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RESEARCH ARTICLE

Cytotoxic triterpene glycosides from the roots of *Sanguisorba officinalis*

Jiang Hu · Yan Song · Hui Li · Benshou Yang · Xia Mao · Yongmao Zhao · Xiaodong Shi

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Abstract Phytochemical investigation of the ethanol extract of the roots of *Sanguisorba officinalis* resulted in the isolation of three new triterpene glycosides, 3*β*-[(α-L-arabinopyranosyl)oxy]-19α,23-dihydroxyolean-12-en-28-oic acid 28-[6-*O*-acetyl-*β*-D-glucopyranosyl] ester (**1**), 2α,3*β*,19α,23-tetrahydroxyurs-12-en-28-oic acid 28-[6-*O*-acetyl-*β*-D-glucopyranosyl] ester (**2**), and 3*β*-[(α-L-arabinopyranosyl)oxy]-19α-hydroxyurs-12,20(30)-dien-28-oic acid 28-[6-*O*-acetyl-*β*-D-glucopyranosyl] ester (**3**). All the triterpene glycosides exhibited the significant cytotoxic potential with low IC_{50} values ($IC_{50} < 5.0$ μM) against six tumor cell lines (MCF-7, HeLa, HepG2, SGC-7901, NCI-H460, and BGC-823).

Keywords *Sanguisorba officinalis* · Rosaceae · Triterpene glycoside · Cytotoxicity

Jiang Hu and Yan Song have contributed equally to this work.

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Introduction

Radix Sanguisorbae, the dried root of *Sanguisorba officinalis* L. (Rosaceae), is a traditionally valuable plant with hemostatic, analgesic, and astringent properties (Liang et al. 2013; Cheng and Cao 1992). In China, Korea, and Japan, this perennial plant has been used for the treatment of inflammatory and metabolic disease such as diarrhea, chronic intestinal infections, duodenal ulcers, and internal hemorrhage (Yu et al. 2011; Cheng and Cao 1992; Zhang et al. 2012a, b). Previous studies reported the isolation of some types of natural products such as a series of hydrolysable tannins, gallic acid, as well as triterpenes and their glycosides (e.g. ziyuglycoside I) which have been reported as characteristic constituents of *S. officinalis* and are considered to be major responsible for the in vitro and in vivo pharmacological effects of this crude drug (Liu et al. 2004; Liu et al. 2005; Mimaki et al. 2001). In search for bioactive constituents from the abundant saponin components (>3 % weight of the dry roots) of *S. officinalis*, we systematically examined the triterpene glycoside components and isolated three new ones, 3*β*-[(α-L-arabinopyranosyl)oxy]-19α,23-dihydroxyolean-12-en-28-oic acid 28-[6-*O*-acetyl-*β*-D-glucopyranosyl] ester (**1**), 2α,3*β*,19α,23-tetrahydroxyurs-12-en-28-oic acid 28-[6-*O*-acetyl-*β*-D-glucopyranosyl] ester (**2**), and 3*β*-[(α-L-arabinopyranosyl)oxy]-19α-hydroxyurs-12,20(30)-dien-28-oic acid 28-[6-*O*-acetyl-*β*-D-glucopyranosyl] ester (**3**). This paper deals with the isolation and structure elucidation of the new triterpene glycosides by spectroscopic methods, and of the results of hydrolytic cleavage. All the isolated compounds were in vitro evaluated for their cytotoxic activities against six tumor cell lines (MCF-7, HeLa, HepG2, SGC-7901, NCI-H460, and BGC-823).

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Materials and methods

General experimental procedures

Optical rotations were taken on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on Nicolet Magna FT-IR 750 spectrophotometer using KBr disks. NMR spectra were recorded on Bruker AM-300, AM-400, and INVOR-600 NMR spectrometers. The chemical shift (δ) values are given in ppm with TMS as internal standard, and coupling constants (J) are in Hz. FAB-MS spectra were recorded on a Finnigan MAT TSQ-700. EI-MS and HR-EI-MS spectra were recorded on a Finnigan MAT-95 mass spectrometer (San Jose, CA, USA). Column chromatographic separations were carried out using silica gel (200–300 mesh and H60, Qingdao Haiyang Chemical Group Corporation, China), MCI gel CHP20P (75–150 μm, Mitsubishi Chemical Industries, Japan), and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden) as packing material. TLC was carried out on precoated silica gel GF_{254} plates (Yantai Chemical Industrials), and the TLC spots were viewed at 254 nm and visualized using 5 % sulfuric acid in alcohol containing 10 mg/mL vanillin. Analytical HPLC was performed on a Waters 2690 instrument with a 996 PAD (photodiode array detector) coupled with an Alltech ELSD 2000 detector. Semipreparative and preparative HPLC was performed on a Varian SD1 instrument with a 320 single-wave detector. Their chromatographic separations were carried out on C-18 columns (250 × 10 mm, 5 μm, Waters; 220 × 25 mm, 10 μm, Merck, respectively), using a gradient solvent system comprised of H_2O and MeOH, with a flow rate of 3.0 and 15.0 mL/min, respectively. All cell lines were purchased from Cell Bank of Shanghai Institute of Biochemistry & Cell Biology, Chinese Academy of Sciences. Other reagents were purchased from Shanghai Sangon Biological Engineering Technology & Services CO., Lt.

