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Cytotoxic 9,19-cycloartane triterpenes from the aerial parts of *Cimicifuga yunnanensis*

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ABSTRACT

Six new 9,19-cycloartane triterpenes (**1**–**6**) were isolated from the aerial parts of *Cimicifuga yunnanensis*. The new chemical structures were determined by extensive analyses of 1D and 2D NMR spectroscopy. Compounds **1** and **2** are the first 9,19-cycloartane triterpenes characterized by CH_3-18 shifting from C-13 to C-12 in the *Cimicifuga* spp. The evaluation of inhibition activity against human HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines indicated that compounds **1**–**6** showed different levels of cytotoxic activities with IC_{50} values ranging from 1.2 to 27.8 μm.

1. Introduction

Cancer has become a major cause of human mortality. By the year 2050, 27 million new cancer cases and 17.5 million cancer deaths are projected worldwide [1]. Natural products represent a rich source of anticancer agents and approximately 60% of the currently available drugs for this purpose are naturally occurring (from 1940 to 2010) [2].

In theory, traditional Chinese medicine defines a tumor as a type of toxin [3], so it is of interest to study the antitumor activity of plants which are used as detoxification agents. Interestingly, we chose several *Cimicifuga* spp. (including *Cimicifuga foetida*, *Cimicifuga dahurica*, and *Cimicifuga heracleifolia*) as targets for the investigation of potential antitumor constituents and a number of cytotoxic 9,19-cycloartane triterpenes are reported both from the aerial parts and roots of these herbal sources [4–12]. Moreover, preliminary structure–activity relationships (SAR) of the compounds with the cimigenol-skeleton were proposed based on the analysis of related bioassay results [12].

C. yunnanensis is only distributed in the south-west area of China. Previous pharmaceutical studies revealed that the roots of *C. yunnanensis* contained three cytotoxic 9,19-cycloartane triterpenes inducing apoptosis of MCF-7 cells via p53-dependent mitochondrial pathway [13]. More recently, a series of cytotoxic 9,19-cycloartane triterpenes against $p53^{N236S}$ mouse embryonic fibroblasts were isolated from the aerial parts of this plant [7]. Motivated by a search for additional bioactive metabolites, the aerial parts of *C. yunnanensis* from Li Tang County, Sichuan Province of China were further studied. As a result, another six new compounds, yunnanterpene G (1), 26-methoxy-acteol-12(18)-en (2), 7β-hydroxy-23-epi-acteol-3-O-α-L-arabinopyranoside (3), 7β-hydroxy-23-epi-acteol-3-O-β-D-xylosepyranoside (4), 25-methoxy-24-O-acetylisohurinol (5), and 15,16-seco-shengmanol C (6) were isolated and identified (Fig. 1). All compounds were evaluated for their cytotoxicities toward human HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines by the MTT method [14,15]. Described herein are the isolation, structure elucidation, and biological activities of the aforementioned compounds.

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2. Experimental

2.1. General

^{1}H and ^{13}C-DEPT-135 NMR spectra were recorded in pyridine-d_5 on Bruker DRX-500 and Avance III-600 MHz spectrometers (Bruker, Zűrich, Switzerland). Optical rotations were measured in MeOH with a Horiba SEAP-300 polarimeter. Mass spectra were performed on a VG Autospec-3000 spectrometer. Infrared spectra were recorded on a Shimadzu IR-450 instrument as KBr pellets. Thin-layer chromatography was performed on precoated TLC plates (200–250 μm thickness, silica gel 60 F_{254}, Qingdao Marine Chemical, Inc.), and spots were visualized by heating after spraying with 10% aqueous H_2SO_4. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a YMC-Pack Pro C_{18} RS 10 mm × 250 mm column. Silica gel (200–300 mesh, Qingdao Marine Chemical, Inc.), LiChroprep RP-18 (40–63 μm, Merck), and Sephadex LH-20 (20–150 μm, Pharmacia) were used for column chromatography (CC).

2.2. Plant materials

The aerial parts of *C. yunnanensis* were collected in Li Tang county, Sichuan Province, People's Republic (PR) of China, in September 2013 and were identified by Prof. Zongyu Wang, Kunming Institute of Botany, Chinese Academy of Science. A voucher specimen has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in the West China, Kunming Institute of Botany, Chinese Academy of Science, RP China.

