28-trihydroxyolean-12-ene 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-xylopyranoside] (2). The structural elucidation was accomplished by using a combination of the 1D-NMR (^{1}H-, ^{13}C-NMR, distortionless enhancement by polarization transfer (DEPT) 90°, and DEPT 135°), 2D-NMR (^{1}H–^{1}H correlation spectroscopy, heteronuclear multiple quantum correlation, heteronuclear multiple bond correlation, and rotating frame Overhouser effect spectroscopy), ESI-MS, and HR-FAB-MS experiments. Glochieriosides A and B exhibited significant cytotoxic activity against HL-60, HT-29, MCF-7 and SK-OV-3 human cancer cell lines with the IC_{50} values of 5.5, 6.8, 29.1, and 22.7 μM for glochierioside A, respectively, and 6.6, 18.6, 36.1, and 16.0 μM for glochierioside B. Glochidone was less active with IC_{50} values greater than 100 μM while lup-20(29)-en-1β,3β-diol was moderately active with IC_{50} values of 43.3, 67.0, 66.1, and 48.0 μM, respectively.

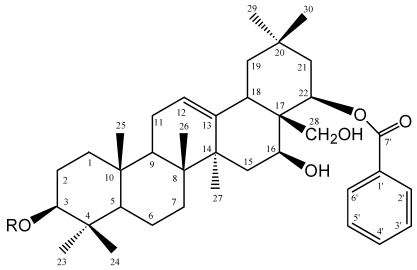
Key words *Glochidion eriocarpum*; Euphorbiaceae; triterpenoid saponin; glochierioside A; glochierioside B; cytotoxic activity

Glochidion J. R. FORST. & G. FORST. (1775) is a relatively large genus of the Euphorbiaceae family, comprising approximately 300 species distributed from Madagascar to the Pacific Islands and distributed mainly in tropical Asia. In Vietnam, this genus comprises 22—24 species, whereby *G. eriocarpum* CHAMP. is a shrub plant and abundantly found throughout.[1)] In Vietnamese folk medicine, the roots and leaves are used to treat enteritis, indigestion, asthma, cholera, and rheumatism. The whole plant decoction showed antimicrobial activity against *Staphylococcus aureus* and *Bacillus pyocyaneus*.[1)] Previous investigation resulted in the isolation of six lupanes from the roots and stem wood: lupenone; 3-epi-lupeol; glochidone; glochidonol; glochidiol; and lup-20(29)-ene-1β,3β-diol. Among them, glochidonol and glochidiol exhibited strong inhibitory effects against three human tumor cell lines, MCF-7, NCI-H-460, and SF-268. In addition, the two compounds exerted their antiproliferative activity through the involvement of apoptosis.[2)]

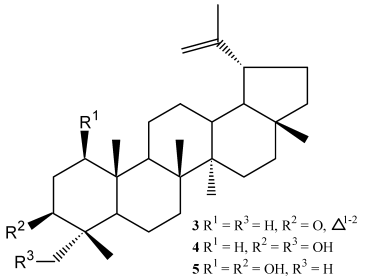
In continuation of our focusing investigation on cytotoxic constituents from Vietnamese medicinal plants, we report herein the isolation, structural elucidation, and cytotoxic evaluation of two new triterpenoid saponins, glochieriosides A and B (**1**, **2**), along with three known triterpenes, glochidone (**3**), lup-20(29)-en-3β,23-diol (**4**), and lup-20(29)-en-1β,3β-diol (**5**), from the aerial parts of *G. eriocarpum*.

