Bioorganic & Medicinal Chemistry Letters 20 (2010) 7110–7115

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Oleanane-type triterpenoids from *Panax stipuleanatus* and their anticancer activities

Chun Liang [a], Yan Ding [a], Huu Tung Nguyen [a], Jeong-Ah Kim [a], Hye-Jin Boo [b], Hee-Kyoung Kang [b], Mahn Cuong Nguyen [c], Young Ho Kim [a,*]

[a] College of Pharmacy, Chungnam National University, Daejeon, Republic of Korea
[b] Department of Pharmacology, School of Medicine, Institute of Medical Sciences, Jeju National University, Jeju, Republic of Korea
[c] Institute of Natural Product Chemistry, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Caugiay, Hanoi, Viet Nam

ARTICLE INFO

Article history:
Received 1 June 2010
Revised 25 August 2010
Accepted 14 September 2010
Available online 21 September 2010

Keywords:
Panax stipuleanatus
Oleanane-type triterpenoid
Apoptosis
ERK1/2

ABSTRACT

One newly (**1**) and 10 known oleanane-type triterpenoids (**2**–**11**) were isolated from the methanol extract of *Panax stipuleanatus* rhizomes. Based on their spectroscopic data, these compounds were identified as spinasaponin A methyl ester (**1**), pesudoginsenoside RP_1 methyl ester (**2**), spinasaponin A 28-*O*-glucoside (**3**), pseudoginsenoside RT_1 methyl ester (**4**), pseudoginsenoside RT_1 (**5**), stipuleanoside R_2 methyl ester (**6**), stipuleanoside R_2 (**7**), araloside A methyl ester (**8**), 3-*O*-β-D-glucopyranosyl (1→3)-β-D-glucuronopyranoside-28-*O*-β-D-glucopyranosyl oleanolic acid methyl ester (**9**), 3-*O*-β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl-28-*O*-β-D-glucopyranosyl oleanolic acid (**10**), and chikusetsusaponin IVa (**11**). When the cytotoxic activities of the isolated compounds were evaluated, compound **1** exhibited significant cytotoxic activity with IC_{50} values of 4.44 and 0.63 μM against HL-60 (leukemia) and HCT-116 (colon cancer) cell lines, respectively. Compound **2** showed potent cytotoxicity with an IC_{50} of 6.50 μM against HCT-116, whereas it was less cytotoxic against HL-60 (IC_{50} = 41.45 μM). After HL-60 and HCT-116 were treated with compounds **1** and **2**, increased production of apoptotic bodies was observed. Furthermore, compounds **1** and **2** in HCT-116 cells activated intrinsic and extrinsic apoptosis pathways by upregulating DR-5 and Bax, downregulating Bcl-2, activating caspase-9, and cleaving poly-ADP-ribose polymerase (PARP). We also observed the activation of ERK1/2 MAPK by both compounds in the HCT-116 cells. Together, compounds **1** and **2** might induce intrinsic and extrinsic apoptosis pathways through the activation of the ERK1/2 MAPK pathway in HCT-116 colon cancer cells. Structure–activity relationship analysis indicated that a carboxyl group at position-28 is potentially responsible for the cytotoxic effects.

Panax stipuleanatus Tsai et Feng (Araliaceae) is an herb that grows in Southeast Yunnan, China, and North Vietnam. In China, this plant has traditionally been used as a tonic and in the treatment of bruises, bleeding, and muscular pain.[1] However, its chemical constituents and biological activities have not been studied in depth. Up until now, oleanane-type triterpenoids have been reported to be the major components of *P. stipuleanatus*[1] and only stipuleanoside R_1 and R_2 was isolated from the methanol extract of this plant.[2] Recently, a biological research on antitumor activity of oleanane-type triterpenoids has been studied.[3]

In our ongoing study to find biologically active compounds from medicinal plants, the rhizomes of *P. stipuleanatus* were collected in Vietnam in 2007 and taxonomically identified by Dr. Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnam. The reference specimen (VHKC-0044) has been deposited at the Institute of Natural Products Chemistry, VAST, Vietnam. The rhizomes of *P. stipuleanatus* (2 kg) were extracted with MeOH at room temperature for 1 day (10 L × 3 times). Evaporation of the combined MeOH extract in vacuo gave a residue (200 g), which was then suspended in H_2O (3 L) and extracted with EtOAc (3 L × 3 times) to give 4 g of an EtOAc-soluble fraction and 146 g of an H_2O-soluble fraction. The H_2O fraction was loaded onto a Diaion HP-20 column and eluted with MeOH–H_2O (0%, 25%, 50%, 75%, and 100% MeOH) to give five fractions (Fr. 1A–1E). Fractions 1D and 1E were combined due to their similar TLC pattern and then separated chromatographically through a silica gel column using a CH_2Cl_2–MeOH (10:1–0:1) gradient to give five fractions (Fr. 3A–3E). Fraction 3B was re-chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (5:1:0.1) to give six sub-fractions (Fr. 4A–4F). Fraction 4D was separated by preparative HPLC eluted with an acetonitrile–water (40:60, 55:45) and gave compounds **1** (44 mg), **2** (15 mg), **3** (41 mg) and **4** (32 mg). Fraction 3C was re-chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (6:1:0.5–4:1:0.5) to give eight sub-fractions (Fr. 5A–5H). Fraction 5H was purified using a YMC RP-18 column (acetone–H_2O, 1:1) to give **6** (40 mg) and **7** (320 mg). The EtOAc fraction was chromatographed on a YMC RP-18 column (acetone–H_2O, 1:1) to give three fractions (Fr. 2A–2C). Fraction 2C was