Plant materials

The dried roots of *S. officinalis* were collected in the suburb of Qujing, Yunnan province of China, in October of 2012 and identified by one of the authors (X. Mao). A voucher specimen (SO20121001) was deposited in the Herbarium of the College of Biological Resources and Environment Science, Qujing Normal University, Qujing, Yunnan province, China.

Extraction and isolation

The air-dried roots of *S. officinalis* (5 kg) were ground into powder and were extracted thrice with 80 % EtOH. After evaporation of the EtOH, the aqueous brownish syrup (1 L) was suspended in H_2O (l L) and then partitioned with petroleum ether (1 L × 3), chloroform (1 L × 3), and ethyl acetate (1 L × 3) gradually to afford three fractions (51.0, 81.0, and 143.0 g respectively). The ethyl acetate fraction was further fractionated through a silica gel column (200–300 mesh, 10 × 80 cm) eluting with a gradient of $CHCl_3$–MeOH (100:1, 50:1, 30:1, 20:1, 15:1, 10:1, 5:1, 2:1, 1:1, each 10 L) to afford 9 fractions. Fraction 3 (5.6 g) was applied to an ODS MPLC column and eluted with MeOH/H_2O (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 80:20, 90:10, each1 L) to yield 3 subfractions. Subfraction 2 (830 mg) was purified by a preparative RP-HPLC using 25 % methanol as mobile phase to obtain **2** (112 mg). Fraction 4 (6.1 g) was applied to an ODS column and eluted with MeOH/H_2O (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 90:10, each 500 mL), followed by a Sephadex LH-20 column eluting with MeOH/H_2O (50:50, 3 L) to obtain **3** (138 mg). Fraction 5 (4.9 g) was applied to an ODS column using MeOH/H_2O (10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 90:10, each 1 L) as mobile phase to yield 4 subfractions. Subfraction 2 (898 mg) was purified by a preparative RP-HPLC eluting with 22 % methanol to get **1** (151 mg).

Acid hydrolysis and sugar analysis of compounds **1**–**3**

A solution of compound **1** (**2** or **3**) (about 10.0 mg) in 1 M HCl (dioxane-H_2O, 1:1, 1 mL) was heated at 95 °C for 2 h based on a previous reference (Zhang et al. 2008). After cooling, the reaction mixture was neutralized by passage through an Amberlite IRA-93ZU (Organo, Tokyo, Japan) column and subjected to silica gel chromatography using a gradient mixture of $CHCl_3$–MeOH (19:1; 9:1; 1:1) to give an aglycone fraction and a sugar fraction (3.0 mg). The aglycone fraction was purified by silica gel CC eluting with hexane-Me_2CO (4:1) to give an aglycone **1a** (2.9 mg) [**2a** (3.3 mg) or **3a** (3.1 mg)]. HPLC analysis of the sugar fraction under the following conditions showed the presence of D-glucose and L-arabinose. Column: Capcell Pak NH_2 UG80 (4.6 mm i.d. × 250 mm, 5 μm, Shiseido); detector: Shodex OR-2 (Showa-Denko, Tokyo, Japan); solvent: MeCN–H_2O (17:3); flow rate: 1.0 mL/min. R_t (min): 8.27 (L-arabinose, positive optical rotation); 13.39 (D-glucose, positive optical rotation). All chemical reagents and standard sugars were purchased from Sigma-Aldrich Corporation.

3β-[(α-L-arabinopyranosyl)oxy]-19α,23-dihydroxyolean-12-en-28-oic acid 28-[6-*O*-acetyl-β-D-glucopyranosyl] ester (**1**): White amorphous powder; $[\alpha]_D^{23.3} = +23.8$ (c 0.64, MeOH); IR (KBr) ν_{max} 3450, 2943, 1731, 1459, 1389, 1073, 860, 781 cm^{-1}; ^1H NMR (pydirine-d_5, 600 MHz) data see Table 1, ^{13}C NMR (pydirine-d_5, 150 MHz) data see Table 2; FAB-MS (pos.) m/z: 847 $[M + Na]^+$; HR-ESI–MS (pos.) m/z: 847.4451 ($[M + Na]^+$, $C_{43}H_{68}O_{15}Na$; calc. 847.4456).