Results and Discussion

From the methanol extract of aerial parts of *G. eriocarpum*, two new triterpenoid saponins, **1** and **2**, were isolated by various chromatographic methods. Compound **1** was obtained as an amorphous white powder. The IR spectrum suggested the presence of hydroxyl groups (br, 3392 cm^{-1}), carbonyl group (1709 cm^{-1}), aromatic ring (1649 cm^{-1}), and ether groups (1073, 1025 cm^{-1}). The molecular formula, $C_{48}H_{72}O_{14}$, was determined on the basis of positive ESI-MS (*m/z* 873 $[M+H]^+$ and 895 $[M+Na]^+$) and HR-FAB-MS data [*m/z*: 895.4826 $[M+Na]^+$ (Calcd for $C_{48}H_{72}O_{14}Na$: 895.4820)]. The spectral features and physicochemical properties suggested that **1** is a triterpenoid saponin having a benzoyl group. The ^{13}C-NMR spectrum showed signals of forty-eight carbons, of which thirty were assigned to the aglycon part, seven to a benzoyl group, and eleven to a disaccharide moiety. All the carbons were assigned to relevant protons by means of a heteronuclear multiple quantum



1 R= β-D-glucopyranosyl-(1➤3)-α-L-arabinopyranoside
2 R= β-D-glucopyranosyl-(1➤3)-β-D-xylopyranoside



3 $R^1 = R^3 = H$, $R^2 = O$, $\Delta^{1,2}$
4 $R^1 = H$, $R^2 = R^3 = OH$
5 $R^1 = R^2 = OH$, $R^3 = H$

Fig. 1. Structures of Compounds **1**—**5**

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Table 1. The NMR Spectral Data of Compounds **1** and **2**

C	**1**		**2**	
	δ_C [a),b)]	δ_H [a),c)] mult. (J in Hz)	δ_C [a),b)]	δ_H [a),b)] mult. (J in Hz)
Aglycon				
1	40.08	1.00[d)]/1.64[d)]	40.02	1.00[d)]/1.64[d)]
2	27.20	1.70[d)]/1.85[d)]	27.22	1.69[d)]/1.83[d)]
3	90.50	3.17 dd (11.0, 4.0)	90.66	3.17 dd (11.0, 4.0)
4	40.40	—	40.38	—
5	57.08	0.82 d (12.0)	57.03	0.83 d (12.0)
6	19.44	1.49[d)]/1.63[d)]	19.44	1.47[d)]/1.62[d)]
7	33.77	1.42[d)]/1.64[d)]	33.77	1.42[d)]/1.65[d)]
8	41.36	—	41.37	—
9	48.31	1.61[d)]	48.30	1.61[d)]
10	37.87	—	37.87	—
11	24.83	1.93[d)]/1.99[d)]	24.83	1.93[d)]/1.99[d)]
12	124.41	5.36 t (4.0)	124.43	5.36 t (4.0)
13	143.61	—	143.62	—
14	44.38	—	44.39	—
15	37.72	1.53[d)]/1.99[d)]	37.74	1.52[d)]/1.99[d)]
16	69.60	4.32 dd (11.2, 4.8)	69.62	4.32 dd (11.2, 4.8)
17	44.97	—	44.99	—
18	43.59	2.46 dd (13.5, 4.0)	43.62	2.46 dd (13.5, 4.0)
19	47.31	1.22 m/1.91[d)]	47.32	1.22 m/1.91[d)]
20	31.15	—	30.99	—
21	38.49	1.76 dd (15.5, 3.0) 1.84[d)]	38.50	1.76 dd (15.5, 3.0) 1.82[d)]
22	72.20	5.92 t (4.0)	72.22	5.92 t (4.0)
23	28.64	1.08 s	28.56	1.08 s
24	17.14	0.87 s	17.11	0.86 s
25	16.31	1.00 s	16.29	1.00 s
26	17.44	1.07 s	17.43	1.08 s
27	28.08	1.31 s	28.05	1.31 s
28	64.86	3.70 d (11.0) 4.01 d (11.0)	64.87	3.69 d (11.0) 4.02 d (11.0)
29	34.48	0.94 s	34.45	0.94 s
30	27.65	1.04 s	27.54	1.04 s
Benzoyl group				
1′	132.31	—	132.34	—
2′	130.61	8.05 d (8.0)	130.62	8.05 d (8.0)
3′	129.79	7.50 t (8.0)	129.79	7.50 t (8.0)
4′	134.24	7.61 t (8.0)	134.24	7.60 t (8.0)
5′	129.79	7.50 t (8.0)	129.79	7.50 t (8.0)
6′	130.61	8.05 d (8.0)	130.62	8.05 d (8.0)
7′	167.33	—	167.36	—
	3-Ara		3-Xyl	
1″	107.25	4.30 d (7.2)	107.20	4.32 d (7.2)
2″	72.24	3.73[d)]	74.99	3.37[d)]
3″	84.00	3.64 dd (10.0, 3.2)	87.84	3.47 t (9.0)
4″	69.66	4.03[d)]	70.16	3.57 m
5″	66.81	3.55 d (12.0)/3.85[d)]	66.47	3.22 t (13.0)/3.88[d)]
	3″-Glc		3″-Glc	
1‴	105.53	4.56 d (7.5)	105.26	4.57 d (7.5)
2‴	75.47	3.30[d)]	75.63	3.28[d)]
3‴	77.80	3.39 t (8.0)	77.96	3.38[d)]
4‴	71.33	3.35[d)]	71.75	3.27[d)]
5‴	78.04	3.29[d)]	78.31	3.32[d)]
6‴	62.52	3.69[d)] 3.83[d)]	62.80	3.63 dd (13.0, 6.5) 3.88[d)]