* Corresponding author. Tel.: +82 42 821 5933; fax: +82 42 823 6566.
E-mail address: yhk@cnu.ac.kr (Y.H. Kim).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.09.074

Table 1
^{13}C NMR data of the compounds **1**–**11** in CD_3OD (δ)

Carbon	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[b]	**6**[b]	**7**[b]	**8**[b]	**9**[b]	**10**[b]	**11**[b]
Aglycon											
C–1	39.8	39.9	39.8	39.5	39.9	39.8	39.8	39.7	39.7	40.1	39.8
2	27.1	27.2	26.9	27.0	27.2	26.9	26.9	26.9	26.9	26.9	26.8
3	91.3	91.4	91.1	91.2	91.3	91.2	90.7	91.2	91.1	90.9	90.8
4	40.3	40.4	40.1	40.2	40.4	40.2	40.1	40.1	40.1	40.3	40.1
5	57.1	57.2	57.0	57.1	57.2	57.0	57.0	57.0	57.0	57.1	57.0
6	19.4	19.4	19.2	19.3	19.4	19.3	19.3	19.3	19.3	19.3	19.3
7	34.1	33.7	33.9	33.9	34.0	33.9	33.9	33.9	33.9	33.9	33.9
8	40.7	40.7	40.6	40.7	40.8	40.7	40.7	40.7	40.7	40.7	40.7
9	49.1	49.1	49.0	49.1	49.1	49.1	49.1	49.1	49.1	49.1	49.1
10	38.0	38.0	37.8	37.8	37.9	37.8	37.8	37.8	37.8	37.8	37.8
11	24.6	24.1	24.5	24.5	24.6	24.5	24.5	24.5	24.5	24.5	24.5
12	123.8	123.8	123.7	123.7	123.9	123.7	123.8	123.8	123.8	123.8	123.8
13	145.3	145.3	144.8	144.8	144.9	144.8	144.7	144.8	144.8	144.8	144.8
14	43.0	43.0	42.9	42.9	42.7	42.9	42.9	42.9	42.9	42.9	42.9
15	29.0	29.0	28.8	28.8	29.0	28.8	28.8	28.9	28.9	28.9	28.9
16	24.1	24.2	24.0	23.9	24.0	23.9	23.9	23.9	23.9	23.7	23.8
17	47.8	47.8	47.9	48.0	48.1	47.9	48.0	48.0	48.0	48.0	48.0
18	42.9	42.9	42.5	42.5	42.7	42.5	42.5	42.6	42.6	42.6	42.6
19	47.4	47.4	47.2	47.2	47.3	47.2	47.2	47.2	47.2	47.2	47.2
20	31.7	31.7	31.5	31.5	31.6	31.5	31.5	31.5	31.5	31.5	31.5
21	35.0	35.0	34.8	34.8	35.0	34.8	34.9	34.9	34.9	34.9	34.9
22	33.7	33.9	33.0	33.1	33.2	33.1	33.1	33.1	33.1	33.1	33.1
23	28.6	28.3	28.5	28.2	28.3	28.4	28.5	28.4	28.4	28.2	28.5
24	17.1	16.7	16.9	16.5	16.6	17.0	17.0	16.9	16.9	16.5	17.0
25	16.0	16.0	16.0	16.0	16.1	16.0	16.0	15.9	15.9	16.0	16.0
26	17.8	17.9	17.7	17.7	17.8	17.7	17.7	17.7	17.7	17.7	17.7
27	26.5	26.5	26.4	26.4	26.4	26.4	26.3	26.2	26.3	26.2	26.2
28	181.9	182.0	177.9	178.0	178.1	178.0	178.0	178.0	178.0	178.0	178.1
29	33.9	34.1	33.5	33.5	33.5	33.5	33.4	33.4	33.4	33.4	33.4
30	24.2	24.6	24.0	23.9	24.1	23.9	24.0	23.9	23.9	23.9	23.9
Sugar(C–3) GlcUA or Glc											
1	106.7	105.7	106.6	105.5	105.6	106.5	106.3	106.9	105.1	105.2	106.6
2	74.8	82.9	74.6	82.7	82.8	76.1	76.4	73.9	74.7	83.3	75.5
3	86.8	77.7	86.5	76.3	76.4	81.5	81.9	75.1	86.6	78.1	78.0
4	71.7	73.1	71.7	72.9	73.0	79.1	79.3	78.7	71.5	71.1	73.7
5	76.4	77.9	76.2	77.4	77.6	75.2	78.1	75.9	76.3	73.5	76.5
6	171.2	171.3	171.0	171.2	171.3	171.2	176.5	171.1	171.1	62.4	176.9
OCH_3	52.9	52.9		52.8		53.1		53.0	52.8		
Xyl (1→2) GlcUA											
1		106.4		106.2	106.3					106.3	
2		76.4		76.2	76.3					76.2	
3		76.5		77.7	77.8					77.7	
4		71.3		71.1	71.2					71.1	
5		67.3		67.1	67.2					67.1	
Glc–I (1→3)GlcUA											
1	105.3		105.0			104.5	104.4		106.6		
2	75.6		75.4			75.5	75.5		75.6		
3	77.9		78.1			77.9	78.3		78.2		
4	71.9		71.5			71.0	71.0		71.1		
5	78.3		77.6			78.1	78.3		77.7		
6	62.8		62.6			62.4	63.2		62.6		
Ara(f)(1→4)GlcUA											
1						108.2	108.3	109.2			
2						82.3	82.1	82.9			
3						74.6	75.3	78.5			
4						86.8	87.1	86.6			
5						62.9	62.4	62.4			
C–28 Glc–II											
1			95.6	95.6	95.8	95.6	95.7	95.7	95.7	95.7	95.7
2			73.8	73.9	74.0	73.9	73.9	75.0	73.9	73.9	73.9
3			78.2	78.2	78.4	78.2	77.9	78.4	78.6	78.3	78.3
4			71.0	71.1	71.2	71.0	71.4	71.1	71.7	71.1	71.2
5			78.6	78.6	78.7	78.6	78.6	78.3	78.3	78.6	78.7
6			62.4	62.4	62.5	62.2	62.2	63.0	62.4	62.4	62.5