2.3. Extraction and isolation

The air-dried and milled aerial parts of *C. yunnanensis* (1.0 kg) were extracted three times with MeOH (3 × 3 L × 24 h) and afforded a residue (119 g) at room temperature and after vacuum evaporation at 50 °C. The extract was subjected to silica gel column chromatography (CC) (2 kg, 200–300 mesh, 10 × 150 cm) and eluted with gradient $CHCl_3$/MeOH [100:0 (2 L), 50:1 (4 L), 20:1 (4 L), 10:1 (4 L), 0:100 (3 L)], to give fractions A (16.7 g), B (22.7 g), C (18.5 g), D (19.3 g), E (20.7 g) after deducting the solvents. Fraction B gave five sub-fractions (B-1 to B-5) after eluting the gradient with MeOH/H_2O (from 50:50 to 100:0) on RP-18 CC (500 g, 200–300 mesh, 6 × 50 cm). Fraction B-2 was further purified on silica gel CC with repeated gradient elution ($CHCl_3$/Me_2CO, from 20:1 to 10:1), subsequently separated by semipreparative HPLC (eluted with CH_3CN/H_2O, gradient from 60:40 to 85:15) to afford **1**(1.7 mg). Fraction B-4 was subjected to silica gel CC (60 g, 200–300 mesh, 5 × 40 cm), which was eluted with $CHCl_3$/Me_2CO (10:1), then subsequently purified by semipreparative HPLC (eluted with CH_3CN/H_2O, gradient from 65:45 to 90:10) to obtain **2** (1.3 mg), **5** (6.7 mg) and **6** (1.6 mg). Fraction C was fractionated with RP-18 CC (500 g, 40–63 μm, 6 × 50 cm), and eluted with MeOH/H_2O (gradient from 50:50 to 90:10) to give four sub-fractions (C-1 to C-4). Fraction C-4 was chromatographed on a silica gel CC (50 g, 200–300 mesh, 5 × 40 cm) with gradient elution (CH_3Cl/Me_2CO) from 10:1 to 8:1, followed by semipreparative HPLC (eluted with CH_3CN/H_2O, gradient from 50:50 to 70:30) to yield **3** (5.5 mg) and **4** (5.3 mg).

Compound **1**: White amorphous powder; $[\alpha]_D^{27}$ + 35.99 (c 0.10, MeOH); HR-ESI-MS m/z 509.3259 $[M + Na]^+$, ($C_{30}H_{46}O_5Na^+$; calcd. 509.3242). IR (KBr) ν_{max} 3441.11, 2961.98, 2928.21, 1632.45, 1065.50, 1020.34 cm^{-1}. For ^{1}H (C_5D_5N, 500 MHz) and ^{13}C–NMR (C_5D_5N, 125 MHz) spectra see Tables 1 and 2.

Compound **2**: White amorphous powder; $[\alpha]_D^{27}$ − 7.3 (c 0.09, MeOH); HR-EI-MS m/z 498.3364 $[M]^+$, ($C_{31}H_{46}O_5^+$; calcd. 498.3345). IR (KBr) ν_{max} 3445.11, 2938.98, 2873.21, 1637.11, 1463.09, 1378.25, 1189.09, 1092.34, 989.32 cm^{-1}. For ^{1}H (C_5D_5N, 600 MHz) and ^{13}C–NMR (C_5D_5N, 150 MHz) spectra see Tables 1 and 2.

Compound **3**: White amorphous powder; $[\alpha]_D^{27}$ − 56.7 (c 0.06, MeOH); HR-EI-MS m/z 618.3757 $[M]^+$, ($C_{35}H_{54}O_9^+$; calcd.618.3768). IR (KBr) ν_{max} 3435.11, 2964.98, 2871.21, 1633.34, 1645.23, 1465.30, 1073.34 cm^{-1}. For ^{1}H (C_5D_5N, 500 MHz) and ^{13}C–NMR (C_5D_5N, 125 MHz) spectra see Tables 1 and 2.

Compound **4**: White amorphous powder; $[\alpha]_D^{27}$ − 60.78 (c 0.06, MeOH); HR-EI-MS m/z 618.3751 $[M]^+$, ($C_{35}H_{54}O_9^+$; calcd. 618.3768). IR (KBr) ν_{max} 3430.23, 2958.55, 2930.18, 1071.02 cm^{-1}. For^{1}H (C_5D_5N, 600 MHz) and ^{13}C–NMR (C_5D_5N, 150 MHz) spectra see Tables 1 and 2.