a) Measured in CD_3OD, *b*) 226 MHz, *c*) 900 MHz, *d*) overlapped signals, assignments were done by HMQC, HMBC, ^1H–^1H COSY and ROESY experiments.

correlation (HMQC) experiment (Table 1). The extensive NMR spectral analyses indicated an olean-12-ene aglycon skeleton, that contained typical signals of seven methyl (δ 16.31, 17.14, 17.44, 27.65, 28.08, 28.64, 34.48), two olefinic (δ 124.41, 143.61), and four oxygen-bearing carbons (δ 64.86, 69.60, 72.20, 90.50). Besides, the two hydroxyl groups were at C-3 and C-28, and the other two groups were identified at C-16 and C-22 by means of the HMBC and ^1H–^1H correlation spectroscopy (COSY) experiments (Fig. 2). The presence of a benzoyl group was indicated by signals at δ 132.31 (C-1′), 130.61 (C-2′, C-6′), 129.79 (C-3′, C-5′), 134.24 (C-4′), and 167.33 (C-7′),[3] observed in the ^{13}C-NMR spectrum of **1**. The attached position of the benzoyl group at C-22 was defined by the HMBC cross peak between proton H-22 at δ 5.92 (1H, t, J=4.0 Hz) and carbon C-7′ at δ 167.33. In addition, the ^{13}C-NMR spectrum of **1** exhibited eleven signals of a disaccharide moiety at δ 107.25 (C-1″), 72.24 (C-2″), 84.00 (C-3″), 69.66 (C-4″), 66.81 (C-5″), 105.53 (C-1‴), 75.47 (C-2‴), 77.80 (C-3‴), 71.33 (C-4‴), 78.04 (C-5‴), and 62.52 (C-6‴). These data, together with two anomeric proton signals at δ 4.30 (1H, d, J=7.2 Hz, H-1″) and 4.56 (1H, d, J=7.5 Hz, H-1‴), were indicative of the presence of a β-D-glucopyranosyl-(1→3)-α-L-arabinopyranoside structure,[4] which was further confirmed by the HMBC and ^1H–^1H COSY experiments (Fig. 2), and by acid hydrolysis of **1** (see Experimental). The HMBC cross peaks from the anomeric proton H-1″ (δ 4.30) to carbon C-3 (δ 90.50) and H-3 (δ 3.17) to C-1″ (δ 107.25) confirmed the attached position at C-3 of the disaccharide moiety. From all the above evidence, the planar structure of **1** was obviously assigned as shown in Fig. 1. The stereochemistry of **1** was established by comparison of its ^1H- and ^{13}C-NMR chemical shifts and the coupling constants with those of similar compounds,[5] and further confirmed by a rotating frame Overhauser effect spectroscopy (ROESY) experiment. The resonance of proton H-3 at δ 3.17 (1H, dd, J=11.0, 4.0 Hz) is typical for its usual α-configuration,[5] which was further confirmed by ROEs correlations from H-3 to H-23 (δ 1.08) and H-5 (δ 0.82). The configuration of the hydroxyl group at C-16 was defined as β-orientation by comparison of the ^{13}C-NMR chemical shifts at C-15, C-16, and C-17 of **1** with the corresponding values of maelaxin A,[5] maetenoside A,[6] and alternoside XI.[7] Moreover, the H-16 proton signal at δ 4.32 (dd, J=11.2, 4.8 Hz) suggested its α-orientation,[8] which was confirmed by the spatial proximity observed between H-16 and H-27 (δ 1.31) from the ROESY. Proton H-22 (δ 5.92) showed ROEs correlation with H-16 (δ 4.32) and did not show any correlation with protons H-18 (δ 2.46) and H-30 (δ 1.04), clearly indicating a β-orientation of the hydroxyl group at C-22 (Fig. 3).[6] Thus, the structure of **1** was determined to be a new compound 22β-benzoyloxy-3β,16β,28-trihydroxyolean-12-ene 3-*O*-[β-D-glucopyranosyl-(1→3)-α-L-arabinopyranoside] (**1**), which was named as glochierioside A.