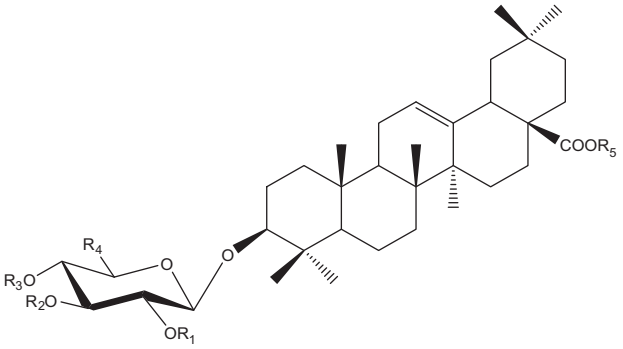
[a] Recorded at 100 MHz.
[b] Recorded at 150 MHz.

re-chromatographed on a silica gel column eluted with $CHCl_3$–MeOH–H_2O (6:1:0.05–2.5:1:0.15) to give 12 sub-fractions (Fr. 6A–6Q). Fractions 6E, 6G, 6M, and 6O were separated by preparative TLC with *n*-BuOH–CH_3COOH–H_2O (4:1:5) (higher phase) elution solvent and gave compound **5s** (11 mg), **8** (6 mg), **9** (11 mg), **10** (16 mg), and **11** (10 mg). Eleven compounds were identified by comparing their physical and spectroscopic data with those reported in Figure 1.

Table 2
Effects of compounds **1**–**11** on the growth of HL-60 and HCT-116 human cancer cells

Compound	IC_{50}[a] (μM)	
	HL-60 (leukemia)	HCT-116 (colon)
1	4.44	0.63
2	41.45	6.50
3	72.99	>100
4	>100	>100
5	91.16	>100
6	67.08	>100
7	>100	>100
8	63.44	>100
9	83.90	>100
10	>100	75.94
11	76.23	78.11
Oleanolic acid	77.48	4.80
Mitoxantrone[b]	7.5	3.96

[a] Results are the means ± SD of three independent experiment in triplicate, and values <100 μM are considered to be active.
[b] Positive control.



	R_1	R_2	R_3	R_4	R_5
1	H	Glc I	H	$COOCH_3$	H
2	Xyl	H	H	$COOCH_3$	H
3	H	Glc I	H	COOH	Glc II
4	Xyl	H	H	$COOCH_3$	Glc II
5	Xyl	H	H	COOH	Glc II
6	H	Glc I	Ara(f)	$COOCH_3$	Glc II
7	H	Glc I	Ara(f)	COOH	Glc II
8	H	H	Ara(f)	$COOCH_3$	Glc II
9	H	Glc I	H	$COOCH_3$	Glc II
10	Xyl	H	H	CH_2OH	Glc II
11	H	H	H	COOH	Glc II

Ara(*f*): α-L-arabinofuranosyl
Xyl: β-D-xylopyranosyl
Glc: β-D-glucopyranosyl

Figure 1. Structures of compounds **1**–**11** isolated from *P. stipuleanatus*.