Table 1 ^{1}H NMR data of compounds **1**–**3** in pydirine-d_5 (δ in ppm and J in Hz)

No.	1	2	3
H-1ax	1.03 (*ddd*, 14.0, 13.6, 3.8)	1.94 (*dd*, 13.6, 13.2)	1.06 (*ddd*, 13.8, 13.6, 2.8)
H-1eq	1.60 (*ddd*, 13.6, 3.6, 3.2)	2.17 (*dd*, 13.2, 3.6)	1.56 (overlapped)
H-2ax	1.95 (*m*)	4.26 (*ddd*, 13.6, 13.2, 3.6)	1.96 (overlapped)
H-2eq	2.22–2.25 (overlapped)	–	2.26 (*m*)
H-3ax	3.40 (*dd*, 13.6, 3.6)	3.72 (*d*, 13.2)	3.45 (*dd*, 13.6, 3.6)
H-5	0.92 (*dd,* 13.8, 3.2)	1.00 (*dd,* 13.8, 3.6)	0.95 (*dd,* 13.8, 3.0)
H-6ax	1.37 (*m*)	2.10–2.12 (overlapped)	1.39 (*m*)
H-6eq	1.57 (*m*)	2.37 (*m*)	1.56 (overlapped)
H-7ax	1.40 (*ddd*, 13.8, 13.6, 3.8)	2.07–2.09 (overlapped)	1.42 (*ddd*, 13.8, 13.6, 3.8)
H-7eq	1.63 (*ddd*, 13.6, 3.6, 3.4)	2.22 (*ddd*, 13.6, 3.6, 3.0)	1.56 (overlapped)
H-9	1.91 (*dd*, 13.8, 3.2)	2.19 (*dd,* 13.6, 3.0)	1.83 (*dd*, 13.8, 3.2)
H-11ax	2.03 (*m*)	2.15 (*m*)	1.93 (*m*)
H-11eq	2.12 (*m*)	2.34 (*m*)	2.07 (overlapped)
H-12	5.62 (*br s*)	5.59 (*br s*)	5.58 (*br s*)
H-15ax	1.33 (*ddd*, 13.8, 13.6, 3.8)	2.48 (*ddd*, 13.8, 13.6, 3.8)	1.31 (*ddd*, 13.8, 13.6, 3.8)
H-15eq	2.16 (*ddd*, 13.6, 3.6, 3.5)	2.57 (*ddd*, 13.6, 3.6, 3.5)	2.32 (*ddd*, 13.6, 3.6, 3.5)
H-16ax	2.14 (*ddd*, 13.8, 13.6, 3.6)	2.10–2.12 (overlapped)	2.09 (*ddd*, 13.8, 13.6, 3.6)
H-16eq	2.90 (*ddd*, 13.6, 3.8, 3.5)	3.07 (*ddd*, 13.6, 3.8, 3.5)	2.82 (*ddd*, 13.6, 3.8, 3.5)
H-18ax	3.70 (*d*, 2.8)	2.99 (*s*)	2.97 (*s*)
H-19eq	3.66 (*d*, 2.8)	–	–
H-20	–	1.37 (*m*)	–
H-21ax	1.25 (*ddd*, 13.6, 13.2, 3.0)	1.32 (*m*)	1.96 (overlapped)
H-21eq	2.22–2.25 (overlapped)	1.43 (*m*)	2.40 (*ddd*, 13.6, 3.2, 3.0)
H-22ax	1.87 (*ddd*, 13.8, 3.6, 2.8)	2.07–2.09 (overlapped)	2.78 (*ddd*, 13.8, 13.2, 3.2)
H-22eq	2.22–2.25 (overlapped)	2.13 (*ddd*, 13.6, 3.6, 3.0)	2.90 (*ddd*, 13.2, 3.6, 3.0)
H-23	4.30 (*d*, 13.0)	4.29 (*d*, 13.0)	1.37 (*s*)
–	3.72 (*d*, 13.0)	3.77 (*d*, 13.0)	–
H-24	1.01 (*s*)	1.03 (*s*)	1.03 (*s*)
H-25	0.96 (*s*)	1.39 (*s*)	0.93 (*s*)
H-26	1.11 (*s*)	1.35 (*s*)	1.10 (*s*)
H-27	1.74 (*s*)	1.72 (*s*)	1.41 (*s*)
H-29	1.28 (*s*)	1.47 (*s*)	1.82 (*s*)
H-30	1.20 (*s*)	1.18 (*d*, 5.1)	4.83 (*s*)
–	–	–	5.03 (*s*)
3-*O*-ara			
H-1′	4.80 (*d*, 7.0)	–	4.88 (*d*, 7.0)
H-2′	4.48 (*m*)	–	4.54 (*m*)
H-3′	4.22–4.25 (overlapped)	–	4.23 (*m*)
H-4′	4.40 (*m*)	–	4.43 (*m*)
H-5′	4.34–4.37 (overlapped)	–	4.40 (*m*)
–	3.89 (*dd*, 4.0)	–	3.91 (*dd*, 4.0)
–	3.92 (*dd*, 4.0)	–	3.93 (*dd*, 4.0)
28-*O*-glc			
H-1″	6.29 (*d*, 8.0)	6.29 (*d*, 8.0)	6.32 (*d*, 8.0)
H-2″	4.22–4.25 (overlapped)	4.20 (*m*)	4.21 (*m*)
H-3″	4.34–4.37 (overlapped)	4.33 (*m*)	4.34 (*m*)
H-4″	4.12 (*m*)	3.35 (*m*)	3.37 (*m*)
H-5″	4.42 (*m*)	4.12 (*m*)	4.11 (*m*)
H-6″	4.26 (*dd*, 13.0, 6.6)	4.23 (*dd*, 13.0, 6.6)	4.25 (*dd*, 13.0, 6.6)
–	4.32 (*dd*, 13.0, 6.6)	4.30 (*dd*, 13.0, 6.6)	4.32 (*dd*, 13.0, 6.6)
Me	2.07 (*s*)	2.05 (*s*)	2.07 (overlapped)