Compound **5**: White amorphous powder; $[\alpha]_D^{27}$ + 32.4 (c 0.08, MeOH); HR-EI-MS m/z 544.3766 $[M]^+$, ($C_{33}H_{52}O_6^+$; calcd. 544.3764). IR (KBr) ν_{max} 3741.71, 2970.18, 2872.39, 1745.71, 1724.16, 1246.88, 1091.49, 1025.24 cm^{-1}. For ^{1}H (C_5D_5N, 500 MHz) and ^{13}C–NMR (C_5D_5N, 125 MHz) spectra see Tables 1 and 2.

Compound **6**: White amorphous powder; $[\alpha]_D^{27}$ − 32.80 (c 0.08, MeOH); HR-EI-MS m/z 504.3453 $[M]^+$, ($C_{30}H_{46}O_5^+$; calcd. 504.3451). IR (KBr) ν_{max} 3436.34, 2957.61, 2874.98, 1716.21, 1453.50, 1368.34, 1202.34 cm^{-1}. For ^{1}H (C_5D_5N, 500 MHz) and ^{13}C–NMR (C_5D_5N, 125 MHz) spectra see Tables 1 and 2.

2.4. Hydrolysis and identification of the sugar residue in compounds 3 and 4

Compounds **3** and **4** (4 mg of each), were individually dissolved in MeOH (5 mL) and refluxed with 0.5 N HCl (3 mL) for 4 h. Each reaction mixture was diluted with H_2O and extracted with $CHCl_3$ (3 × 10 mL). Each aqueous layer was then neutralized with Ag_2CO_3 and filtered the precipitate to afford a monosaccharide, which had an R_f (EtOAc/$CHCl_3$/MeOH/H_2O, 3:2:2:1), and measurement of optical rotations afforded specific rotation $[\alpha]_D^{27}$ + 82.78 (c 0.05, MeOH), $[\alpha]_D^{27}$ + 24.3 (c 0.10, H_2O), corresponding to L-arabinose and D-xylose (Sigma-Aldrich), respectively.

2.5. Cytotoxicity bioassay

Five kinds of human cancer cell lines, including human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7 and colon cancer

Table 1
^{1}H NMR data of compounds in pyridine-d_5 at 500 MHz (compounds **1**, **3**, **5**–**10**) and 600 MHz (compound **2**, **4**) (δ ppm, J Hz, [a] signals overlapped).