Spinasaponin A methyl ester (**1**) was obtained as a white powder, mp 261–263 °C, $[\alpha]_D20$ +14.30 (*c* 0.6 MeOH) and gave positive result for the Liebermann-Burchard test. Its negative ESI-MS gave a quasi-molecular ion peak at *m*/*z* 807 $[M–H]^-$, corresponding to the molecular formula of $C_{43}H_{68}O_{14}$. Further fragment ion peaks were observed in the ESI-APCI-MS spectrum at *m*/*z* 645 $[M–H–162]^-$, 455 $[M–H–162–190]^-$ corresponding to the successive loss of glucosyl and glucuronic acid methyl ester moiety. This result suggested that saponin **1** contained two sugar units, one of them being a glucuronic acid methyl ester and another was glucose attached at terminal site. The acid hydrolysis of **1** liberated D-glucose as confirmed in a gas chromatography (GC) experiment.[4] Its ^1H NMR spectrum showed seven angular methyl signals at δ_H 1.11 (3H, s), 1.00 (3H, s), 0.89 (3H, s), 0.89 (3H, s), 0.86 (3H, s), 0.79 (3H, s), and 0.76 (3H, s), one olefinic proton at δ_H 5.19 (1H, t, *J* = 4.0 Hz), and sugar proton signals at δ_H 3.0–4.6. The ^{13}C NMR spectrum (Table 1) revealed seven methyl signals at δ_C 16.0, 17.1, 17.8, 24.2, 26.5, 28.6, and 33.9, and a pair of olefinic carbon signals at δ_C 123.8 and 145.3, suggesting that **1** was an oleanane-type triterpenoidal saponin. Anomeric signals in the ^{13}C NMR spectrum indicated the presence of a glucuronic acid signal at δ_C 106.7 and a glucose signal at δ_C 105.3 in **1**. Two anomeric proton signals in the ^1H NMR spectrum of **1** were observed at δ_H 4.52 (1H, d, *J* = 8.0 Hz) and 4.39 (1H, d, *J* = 8.0 Hz), indicating that the glucuronyl and glucosyl moieties were all linked in β configuration. The C-3 was shifted down-field to δ_C 91.3, while the signal for C-28 was not, suggesting that the sugar chain is located at the C-3 position of the aglycone. The ^1H and ^{13}C NMR spectra of **1** were almost superimposable with those of spinasaponin A,[5,6] except that the former showed additional *O*-methyl signals at δ_H 3.72 (3H, s) and δ_C 52.9, suggesting that **1** was a methyl ester derivative of spinasaponin A. The *O*-methyl group was located at the C-6 carboxyl group of a glucuronopyranoside moiety, since **1** exhibited similar ^{13}C NMR signals at C-4′, C-5′, C-6′, and for an *O*-methyl group, consistent with the corresponding signals of **9**. Hence, the structure of **1** was determined to be that of spinasaponin A methyl ester [oleanolic acid 3-*O*-{β-D-glucopyranosyl-(1→3)-β-D-glucuronopyranoside}methyl ester]. Compound **1** was previously synthesized from taibaienoside VI on partial methanolysis,[5] but no information on its structure was reported. From all the above information, we concluded that compound **1** was isolated for the first time from nature. Compound **1** has been checked with 70% EtOH extract (70% EtOH 100 ml at room temperature for 1 day × 3 times), MeOH extract, and two subfractions (Fr. 1D–1E) using TLC method (*n*-BuOH–H_2O–EtOAc = 4:1:5, R_f = 0.42). The presence of identical spots among 70% EtOH extract, MeOH extract, Fr. 1D-1E, and compound **1** was responsible for a natural occurrence of spinasaponin A methyl ester in the rhizomes of *P. stipuleanatus*.

The other known compounds were identified by comparing their physical and spectroscopic data with those reported in the literature. These compounds were determined as pesudoginsenoside RP_1 methyl ester (**2**),[7] spinasaponin A 28-*O*-glucoside (**3**),[8] pseudoginsenoside RT_1 methyl ester (**4**),[7] pseudoginsenoside RT_1 (**5**),[9] stipuleanoside R_2 methyl ester (**6**),[10] stipuleanoside R_2 (**7**),[11] araloside A methyl ester (**8**),[12] 3-*O*-β-D-glucopyranosyl (1→3)-β-D-glucuronopyranoside-28-*O*-β-D-glucopyranosyl oleanolic acid methyl ester (**9**),[13] 3-*O*-β-D-xylopyranosyl (1→2)-β-D-glucopyranosyl-28-*O*-β-D-glucopyran-osyl oleanolic acid (**10**),[14] and chikusetsusaponin IVa (**11**).[15]

To investigate the anticancer effect of the isolated compounds, the cytotoxic activities of compounds **1**–**11** and oleanolic acid were tested against human acute promyelocytic leukemia cancer (HL-60) and human colon cancer (HCT-116) cells using the 3-(dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as described by Mosmann.[16] Among them, compound **1** showed significant cytotoxic activity with IC_{50} values of 4.44 and 0.63 μM against HL-60 and HCT-116 cells, respectively, while compound **2** showed medium cytotoxic activity with IC_{50} values of 41.45 and 6.50 μM. Compounds **10** and **11** showed moderate cytotoxic activity with IC_{50} values of 75.94 and 78.11 μM against HCT-116 cells, and compounds **3**, **5**, **6**, **8**, **9** and **11** showed moderate cytotoxic activity with IC_{50} values of 72.99, 91.16, 67.08, 63.44, 83.90, 76.23 μM against HL-60 cells, respectively, while others lacked cytotoxic activity up to 100.00 μM. Oleanolic acid showed potent cytotoxic activity with IC_{50} values of 4.80 and 77.48 μM against HCT-116 and HL-60 cells, respectively (Table 2).