2α,3β,19α,23-tetrahydroxyurs-12-en-28-oic acid 28-[6-*O*-acetyl-β-D-glucopyranosyl] ester (**2**): White amorphous powder, $[\alpha]_D^{23.3} = +33.8$ (*c* 0.99, MeOH); IR (KBr) ν_{max} 3423, 2933, 1720, 1459, 1381, 1070, 932, 773 cm^{-1}; ^{1}H NMR (pydirine-d_5, 600 MHz) data see Table 1, ^{13}C NMR (pydirine-d_5, 150 MHz) data see Table 2; FAB-MS (pos.) *m/z*: 731 $[M + Na]^+$; HR-ESI–MS (pos.) *m/z*: 731.3981 ($[M + Na]^+$, $C_{38}H_{60}O_{12}Na$; calc. 731.3982).

3β-[(α-L-arabinopyranosyl)oxy]-19α-hydroxyurs-12,20(30)-dien-28-oic acid 28-[6-*O*-acetyl-β-D-glucopyranosyl] ester (**3**): White amorphous powder, $[\alpha]_D^{23.3} = +29.8$ (*c* 0.51, MeOH); IR (KBr) ν_{max} 3441, 2939, 1712, 1455, 1389, 1086, 866, 781 cm^{-1}; ^{1}H NMR (pydirine-d_5, 600 MHz) data see Table 1, ^{13}C NMR (pydirine-d_5, 150 MHz) data see Table 2; FAB-MS (pos.) *m/z*: 829 $[M + Na]^+$; HR-ESI–MS (pos.) *m/z*: 829.4347 ($[M + Na]^+$, $C_{43}H_{66}O_{14}Na$; calc. 829.4350).

Cytotoxicity assay in vitro

The revised MTT method (Chen et al. 2011) was used for in vitro evaluation of the cytotoxic potential of the isolated

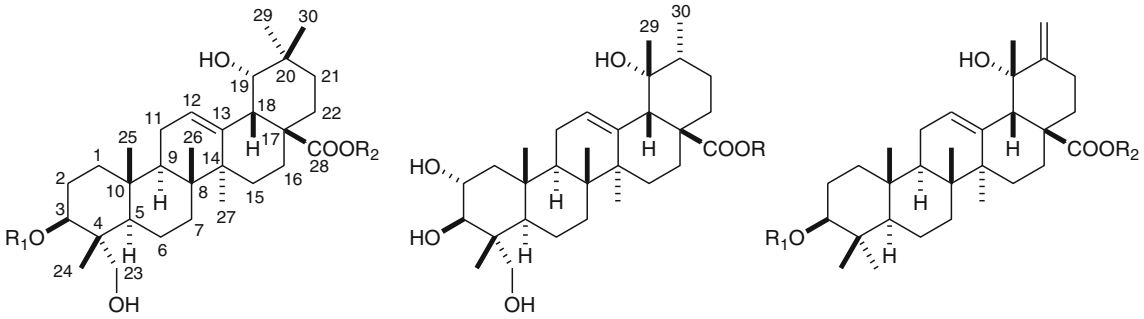
Table 2 ^{13}C NMR data of compounds **1**–**3a** in pydirine-d_5 (δ in ppm)