No.	1	2	3	4	5	6	7	8	9	10
1	1.39 m	1.60 m	1.55 m	1.57 m	1.60 m (2H)	1.65 m	1.71 m	1.11	1.53 m	1.61
	0.80	1.05	1.23 m	1.26 m		1.32 m	1.43	1.52	1.14 m	1.32 m
2	1.88 m	2.05 m	2.35 m	2.34 m	1.98 m	2.02 m	2.28 m	1.84	1.99 m	2.32 brd (9.6)
	1.74 m	1.97 m	1.91 m	1.96 m	1.86 brd (12.4)	1.89 dd (13.1, 4.9)	2.62 m	2.28	1.86 dd (3.2, 10.0)	1.89 t (13.8)
3	3.43 brd (8.0)	3.57 brd (8.4)	3.48 dd (11.6, 3.9)	3.50 dd (11.7, 4.1)	3.53 brd (11.1)	3.56 m		3.44 dd (3.4, 10.8)	3.48 m	3.49 dd (11.6, 4.1)
5	1.09[a]	1.17 brd (12.2)	1.56 m	1.59 m	1.25 dd (12.3, 3.7)	1.52 m	1.56 dd (12.1, 4.1)	1.22 brd (10.1)	1.25 dd (3.2, 10.0)	1.51
6	1.43 m	1.37 m	1.87 m	1.90 m	1.51 m	1.44 m	1.37 m	0.60 brdd (13.6, 11.1)	1.51 m	1.38 m
	0.56 m	0.67 m	1.19 m	1.22 m	0.65 q (12.5)	1.00	0.84	1.40	0.65 m	0.91 m
7	1.39 m	1.46 m	3.72 m	3.75 brt (10.6)	2.28 m	1.41 m	0.97	0.91	2.27 m	1.38 m
	0.69 m	0.79 m			1.05[a]	1.20 m	1.30 m	1.21	1.95 m	1.17 m
8	1.38 m	2.07 m	1.87 m	1.90 m	1.73 dd (12.9, 3.9)	2.45 brt (8.8)	1.65	1.59 brd (12.4)	1.72 brd(10.4)	2.41 m
11	2.07 d (18.2)	1.97 m	1.95 m	1.99 m	1.97 m	1.62[a] 1.62[a]	1.84	1.17 brd (15.5)	2.23 m	1.47 m (2H)
	1.58 d (15.0)	1.76 m	1.15 m	1.19 m	1.05[a]		2.57 dd (8.4, 15.0)	2.70 dd (8.6, 15.5)	1.05[a]	
12			1.58 m	1.59 m	1.52 m	1.67 m	4.06 m	5.10 brd (5.3)	1.58 m	1.63
			1.49 m	1.52 m	1.15 m	1.42 m			1.37 m	
13	2.16 brd (6.2)	2.23 m								1.40 m
15	2.74 t (13.2)	2.01	2.68 dd (13.5, 7.9)	2.68 dd (13.5, 7.9)		9.97 s	1.88 dd (8.1, 12.7)	1.78		9.93 s
	1.51 m	1.64 m	2.19 dd (13.6, 6.2)	2.20 dd (13.7, 6.4)			1.64 dd (5.4, 11.2)	1.89 dd (6.7, 11.8)		
16	4.16 m	4.57 brs	4.26 m	4.28 m	3.79 d (11.6)		4.71 dd (7.8, 14.7)	4.23 brd (6.7)	3.79 d (9.6)	
17	2.05 m	2.16 m	1.55 m	1.59 m	1.55 m	2.76 d (5.7)	1.90 dd (15.2, 7.0)	1.76	1.52 m	2.75 d (5.2)
18	4.85 d (2.0)	5.28 brs	1.32 s	1.32 brs	1.18 s	1.56 s	1.39 s	1.40 s	1.18 s	1.52 s
	4.73 d (2.0)	5.09 brs								
19	0.45 d (4.2)	0.67 brs	0.67 d (3.9)	0.69 d (3.8)	0.53 d (3.8)	0.70 d (4.3)	0.72 d (4.1)	0.19 d (3.8)	0.52 d (3.6)	0.60 d (4.3)
	0.01 d (4.2)	0.28 brs	0.20 d (3.9)	0.21 d (4.1)	0.28 d (4.1)	0.06 d (4.6)	0.47 d (4.1)	0.52 d (3.8)	0.29 d (3.6)	−0.03 d (4.5)
20	2.51 m	2.30 m	2.23 m	2.25 m	1.79 m	2.08 m	2.13 m	2.23	1.80 m	2.06 m
21	0.75 d (6.5)	1.23 brd (7.1)	0.98 d (7.0)	0.99 d (6.5)	0.91 d (6.4)	1.01 d (6.4)	1.46 d (6.5)	1.00 d (6.7)	0.91 d (7.2)	0.99 d (6.0)
22	1.84 m	2.43 dd (13.9, 5.8)	1.55 m	1.58 m	1.66 brd (13.3)	2.56 dd (14.1, 5.8)	1.70 m	1.44 brd (12.4)	1.72 brd (10.4)	2.04 m
	1.55 m	1.65 m	1.38	1.42	1.40	1.78 m	2.85 dd (13.8, 2.7)	1.59 brd (12.4)	1.44 m	1.87 m
23					4.02 d (11.6)	5.00 brd (10.8)			4.25 brd (11.2)	5.04 d (11.5)
24	4.19 brs	3.74 s	3.61 s	3.63 s	5.28 d (2.1)	3.98 s	4.60 d (6.1)	4.04 s	5.31 d (1.6)	3.73 s
26	4.14 brs (2H)	5.05 s	3.99 d (10.2)	4.01 d (10.3)	1.42 s	1.55 s	4.32 d (8.6)	3.62 d (10.4)	1.61 s	1.55 s
			3.57 d (10.2)	3.59 d (10.5)			4.10 d (8.6)	4.05 d (10.4)		
27	1.71 s	1.50 s	1.42 s	1.44 s	1.43 s	1.60 s	1.76 s	1.45 s	1.61 s	1.59 s
28	0.78 s	0.89 s	1.06 s	1.06 s	1.01 s	1.61 s	0.82 s	0.83 s	1.01 s	1.60 s
29	0.91 s	1.08 s	1.00 s	1.02 s	1.05 s	1.06 s	0.98 s	1.29 s	1.19 s	0.97 s
30	1.09[a] s	1.21 s	1.28 s	1.32 brs	1.18 s	1.16 s	1.12 s	0.99 s	1.05[a] s	1.23 s
			−Arabinose	−Xylose			−Xylose	−Xylose		−Xylose
1′			4.78 d (7.1)	4.85 d (7.5)			4.86 d (7.6)	4.83 d (7.2)		4.90 d (7.6)
2′			4.45 t (7.9)	4.04 t (8.5)			4.05 t (7.8)	4.01 t (8.6)		4.06 t (8.1)
3′			4.16 brd (8.8)	4.17 t (8.7)			4.18 t (8.7)	4.14 t (8.6)		4.20 t (8.8)
4′			4.32 brs	4.27 m			4.24 m	4.21 m		4.26 m
5′			4.30 m	4.38 dd (11.2, 5.1)			3.77 t (10.0)	3.73 t (10.2)		4.43 dd (11.2,5.2)
			3.80 brd (10.8)	3.75 brt (10.6)			4.38 dd (11.2, 5.1)	4.35 dd (10.5, 4.2)		
12-OCH_3					2.21 s		2.15 s	2.13 s		3.83 t (10.7)
24-OAc					3.21 s			2.16 s		
25-OCH3										
26-OCH3		3.47 s								