In order to elucidate the cytotoxic mechanism, we investigated whether the inhibitory effects of compounds **1** and **2** on the proliferation of HL-60 and HCT-116 cells might arise from the induction

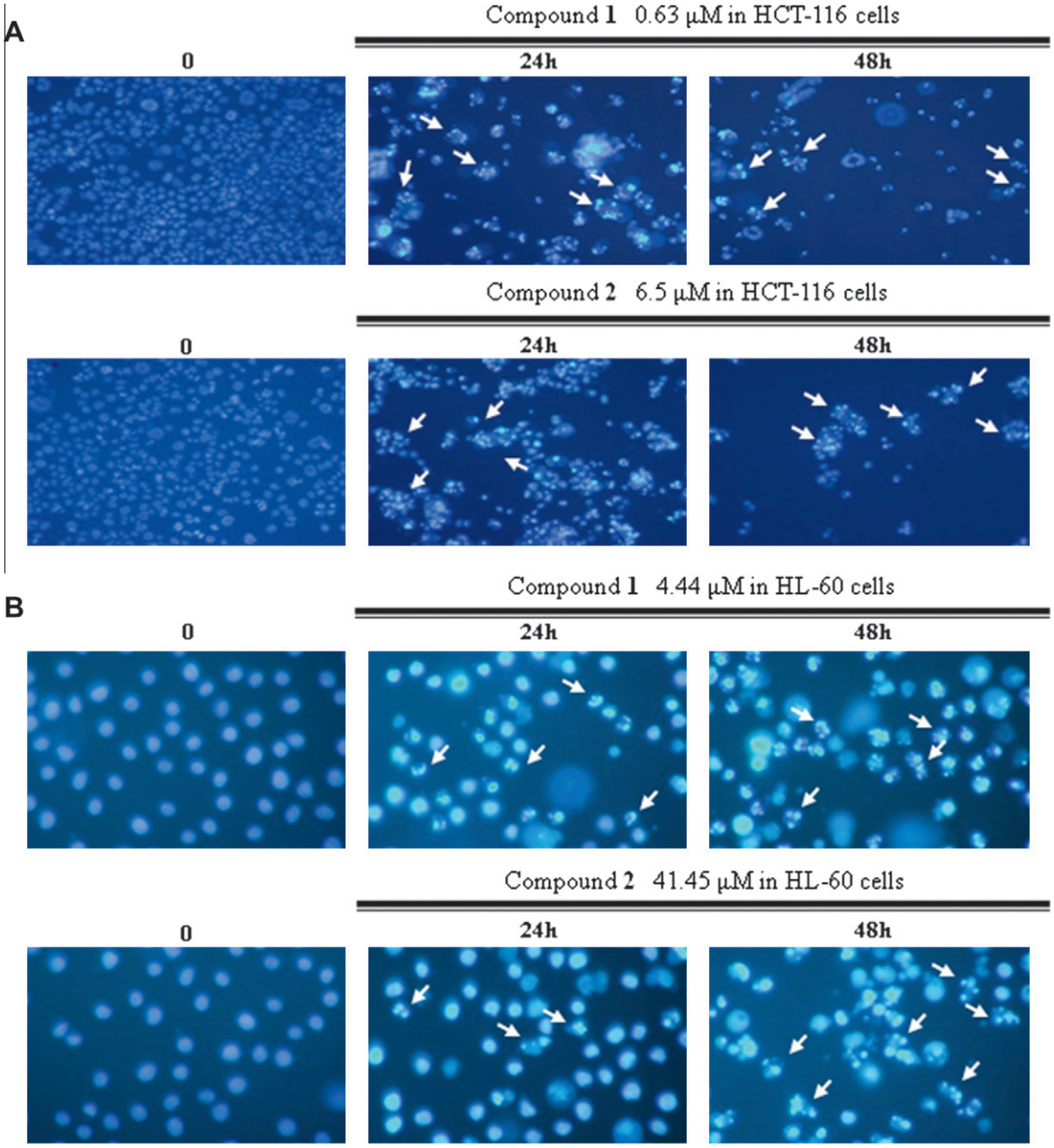


Figure 2. The degree of apoptosis represented as the fluorescent image of nuclei in HL-60 and HCT-116 cells by Hoechst 33342 staining. (A) The HCT-116 cells were treated with 0.63 μM of compound **1** and 6.5 μM of compound **2** for 24 and 48 h. (B) The HL-60 cells were treated with 4.44 μM of compound **1** and 41.45 μM of compound **2** for 24 and 48 h. DNA-specific fluorescent dye, Hoechst 33342 (culture medium at a final concentration of 10 μg/mL) was directly added to media and apoptotic bodies were observed with an inverted fluorescent microscope equipped with an IX-71 Olympus camera and photographed (magnification ×200).

of apoptosis. After HL-60 and HCT-116 cells were treated with the IC_{50} concentration of compounds **1** and **2** for 24 and 48 h, the apoptotic characteristics were examined by Hoechst 33342 staining. Nuclear morphological changes, such as chromatin condensation, membrane blebbing, and cell shrinkage, are critical markers of cell apoptosis. Compounds **1** and **2** induced the production of these apoptotic bodies in Hoechst-stained HCT-116 and HL-60 cells (Fig. 2). The results indicated that compounds **1** and **2** could markedly inhibit the proliferation of HL-60 and HCT-116 cells by inducting apoptosis.