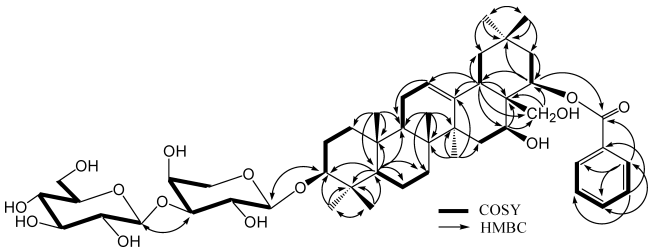

Fig. 2. Selected HMBC and ^1H–^1H COSY Correlations of **1**

Compound **2** was also obtained as an amorphous white powder. Its IR spectrum showed typical absorptions of hy-

droxyl (3414 cm^{-1}), carbonyl (1717 cm^{-1}), and ether (1076, 1025 cm^{-1}) functionalities. The molecular formula was identified to be the same as that of **1** by HR-FAB-MS [Found *m/z*: 895.4860 $[M+Na]^+$ (Calcd for $C_{48}H_{72}O_{14}Na$: 895.4820)]. This data, together with the excellent agreement of the NMR spectral data between the two compounds (Table 1), indicated **1** to be a derivative of **2**. The differences between the two compounds were only observed in the NMR spectral data of the disaccharide moieties. In the ^{13}C-NMR spectrum of **2**, the signals at δ 105.26 (C-1‴), 75.63 (C-2‴), 77.96 (C-3‴), 71.75 (C-4‴), 78.31 (C-5‴), and 62.80 (C-6‴) were similar to those of **1**, indicating the presence of a β-D-glucopyranosyl unit. The remaining signals of the other saccharide moiety at δ 107.20 (C-1″), 74.99 (C-2″), 87.84 (C-3″), 70.16 (C-4″), and 66.47 (C-5″) were typical of a β-xylopyranosyl unit having an ether linkage at C-3″,[9] which was further confirmed by the HSQC, HMBC, and ^1H–^1H COSY experiments, and by acid hydrolysis of **2** (see Experimental). Accordingly, the structure of 22β-benzoyloxy-3β,16β,28-trihydroxyolean-12-ene 3-*O*-[β-D-glucopyranosyl-(1→3)-β-D-xylopyranoside] was deduced for **2**, a new compound named as glochierioside B.

Compounds **3**—**5** were identified as glochidone,[10] lup-20(29)-en-3β,23-diol,[11] and lup-20(29)-en-1β,3β-diol,[12] respectively, by comparison of the NMR and mass spectral data with the literature values.