No.	1	1a	2	2a	3	3a
1	38.4 (*t*)	38.8 (*t*)	47.0 (*t*)	46.9 (*t*)	38.9 (*t*)	38.8 (*t*)
2	26.2 (*t*)	28.2 (*t*)	68.6 (*d*)	68.5 (*d*)	26.7 (*t*)	27.5 (*t*)
3	88.5 (*d*)	78.3 (*d*)	79.3 (*d*)	79.5 (*d*)	88.8 (*d*)	79.0 (*d*)
4	43.4 (*s*)	43.2 (*s*)	43.3 (*s*)	43.4 (*s*)	39.6 (*s*)	39.1 (*s*)
5	55.8 (*d*)	56.0 (*d*)	48.3 (*d*)	48.4 (*d*)	56.1 (*d*)	55.7 (*d*)
6	18.7 (*t*)	18.8 (*t*)	21.0 (*t*)	19.8 (*t*)	18.6 (*t*)	18.9 (*t*)
7	33.0 (*t*)	33.3 (*t*)	33.9 (*t*)	33.6 (*t*)	33.5 (*t*)	33.2 (*t*)
8	40.0 (*s*)	40.1 (*s*)	40.8 (*s*)	40.6 (*s*)	40.1 (*s*)	39.8 (*s*)
9	48.0 (*d*)	48.2 (*d*)	47.6 (*d*)	48.0 (*d*)	48.1 (*d*)	47.9 (*d*)
10	37.0 (*s*)	37.3 (*s*)	39.3 (*s*)	39.0 (*s*)	37.1 (*s*)	37.3 (*s*)
11	24.0 (*t*)	24.1 (*t*)	24.8 (*t*)	24.6 (*t*)	24.0 (*t*)	24.4 (*t*)
12	123.1 (*d*)	123.3 (*d*)	128.8 (*d*)	128.7 (*d*)	128.6 (*d*)	128.3 (*d*)
13	144.0 (*s*)	144.4 (*s*)	140.0 (*s*)	143.9 (*s*)	138.0 (*s*)	138.6 (*s*)
14	41.9 (*s*)	42.1 (*s*)	42.3 (*s*)	42.0 (*s*)	43.2 (*s*)	43.0 (*s*)
15	28.8 (*t*)	29.8 (*t*)	29.3 (*t*)	28.9 (*t*)	29.3 (*t*)	28.9 (*t*)
16	27.8 (*t*)	28.0 (*t*)	26.9 (*t*)	27.0 (*t*)	26.5 (*t*)	27.0 (*t*)
17	46.3 (*s*)	46.0 (*s*)	48.9 (*s*)	49.3 (*s*)	49.0 (*s*)	49.2 (*s*)
18	44.2 (*d*)	44.6 (*d*)	54.6 (*d*)	54.3 (*d*)	54.0 (*d*)	54.2 (*d*)
19	80.8 (*d*)	81.1 (*d*)	72.8 (*s*)	72.8 (*s*)	73.0 (*s*)	72.9 (*s*)
20	35.2 (*s*)	35.6 (*s*)	42.4 (*d*)	42.2 (*d*)	150.2 (*s*)	150.4 (s)
21	28.8 (*t*)	29.0 (*t*)	27.2 (*t*)	27.0 (*t*)	31.8 (*t*)	32.0 (*t*)
22	32.7 (*t*)	33.1 (*t*)	37.7 (*t*)	37.7 (*t*)	32.5 (*t*)	32.6 (*t*)
23	64.5 (*t*)	64.9 (*t*)	67.0 (*t*)	66.5 (*t*)	28.4 (*q*)	28.5 (*q*)
24	16.6 (*q*)	16.4 (*q*)	14.8 (*q*)	15.0 (*q*)	16.9 (*q*)	16.8 (*q*)
25	15.2 (*q*)	15.3 (*q*)	15.6 (*q*)	15.4 (*q*)	15.9 (*q*)	15.7 (*q*)
26	17.6 (*q*)	17.4 (*q*)	17.8 (*q*)	17.6 (*q*)	17.6 (*q*)	17.4 (*q*)
27	24.8 (*q*)	24.9 (*q*)	24.8 (*q*)	24.7 (*q*)	25.9 (*q*)	25.8 (*q*)
28	177.0 (*s*)	180.8 (*s*)	177.2 (*s*)	180.6 (*s*)	178.9 (*s*)	181.0 (*s*)
29	28.6 (*q*)	28.7 (*q*)	27.6 (*q*)	27.7 (*q*)	24.0 (*q*)	28.6 (*q*)
30	24.6 (*q*)	24.8 (*q*)	17.0 (*q*)	16.9 (*q*)	106.3 (*t*)	106.1 (*t*)
3-O-ara						
1′	107.5 (*d*)	–	–	–	107.6 (*d*)	–
2′	73.0 (*d*)	–	–	–	72.8 (*d*)	–
3′	74.6 (*d*)	–	–	–	74.7 (*d*)	–
4′	69.6 (*d*)	–	–	–	69.8 (*d*)	–
5′	66.9 (*t*)	–	–	–	66.9 (*t*)	–
28-*O*-glc						
1′	95.8 (*d*)	–	96.6 (*d*)	–	98.6 (*d*)	–
2′	74.2 (*d*)	–	74.5 (*d*)	–	74.1 (*d*)	–
3′	78.8 (*d*)	–	79.5 (*d*)	–	79.0 (*d*)	–
4′	71.1 (*d*)	–	71.6 (*d*)	–	71.0 (*d*)	–
5′	78.8 (*d*)	–	79.6 (*d*)	–	79.3 (*d*)	–
6′	64.7 (*t*)	–	64.6 (*t*)	–	64.5 (*t*)	–
C = O	172.8 (*s*)	–	172.6 (*s*)	–	172.8 (*s*)	–
Me	20.8 (*q*)	–	20.6 (*q*)	–	20.6 (*q*)	–