Compounds **1** and **2** exhibited significant cytotoxic activity with an IC_{50} of 0.63 and 6.50 μM, respectively, against HCT-116, rather than HL-60 cells. We selected the HCT-116 cells and investigated the activity mechanisms of compounds **1** and **2** on apoptosis-induction using Western blot analysis.[17] Apoptosis occurs through the interplay of pro-and anti-apoptotic molecules, which eventually leads to the activation of caspases by extrinsic or intrinsic apoptosis pathways.[18] The two compounds increased the expression of DR5, an extrinsic pathway-related protein in HCT-116 cells (Fig. 3A and B). The two compounds also activated the intrinsic pathway through a decrease in Bcl-2, an anti-apoptotic protein, and an increase in Bax, a pro-apoptotic protein (Fig. 3A and B). The two compounds also increased caspase-9 activation and induced PARP cleavage, an effector caspase substrate.

Mitogen-activated protein kinase (MAPK) pathways play critical roles in various biological processes, such as differentiation, cell cycle arrest, proliferation, mitosis, and apoptosis.[19] The ERK MAPK pathway is one of the best understood MAPK signaling pathways.[20] In order to establish the MAPK mechanism of apoptosis induced by compounds **1** and **2** in HCT-116 cells, the expression ERK1/2

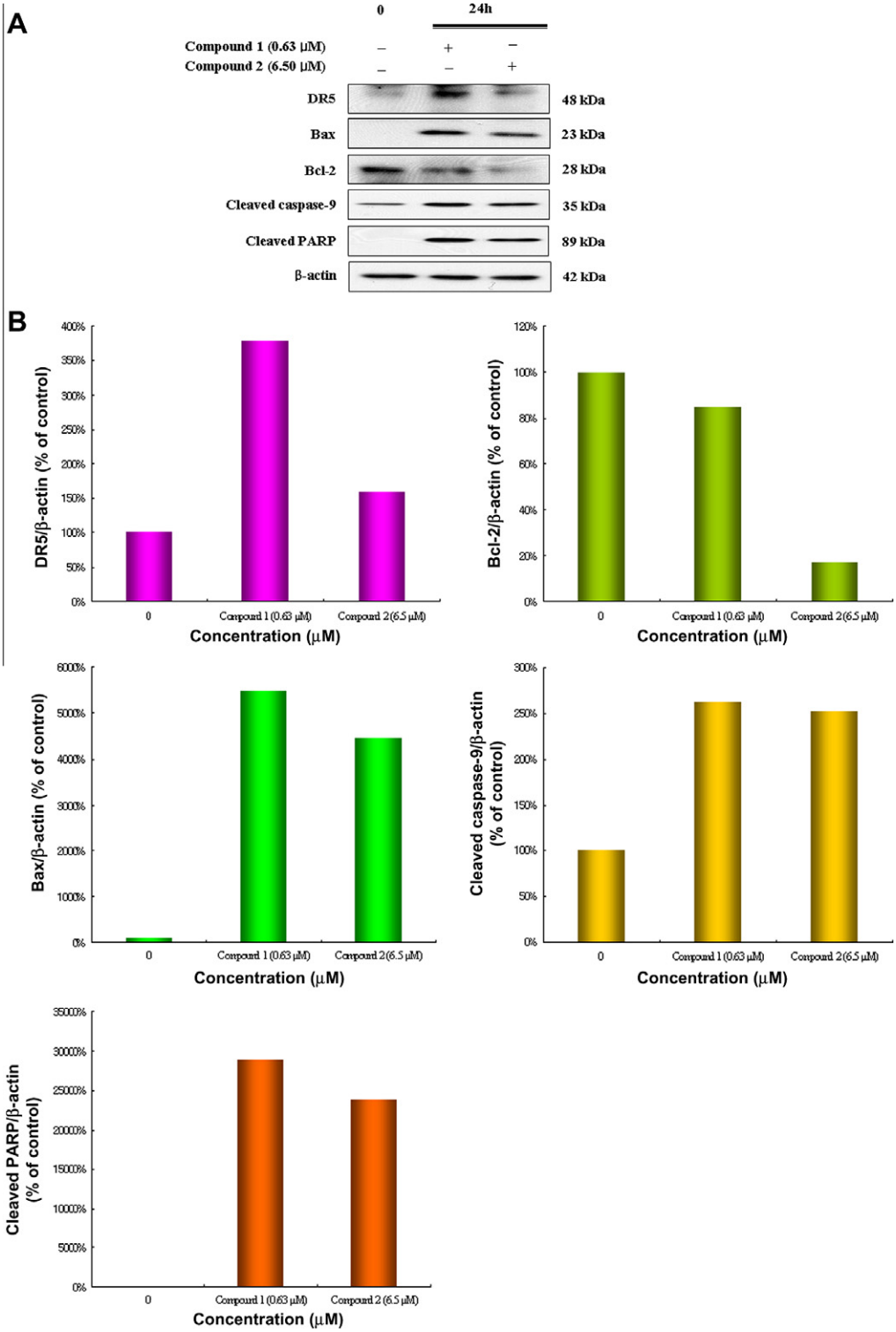


Figure 3. Compounds **1** and **2** induced apoptosis via extrinsic and intrinsic apoptosis pathways in HCT-116 cells. (A) Effects of compound **1** and **2** on the expressions of apoptosis-related proteins in HCT-116 cells. Lysates were prepared from these cells, and then analyzed the expressions of DR5, Bax, Bcl-2, cleaved caspase-9, and cleaved PARP by Western blot analysis using specific antibodies. β-Actin used as loading control of whole protein. (B) Data represent the percentage of DR5, Bax, Bcl-2, cleaved caspase-9, and cleaved PARP in HCT-116 cells.

following treatment with compounds **1** and **2** was examined using Western blot analysis. As shown in Figure 4A and B, compounds **1** and **2** activated ERK1/2 in HCT-116 cells. This indicated that compounds **1** and **2** could induce apoptosis via the upregulation of ERK1/2, as well as activation of the extrinsic and intrinsic apoptosis pathways.