Table 2
^{13}C-DEPT data of compounds **1**–**10** in pyridine-d_5 at 125 MHz (**1**, **3**, **5**–**10**) and 150 MHz (**2**, **4**) (δ ppm).

NO.	1	2	3	4	5	6	7	8	9	10
1	34.46 t	33.66 t	31.74 t	31.77 t	31.45 t	30.63 t	33.30 t	32.0 t	32.7 t	30.42 t
2	31.33 t	31.63 t	29.83 t	29.94 t	31.31 t	29.98 t	37.40 t	30.0 t	31.3 t	29.49 t
3	78.16 d	78.54 d	88.25 d	88.26 d	77.90 d	77.62 d	214.97 s	88.1 d	77.9 d	88.00 d
4	40.95 s	41.28 s	41.02 s	41.07 s	41.07 d	41.13 s	50.07 s	41.2 s	41.1 s	41.30 s
5	47.39 d	47.79 d	46.25 d	46.28 d	47.31 d	43.86 d	48.04 d	47.0 d	47.3 d	44.15 d
6	20.58 t	20.38 t	26.65 t	26.70 t	21.08 t	18.79 t	21.00 t	20.4 t	21.0 t	18.55 t
7	28.03 t	24.94 t	70.03 d	70.07 d	26.18 t	21.75 t	25.64 t	25.7 t	26.2 t	21.68 t
8	55.25 d	47.08 d	55.11 d	55.13 d	43.71 d	38.98 d	45.64 d	45.6 d	43.7 d	38.70 d
9	25.57 s	25.74 s	20.03 s	20.03 s	20.32 s	20.76 s	21.71 s	20.2 s	20.0 s	20.79 s
10	27.56 s	33.70 s	27.06 s	27.10 s	27.38 s	25.91 s	26.34 s	26.8 s	27.4 s	25.71 s
11	36.33 t	33.70 t	31.89 t	31.93 t	26.01 t	26.83 t	40.58 t	36.7 t	26.0 t	26.95 t
12	147.80 s	147.48 s	33.26 t	33.30 t	33.23 t	32.52 t	72.14 d	77.1 d	31.4 d	32.64 t
13	54.17 d	54.37 d	45.27 s	45.30 s	55.08 s	47.43 s	50.46 s	48.8 s	40.0 s	47.20 s
14	45.31 d	46.17 s	46.49 s	46.52 s	39.83 s	55.02 s	47.59 s	47.9 s	55.1 s	55.30 s
15	39.41 t	38.48 t	46.44 t	46.48 t	213.76 s	207.78 s	44.22 t	44.2 t	213.9 s	207.38 s
16	73.64 d	68.68 d	75.22 d	75.25 d	84.30 d	175.10 s	72.84 d	74.5 d	84.3 d	174.86 s
17	52.45 d	46.49 d	56.18 d	56.21 d	52.40 d	55.55 d	57.01 d	56.2 d	52.4 d	55.49 d
18	116.07 t	110.90 t	20.25 q	20.29 q	20.32 q	18.03 q	12.97 q	13.5 q	20.3 q	18.21 q
19	23.94 t	25.72 t	29.03 t	29.07 t	31.31 t	22.24 t	29.07 t	29.5 t	31.3 t	22.07 t
20	26.54 d	27.49 d	23.62 d	23.65 d	33.23 d	28.35 d	26.37 d	23.3 d	33.3 d	28.48 d
21	19.60 q	21.33 q	20.91 q	20.95 q	19.99 s	24.87 q	21.75 q	21.7 q	20.0 q	24.80 q
22	41.82 t	34.37 t	37.78 t	37.81 t	38.78 t	34.60 t	37.47 t	37.6 t	38.8 t	36.71 t
23	105.07 s	105.69 s	106.15 s	106.18 s	78.48 d	80.47 d	110.87 s	105.9 s	79.1 d	78.57 d
24	86.86 d	65.29 d	62.12 d	62.16 d	77.12 d	78.71 d	83.67 d	62.5 d	79.8 d	79.86 d
25	78.28 s	62.87 s	62.41 s	62.65 s	77.01 s	71.85 s	79.78 s	62.3 s	72.1 s	72.43 q
26	78.77 t	105.30 d	68.02 t	68.05 t	21.51 q	27.40 q	77.19 t	67.1 t	26.8 q	26.02 q
27	23.51 q	12.90 q	14.25 q	14.29 q	23.30 q	27.57 q	20.83 q	14.3 q	28.4 q	28.98 q
28	20.00 q	25.97 q	19.39 q	19.43 q	17.53 q	14.43 q	19.58 q	19.7 q	17.6 q	14.73 q
29	14.88 q	15.39 q	15.25 q	15.32 q	14.81 q	14.13 q	20.78 q	25.7 q	26.1 q	14.38 q
30	26.26 q	26.87 q	25.76 q	25.79 q	26.18 q	25.18 q	22.58 q	15.3 q	14.9 q	25.35 q
			3-Ara	3-Xyl				3-Xyl		3-Xyl
1′			107.29 d	107.50 d				107.5 d		107.48 d
2′			72.92 d	75.54 d				75.6 d		75.60 d
3′			74.59 d	78.57 d				78.7 d		78.61 d
4′			69.43 d	71.26 d				71.3 d		71.31 d
5″			66.62 t	67.10 t				67.2 t		67.14 t
12-O$\underline{C}$OCH$_3$								170.7 t		
12-OC$\underline{O}$CH$_3$								21.4 q		
24O$\underline{C}$OCH$_3$									171.1 s	
24-OC$\underline{O}$CH$_3$									21.0 q	
25-OCH$_3$					49.25 q					
26-OCH$_3$		55.68 q								