compounds against six cultured human tumor cell lines. All tumor cell lines (MCF-7, HeLa, HepG2, SGC-7901, NCI-H460, and BGC-823) were cultured on RPMI–1640 medium supplemented with 10 % foetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin in 25 cm^2 culture flasks at 37 °C in humidified atmosphere with 5 % CO_2. For the cytotoxicity tests, cells in exponential growth stage were harvested from culture by trypsin digestion and centrifuging at 180 × g for 3 min, then resuspended in fresh medium at a cell density of 5 × 10^4 cells per mL. The cell suspension was dispensed into a 96–well microplate at 100 μL per well, and incubated in humidified atmosphere with 5 % CO_2 at 37 °C for 24 h, and then treated with the compounds at various concentrations (0, 1, 10, 100 μM). After 48 h of treatment, 50 μL of 1 mg/mL MTT solution was added to each well, and further incubated for 4 h. The cells in each well were then solubilized with DMSO (100 μL for each well) and the optical density (OD) was recorded at 570 nm. All drug doses were tested with doxorubicin as positive control in triplicate and the IC_{50} values were derived from the mean OD values of the triplicate tests versus drug concentration curves.

Results and discussion

Compound **1** was obtained as a white amorphous solid, and its molecular formula was indicated as $C_{43}H_{68}O_{15}$ by HR-ESI–MS, corresponding to 10 unsaturation degrees. The IR spectrum showed a characteristic absorption attributable to an ester carbonyl group at 1,731 cm^{-1}, as well as a broad absorption due to hydroxyl groups near 3,450 cm^{-1}. Acid hydrolysis of **1** with 1 M HCl in dioxane-H_2O (1:1) gave a triterpene (3*β*,19*α*,23-trihydroxyolean-12-en-28-oic acid, $C_{30}H_{48}O_5$) (**1a**) (Zhang et al. 2012a, b), D-glucose and L-arabinose, which was identified by direct HPLC analysis and detected by using an optical rotation (OR) detector (Hara et al. 1987). Besides two anomeric proton signals at δ 6.29 (*d*, J = 8.0 Hz) and 4.80 (*d*, J = 7.0 Hz), the ^1H NMR spectrum of **1** exhibited signals for six tertiary methyl groups at δ 1.74, 1.28, 1.20, 1.11, 1.01, and 0.96 (each, *s*), an olefinic proton at δ 5.62 (*br s*), and an oxygenated methylene at δ 4.30 and 3.72 (each, *d*, J = 13.0), which were characteristic of the oleanolic acid skeleton with a OH group at C-23 (Liu et al. 2004; Liu et al. 2005; Mimaki et al. 2001) (Table 1). In HMBC spectrum, the