Compounds **1**—**5** were evaluated for their cytotoxic activity against HL-60, HT-29, MCF-7, and SK-OV-3 human cancer cell lines, after continuous exposure for 72 h (Table 2). Compounds **1** (glochierioside A) and **2** (glochierioside B) showed significant cytotoxic activity against HL-60, HT-29, MCF-7, and SK-OV-3 cells with IC_{50} values from 5.5 to 36 μM. Compound **4** (lup-20(29)-en-3β,23-diol) showed growth inhibitory effects with IC_{50} values of 13.1, 71.7, and 46.1 μM against HL-60, HT-29 cells, and MCF-7 cell lines, respectively. Compound **5** (lup-20(29)-en-1β,3β-diol) showed less effects with IC_{50} values of 43.3, 67.0, 66.1, and 48.0 μM against HL-60, HT-29, MCF-7, and SK-OV-3 cell lines, respectively. However, compound **3** (glochidone) was ineffective against four tested cell lines even when treated at concentration of 100 μM.

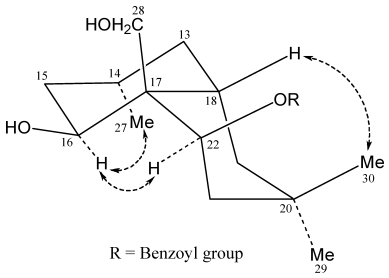

Fig. 3. Probable Configuration of D and E Rings and Key ROEs Correlations of **1** and **2**

Table 2. The Effects of Triterpenes on the Growth of Human Cancer Cells

Compounds	IC_{50} [μM][a]			
	HL-60 (Leukemia)	HT-29 (Colon)	MCF-7 (Breast)	SK-OV-3 (Ovary)
1	5.5	6.8	29.1	22.7
2	6.6	18.6	36.1	16.0
3	>100	>100	>100	>100
4	13.7	71.7	46.1	>100
5	43.3	67.0	66.1	48.0
MX[b]	7.2	8.4	10.3	12.1

a) IC_{50} (concentration that inhibits 50% of cell growth). Compounds were tested at a maximum concentration of 100 μM. Data are presented as the mean of experiments performed in triplicate. *b*) Mitoxantrone (MX), an anticancer agent, was used as reference compound.

Experimental

Optical rotations were determined on a Jasco DIP-370 digital polarimeter. The electrospray ionization (ESI) mass spectra were obtained using an AGILENT 1200 LC-MSD Trap spectrometer. The HR-FAB mass spectra were obtained using a JEOL JMS-AX700 spectrometer. The ^1H-NMR (900 MHz) and ^{13}C-NMR (226 MHz) spectra were recorded on a Bruker AM900 FT-NMR spectrometer and TMS was used as an internal standard. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70—230 mesh and 230—400 mesh, Merck) and YMC RP-18 resins.

Plant Material The aerial parts of *G. eriocarpum* were collected at Tamdao National Botanical Park, Vinhphuc, Vietnam during December 2006 and identified by Dr. Tran Huy Thai, Institute of Ecology and Biological Resources, VAST, Vietnam. An authentic sample (N° VP14) was deposited at the herbarium of the Institute of Natural Products Chemistry, VAST, Vietnam.