Oleanane-type saponins are the main constituents of *P. stipuleanatus* and are believed to play a pharmacologically important role, including anticancer activity. The oleanane-type saponin Durantanin IV was isolated from the leaves of *Duranta repens*, showed significant cytotoxic activity against a HepG2 cell line.[21] The oleanane-type saponin achyranthoside H methyl ester,

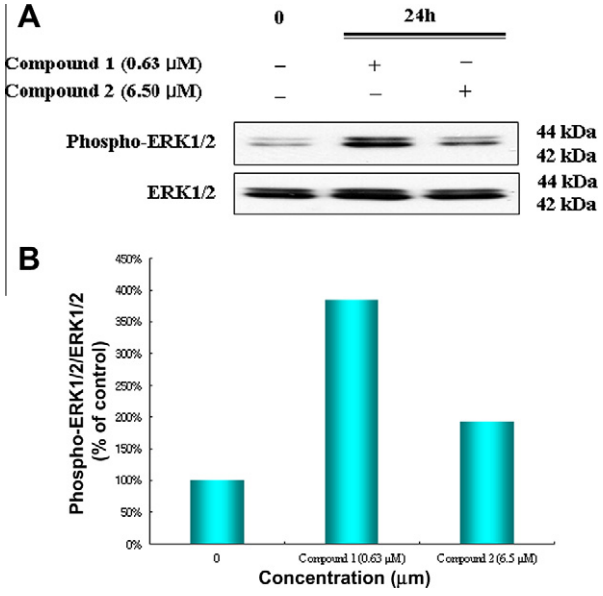


Figure 4. Effects of compounds **1** and **2** on the phosphorylation of ERK1/2 MAP kinase in HCT-116 cells. (A) Effects of compounds **1** and **2** on the expressions of phosphor-ERK1/2 in HCT-116 cells. Lysates were prepared from these cells, and then analyzed the expression of phosphor-ERK1/2 by Western blot analysis using specific antibodies. (B) Data represent the percentage of Phospho-ERK1/2 in HCT-116 cells.

isolated from the roots of *Achyranthes fauriei* induces apoptosis in human breast cancer MCF-7 and MDA-MB-453 cells via a caspase activation pathway.[22]

Compound **1** showed the strongest cytotoxic activity and compound **2** exhibited the potent cytotoxicity against both of HL-60 and HCT-116 cell lines, compared with a positive control. As we looked into the result, compound **1** was an oleanolic acid 3-*O*-glucuronopyranoside methyl ester which showed significantly increased cytotoxic effects against both of cell lines, whereas oleanoic acid showed decreased activity. It is noteworthy that compounds **3**–**11**, which have 28-*O*-glucopyranosyl moiety in its structure, clearly showed decreased cytotoxicity. In addition, compounds **3**, **6**, **8**, **9** showed moderate cytotoxic effects against HL-60 and have glucopyranosyl and arabinofuranosyl moiety at 3′ and 4′-position of the glucuronic acid methyl ester part. Compounds **4**, **5**, and **10**, which possess xylopyranosyl moiety at 2′-position of the glucuronic acid methyl ester part, showed no cytotoxic activity. In terms of structure–activity relationships in our experiments: (1) the 3-*O*-glucuronic acid methyl ester moiety is essential to the activity; (2) and 28-ester glucoside moiety significantly reduces the activity; (3) and the glucopyranosyl and arabinofuranosyl moiety at 3′ and 4′-position of the glucuronic acid methyl ester part tends to increase the activity; (4) and the xylopyranosyl moiety at 2′-position of the glucuronic acid methyl ester part tends to decrease the activity.