	R_1	R_2
1	Ara	6-Ac-Glc
1a	H	H

	R
2	6-Ac-Glc
2a	H

	R_1	R_2
3	Ara	6-Ac-Glc
3a	H	H

Fig. 1 The structures of compounds **1**–**3a**

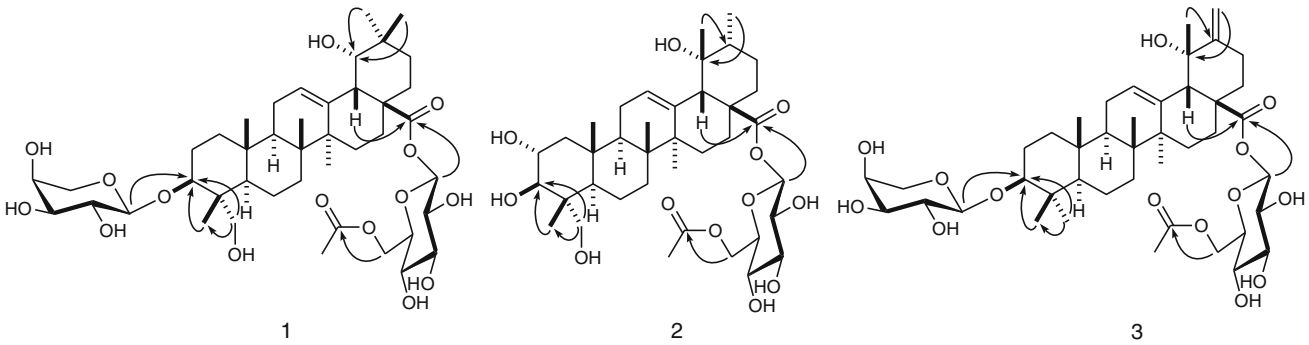


Fig. 2 Key HMBCs (↷) of compounds **1**–**3**

correlations of the proton signals at δ 4.30 and 3.72 with C-3 (δ 88.5), C-5 (δ 55.8), and C-24 (δ 16.6) established the position of the hydroxyl group at C-23, which was further verified by the ROESY correlations of H-2β/H-24/H-25. Furthermore, The HMBC correlations of the proton signal at δ 3.66 with C-13 (δ 144.0), C-17 (δ 46.3), C-29 (δ 28.6), and C-30 (δ 24.6) indicated that the hydroxyl group was located at C-19. The $^{3}J_{H,H}$ value of 2.8 Hz between H-18 and H-19 and the ROESY correlations from H-19 to H-18 and Me-30 (δ 1.20) confirmed the β-oriented configuration of H-19. The ^{1}H and ^{13}C NMR signals of the glycoside moieties were readily assigned by interpretation of the ^{1}H–^{1}H COSY and HMQC spectra. Proton multiplet patterns and coupling constants, as well as the proton and carbon chemical shifts revealed that **1** contained an α-L-arabinopyranosyl and a β-D-glucopyranosyl units (Fig. 1). In the HMBC spectrum of **1**, correlation peaks were observed from δ 4.80 (anomer of arabinosyl) to δ 88.5 (C-3 of aglycone), and from δ 6.29 (anomer of glucosyl) to δ 177.0 (C-28 of aglycone), indicating that the α-L-arabinopyranosyl and a β-D-glucopyranosyl units were positioned at C-3 and C-28 of the aglycone respectively. The C-6″ location of the AcO group in the glucopyranosyl unit was established by the HMBC correlations of H-6″ (δ 4.32 and 4.25) with the C = O (δ 172.8) of the AcO group (Fig. 2). The ROESY correlations of H-3/H-1α and H-3/H-5 indicated that H-3 was α-oriented. Accordingly, the structure of **1** was determined as 3β-[(α-L-arabinopyranosyl)oxy]-19α,23-dihydroxyolean-12-en-28-oic acid 28-[6-*O*-acetyl-β-D-glucopyranosyl] ester.

Compound **2** was obtained as a white amorphous solid, showed a positive-ion at *m/z* 731 $[M + Na]^{+}$, and the molecular formula $C_{38}H_{60}O_{12}$ was established by HR-ESI–MS (found *m/z* 731.3981 $[M + H]^{+}$; calc. 731.3982). Acid hydrolysis with 1 M HCl in dioxane-H_2O (1:1) afforded a triterpene (2α,3β,19α,23-tetrahydroxyurs-12-en-28-oic acid, $C_{30}H_{48}O_6$) (**2a**) (Shu et al. 2012), and D-glucose which was identified by direct HPLC analysis and detected by using an OR detector. ^{1}H NMR spectrum exhibited the presence of five tertiary methyl groups (δ 1.72, 1.47, 1.39, 1.35, and 1.03), one secondary methyl group at δ 1.18 (*d*, $J = 5.1$ Hz), one characteristic methine proton (H-18) of pomolic acid (δ 2.99, s), one trisubstituted olefinic proton (δ 5.59, *br s*), and one anomeric proton (δ 6.29, *d*, $J = 8.0$ Hz). Except six signals assignable to the sugar moiety, ^{13}C NMR spectrum showed 30 carbons of the aglycone, including six methyl carbons, two olefinic carbons at δ 128.8 (C-12) and 140.0 (C-13), one characteristic

oxygenated quarternary C-atoms at δ 72.8 (C-19), and two oxygenated methine at δ 68.6 (C-2) and 79.3 (C-3), and one oxygenated methylene 67.0 (C-23) respectively, indicating that the aglycone was a pomolic acid derivative with four hydroxyl groups. The HMBC correlations between the proton signals at δ 4.29 and 3.77 with C-3 (δ 79.3), C-5 (δ 48.3), and C-24 (δ 14.8), indicating that the proton signals of the oxygenated methine was assigned as H-23. The proton signal at δ 3.72 was assigned as H-3 by the HMBC correlations of the proton signal with C-5, C-23, and C-24. The ^{1}H–^{1}H COSY correlations of the proton signal at δ 4.26 with H-3 indicated that a hydroxyl group was substituted at C-2. Furthermore, the HMBC correlations from the anomeric H-atom (δ 6.29) (anomer of arabinosyl) to δ 177.2 (C-28 of aglycone) suggested that the glucopyranosyl unit was linked to C-28. The HMBC correlations of H-6″ (δ 4.30 and 4.23) with the C = O (δ 172.6) of the AcO group (Fig. 2) indicated AcO group in the glucopyranosyl unit was located at C-6″. The ROESY correlations of H-2 with H-24 and H-25, and of H-3 with H-5 and H-1α suggested that the hydroxyl group at C-2 and C-3 should be α- and β-oriented respectively. The ROESY correlations of H-29/H-20 and H-29/H-21β indicated the α-oriented of the hydroxyl group at C-19. All of these data for **2** were consistent with the structure of 2α,3β,19α,23-tetrahydroxyurs-12-en-28-oic acid 28-[6-*O*-acetyl-β-D-glucopyranosyl] ester.