Extraction and Isolation The dried aerial parts of *G. eriocarpum* (7.0 kg) were powdered and extracted three times with hot MeOH (50 °C) to give the methanol extract (500 g), which was then suspended in water and extracted in turn with *n*-hexane, chloroform, and ethyl acetate, giving corresponding extracts with amounts as 10 g, 113 g, and 56 g, respectively, and water layer. The *n*-hexane (10 g) and chloroform (113 g) extracts were combined and crudely separated on a silica gel CC using stepwise gradient elution with the solvents *n*-hexane–acetone (20 : 1, 10 : 1, 5 : 1, 2.5 : 1, 1 : 1, and 0 : 1, v/v) to yield six sub-fractions, F1—F6. Sub-fraction F2 was chromatographed on a silica gel column eluting with chloroform–*n*-hexane (2 : 1, v/v) to obtain compound **3** (63 mg, white needles). Sub-fraction F3 was further chromatographed on a silica gel column eluting with *n*-hexane–ethyl acetate (2.5 : 1, v/v) to give five smaller fractions, F3—F3E. Fraction F3B was chromatographed on a YMC RP-18 column eluting with acetone–water (5 : 1, v/v) to yield **4** (20 mg, white wax). Fraction F3E was chromatographed on a silica gel column using chloroform–acetone (5 : 1, v/v) as an eluent to obtain compound **5** (10 mg, white needles). Sub-fraction F6 was further separated on a YMC RP-18 column eluting with acetone–methanol–water (1 : 2 : 1, v/v/v) to give four fractions F6A—F6D. The new compound **2** (7.5 mg, amorphous white powder) was purified from fraction F6A through chromatography on a YMC RP-18 column eluting with acetone–methanol–water (1 : 3 : 1, v/v/v). The ethyl acetate extract (56 g) was directly separated on a silica gel column using stepwise gradient elution with chloroform–methanol (20 : 1, 10 : 1, 5 : 1, 2.5 : 1, and 0 : 1, v/v) to obtain five sub-fractions F7—F11. Fraction F9 was further separated on a silica gel column eluting with chloroform–methanol (4 : 1, v/v) to give the new compound **1** (30 mg) as an amorphous white powder.

Glochierioside A (**1**): Amorphous white powder, $[\alpha]_D^{30}$ +17° (*c*=1.00, MeOH); IR (KBr) cm^{-1}: 3392 (OH), 2954 (CH), 1709 (C=O), 1649 (aromatic ring), 1073 and 1025 (C–O–C); positive ESI-MS *m/z*: 873 $[M+H]^+$, 895 $[M+Na]^+$; HR-FAB-MS *m/z*: 895.4826 $[M+Na]^+$ (Calcd for $C_{48}H_{72}O_{14}Na$: 895.4820); ^1H- and ^{13}C-NMR are given in Table 1.

Glochierioside B (**2**): Amorphous white powder, $[\alpha]_D^{30}$ +39° (*c*=1.00, MeOH); IR (KBr) cm^{-1}: 3414 (OH), 2922 (CH), 1717 (C=O), 1648 (aromatic ring), 1076 and 1025 (C–O–C); positive ESI-MS *m/z*: 873 $[M+H]^+$, 895 $[M+Na]^+$; HR-FAB-MS *m/z*: 895.4860 $[M+Na]^+$ (Calcd for $C_{48}H_{72}O_{14}Na$: 895.4820); ^1H- and ^{13}C-NMR are given in Table 1.

Acid Hydrolysis of 1 and 2 Compounds **1** and **2** (2 mg, each) were dissolved in 1 N HCl (dioxane–H_2O, 1 : 1, 1 ml) and heated to 80 °C in a water bath for 3 h. The acidic solution was neutralized with silver carbonate and the solvent thoroughly driven out under N_2 gas overnight. After extraction with $CHCl_3$, the aqueous layer was concentrated to dryness using N_2 gas. The residue was dissolved in 0.1 ml of dry pyridine, followed by addition of L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 ml). The reaction mixture was heated at 60 °C for 2 h. Trimethylsilylimidazole solution (0.1 ml) was then added, followed by heating at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and water (0.1 ml each), and the organic layer was analyzed by gas chromatography (GC): column SPB-1

(0.25 mm×30 m), detector FID, column temp 210 °C, injector temp 270 °C, detector temp 300 °C, carrier gas He (30 ml/min). Under these conditions, standard sugars gave peaks at t_R (min) 8.55 and 9.25 for D- and L-glucose, 4.72 and 9.16 for D- and L-arabinose, and 4.02 and 9.17 for D- and L-xylose, respectively. Peaks at t_R (min) 8.55 and 9.16 of D-glucose and L-arabinose for **1**, and 8.55 and 4.02 of D-glucose and D-xylose for **2**, respectively, were observed.