Acknowledgments

This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093815) in Korea. We are grateful to the KBSI for the NMR analysis.

Reference and notes

1. Zou, K.; Zhu, S.; Komatsu, K. *J. Chin Three Gorges Univ.* **2002**, *24*, 355.
2. Yang, C. R.; Jiang, Z. D.; Zhou, J.; Kasai, R.; Tanaka, O. *Yunnan Zhiwu Yanjiu* **1985**, *7*, 103.
3. Zhang, L. T.; Zhang, Y. W.; Takaishi, Y.; Duan, H. Q. *Chin. Chem. Lett.* **2008**, *19*, 190.
4. *Acid hydrolysis of spinasaponin A methyl ester* (**1**): A solution of compound **1** (2.0 mg) in HCl 1.0 M (3.0 ml) was heated under reflux for 2 h. Then, the reaction mixture was concentrated in vacuo to dryness. The residue was extracted with EtOAc and H_2O (5 ml each, 3 times). Next, the sugar residue, obtained by concentration of the water layer, was dissolved in dry pyridine (0.1 ml). Then L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 ml) was added to the solution. After heating the reaction mixture at 60 °C for 2 h, 0.1 ml of trimethylsilylimidazole was added. Heating at 60 °C was continued for a further 2 h, and the mixture was evaporated in vacuo to give a dried product, which was partitioned between hexane and H_2O.[23] The hexane layer was analyzed using the GC procedure (General Procedures). The peak of hydrolysate of the spinasaponin A methyl ester was detected at t_R 14.38 min for D-glucose. The absolute configuration of glucuronic acid was determined to be D by the measurement of optical rotation after its separation by prep. TLC from the crude sugar mixture. $[\alpha]_D$ +5.0 (*c* 0.1, H_2O).
5. Tang, H. F.; Wang, Z. Z.; Yi, Y. H.; Jiang, Y. P.; Hu, W. J.; Li, Y. Q. *J. Chin. Pharm. Sci.* **1997**, *6*, 75.
6. Shao, C. J.; Kasai, R.; Xu, J. D.; Tanaka, O. *Chem. Pharm. Bull.* **1989**, *37*, 311.
7. Sakai, S.; Katsumata, M.; Satoh, Y.; Nagasao, M.; Miyakoshi, M.; Ida, Y.; Shoji, J. *Phytochemistry* **1994**, *35*, 1319.
8. Paphassarang, S.; Raynaud, J.; Lussignol, M.; Becchi, M. *Phytochemistry* **1989**, *28*, 1541.
9. Tanaka, O.; Morita, T.; Kasai, R.; Kinouchi, J.; Sanada, S.; Ida, Y.; Shoji, J. *Chem. Pharm. Bull.* **1985**, *33*, 2323.
10. Satoh, Y.; Sakai, S.; Katsumata, M.; Nagasao, M.; Miyakoshi, M.; Ida, Y.; Shoji, J. *Phytochemistry* **1994**, *36*, 147.
11. Tang, H. F.; Yi, Y. H.; Wang, Z. Z.; Jiang, Y. P.; Li, Y. Q. *Acta Pharm. Sini.* **1997**, *32*, 685.
12. Jiang, Y. T.; Xu, S. X.; Gu, X. H.; Ren, L.; Cheng, Y. J.; Yao, X. S.; Miao, Z. C. *Acta Pharm. Sini.* **1992**, *27*, 528.
13. Li, J. X.; Hareyama, T.; Tezuka, Y.; Zhang, Y.; Miyahara, T.; Kadota, S. *Planta Med.* **2005**, *71*, 673.
14. Jayasinghe, U. L. B.; Jayasooriya, C. P.; Fujimoto, Y. *Fitoterapia* **2002**, *73*, 406.
15. Magalhaes, A. F.; Goulart de Azevedo Tozzi, A. M.; Santos, C. C.; Serrano, D. R.; Zanotti-Magalhaes, E. M.; Magalhaes, E. G.; Magalhaes, L. A. *Mem. Inst. Oswaldo Cruz* **2003**, *98*, 713.
16. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.
17. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
18. Debatin, K. M. *Cancer Immunol. Immunother.* **2004**, *53*, 153.
19. Wada, T.; Penniger, J. M. *Oncogene* **2004**, *23*, 2838.
20. Kohno, M.; Pouyssegur, J. *Ann. Med.* **2006**, *38*, 200.
21. Ahmed, W. S.; Mohamed, M. A.; El-Dib, R. A.; Hamed, M. M. *Molecules* **2009**, *14*, 1952.
22. Fukkumura, M.; Ando, H.; Hirai, Y.; Toriizuka, K.; Ida, Y.; Kuchino, Y. *J. Nat. Med.* **2009**, *63*, 181.
23. Min, B. S.; Nakamura, N.; Miyashiro, H.; Kim, Y. H.; Hattori, M. *Chem. Pharm. Bull.* **2000**, *48*, 194.