Compound **3** was obtained as a white amorphous solid, with a molecular formula, $C_{43}H_{66}O_{14}$, as determined by the positive-ion FAB-MS (m/z 829 $[M + Na]^+$) and HR-ESI–MS (found *m/z* 829.4347 $[M + Na]^+$; calc. 829.4350). Acid hydrolysis of **3** with 1 M HCl in dioxane-H_2O (1:1) yielded a triterpene (3β,19α-dihydroxyurs-12,20(30)-dien-28-oic acid, $C_{30}H_{46}O_4$) (**3a**) (Thuong et al. 2008), D-glucose and L-arabinose which were identified by direct HPLC analysis and detected by using an OR detector. Its ^{1}H NMR spectrum exhibited the presence of six tertiary methyl groups (δ 1.82, 1.41, 1.37, 1.10, 1.03, and 0.93), one characteristic methine proton of H-18 in pomolic acid (δ 2.97, s), two anomeric protons (δ 4.88, d, J = 7.0 Hz and 6.32, *d*, J = 8.0), one trisubstituted olefinic proton (δ 5.58, br s), and two exocyclic olefinic protons at δ 4.83 and 5.03 (each, *s*). Its ^{13}C NMR spectrum showed six signals assignable to a sugar moiety and 30 signals to the aglycone which contained six methyl carbons, two olefinic carbons, one characteristic oxygenated quarternary carbon at δ 73.0 indicating that the aglycone was a pomolic acid derivative. Comparison the NMR data of the aglycone of **3** with those of pomolic acid (Liu et al. 2004; Liu et al. 2005; Mimaki et al. 2001), the mere ^{13}C NMR difference was that a methyl carbon was replaced by the signals for the exocyclic olefinic carbon. The HMBC correlations of the exocyclic olefinic proton signals (δ 4.83 and 5.03) with C-19 (δ 73.0), and C-21 (δ 31.8), and of H-18 (δ 2.97, *s*), H-22 (δ 2.90 and 2.78) with the olefinic carbon C-20 (δ 150.2) indicated the methyl at C-30 was oxygenated to exocyclic olefinic carbon. In the HMBC spectrum of **3**, correlation of δ 4.88 (anomer of arabinosyl) to δ 88.8 (C-3 of aglycone), and of δ 6.32 (anomer of glucosyl) to δ 178.9 (C-28 of aglycone) were observed. The HMBC correlations between H-6″ (δ 4.32 and 4.25) with the C = O (δ 172.8) of the AcO group indicated AcO group in the glucopyranosyl unit was linked at C-6″ (Fig. 2). The ROESY correlations of H-3/H-1α and H-3/H-5 and H-29/H-18 and H-29/H-21β confirmed that the arabinosyl unit at C-3 and the hydroxyl group at C-19 should be β- and α-oriented respectively. Accordingly, the structure of **3** was characterized as 3β-[(α-L-arabinopyranosyl)oxy]-19α-hydroxyurs-12,20(30)-dien-28-oic acid 28-[6-*O*-acetyl-β-D-glucopyranosyl] ester.

Table 3 Cytotoxicity of compounds **1**–**3** against six human tumor cell lines (IC_{50}, μM)

	Cell lines					
	MCF-7	HeLa	HepG2	SGC-7901	NCI-H460	BGC-823
1	2.6	2.4	2.7	3.1	2.7	3.3
2	4.3	4.1	4.6	4.5	4.8	4.7
3	3.2	3.6	2.9	3.7	3.0	3.7
Doxorubicin	0.02	0.05	0.04	0.01	0.01	0.02

The cytotoxic activities of the isolated compounds were determined by using the revised MTT method (Chen et al. 2011) against BGC-823 cells (human gastric carcinoma), HeLa cells (human cervical cancer), HepG2 cells (human hepatocellular carcinoma), MCF-7 cells (human breast cancer), NCI-H460 (human large cell lung cancer), and SGC-7901 cells (human gastric adenocarcinoma). The IC_{50} value (Table 3) indicated that all triterpene glycosides showed significant cytotoxic properties and, compound **1** and **3** possessed higher cytotoxic potential than **2**. These results indicated that the sugar moiety which could assist in the influx of ex-cellular compounds into cell maybe enhance the cytotoxic activities.

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