Cytotoxicity Tests The effects of compounds **1**—**5** on the growth of human cancer cells were determined by measuring metabolic activity using a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay.[13] Four human cancer cell lines were used, HL-60 (human acute promyeloid leukemia), HT-29 (human colon cancer), MCF-7 (human breast cancer), and SK-OV-3 (human ovarian cancer). The cell lines were obtained from the Korea Cell Line Bank (KCLB) and grown in RPMI 1640 medium supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 U/ml and 100 g/ml, respectively) at 37 °C in a humidified 5% CO_2 atmosphere. The exponentially growing cells were used throughout the experiments.

The MTT assays were performed as follows: human cancer cell lines ($1.5—2.5\times10^5$ cells/ml) were treated for 3 d with 1, 10, 30, and 100 μM of the compounds. After incubation, 0.1 mg (50 μl of a 2 mg/ml solution) MTT (Sigma, Saint Louis, MO, U.S.A.) was added to each well and the cells were then incubated at 37 °C for 4 h. The plates were centrifuged at 1000 rpm for 5 min at room temperature and the media was then carefully aspirated. Dimethylsulfoxide (150 μl) was then added to each well to dissolve the formazan crystals. The plates were read immediately at 540 nm on a microplate reader (Amersham Pharmacia Biotech., U.S.A.). All the experiments were performed three times with the mean absorbance values calculated. The results were expressed as the percentage of inhibition that produced a reduction in the absorbance by the treatment of the compounds compared to the untreated controls. A dose–response curve was generated and the inhibitory concentration of 50% (IC_{50}) was determined for each compound as well as each cell line. Mitoxantrone (MX), an anticancer agent, was used as positive control.

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References

1) Bich D. H., Chung D. Q., Chuong B. X., Dong N. T., Dam D. T., Hien P. V., Lo V. N., Mai P. D., Man P. K., Nhu D. T., Tap N., Toan T., "The Medicinal Plants and Animals in Vietnam," Hanoi Science and Technology Publishing House, Hanoi, 2004, pp. 224—225.
2) Puapairoj P., Naengchomnong W., Kijjoa A., Pinto M. M., Pedro M., Nascimento M. S., Silva A. M., Herz W., *Planta Med.*, **71**, 208—213 (2005).
3) Ye W., Liu X., Zhang Q., Che C. T., Zhao S., *J. Nat. Prod.*, **64**, 232—235 (2001).
4) Chakravarty A. K., Garai S., Masuda K., Nakane T., Kawahara N., *Chem. Pharm. Bull.*, **51**, 215—217 (2003).
5) Jiang Z., Gallard J. F., Adeline M. T., Dumontet V., Tri M. V., Sévenet T., Païs M., *J. Nat. Prod.*, **62**, 873—876 (1999).
6) Koike K., Jia Z., Kikaido T., *Chem. Pharm. Bull.*, **49**, 758—761 (2001).
7) Yoshikawa K., Takahashi K., Matsuchika K., Arihara S., Chang H. C., Wang J. D., *Chem. Pharm. Bull.*, **47**, 1598—1603 (1999).
8) Fei D. Q., Wu G., Liu C. M., Gao K., *Chem. Pharm. Bull.*, **55**, 577—579 (2007).
9) Gonzalez-Laredo R. F., Chen J., Karchesy Y. M., Karchesy J. J., *Nat. Prod. Res.*, **13**, 75—80 (1999).
10) Tanaka R., Matsunaga S., *Phytochemistry*, **27**, 2273—2277 (1988).
11) Peraza-Sánchez S. R., Salazar-Aguilar N. E., Pena-Rodríguez L. M., *J. Nat. Prod.*, **58**, 271—274 (1995).
12) Savona G., Bruno M., Rodriguez B., Marco J. L., *Phytochemistry*, **26**, 3305—3308 (1987).
13) Carmichael J., DeGraff W. G., Gazdar A. F., Minna J. D., Mitchell J. B., *Cancer Res.*, **47**, 943—946 (1987).