SW480, were used in the cytotoxic assay. Cells were cultured in DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA), in 5% CO_2 at 37 °C. The cytotoxicity assay was conducted according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates [14,15]. Exactly 100 μL of adherent cells was seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before addition of test compounds, while suspended cells were seeded just before drug addition with initial density of 1 × 105 cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.064, 0.32, 1.6, 8 and 40 μm triplicates for 48 h, with cisplatin (Sigma, USA) as a positive control. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method [16].

3. Results and discussion

Compound **1** was obtained as white amorphous powder. The HR-ESI-MS of **1** showed an ion peak at *m/z* 509.3259 $[M + Na]^+$ (calcd. 509.3242), consistent with the molecular formula of $C_{30}H_{46}O_5$, requiring eight rings or sites of unsaturation. The IR spectrum showed absorptions for the hydroxyl group at 3441.11 cm^{-1} and double bond at 1632.45 cm^{-1}, respectively. The ^{1}H NMR spectrum (Table 1) showed the presence of the characteristic cyclopropane methylene signals at δ_H 0.45 and 0.01(1H each, d, $J = 4.2$ Hz), a sec-methyl signal at δ_H 0.75 (d, $J = 6.5$ Hz) and four tert-methyl groups at δ_H 1.09–1.71. Moreover, the ^{13}C NMR spectrum (Table 3) of **1** displayed 30 carbon resonances, which were assigned by DEPT and HSQC experiments as 5 methyls, 10 methylenes (including a terminal olefinic methylene), 8 methines (including three oxygenated ones) and 7 quaternary carbons (including two oxygenated ones and a terminal olefinic one). The above data suggested that **1** was a highly oxygenated 9,19-cycloartane triterpene with seven rings and an exocyclic double bond.

Comparison of the NMR data of **1** with those of yunnanterpene A (**7**) [7] suggests that they might be structurally similar, except for the major differences at rings A and C. A methine carbon resonance at δ_C 72.14, a quaternary carbon at δ_C 50.46, and a carbonyl group at δ_C 214.97, ascribed

Table 3
Cytotoxicity[a] (IC_{50}, μm ± SD) of compounds isolated from the aerial parts of *C. yunnanensis*.

Compounds	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	3.8 ± 0.4	6.7 ± 0.4	8.3 ± 0.7	8.6 ± 0.5	10.3 ± 1.2
2	27.8 ± 1.5	>40	>40	>40	>40
3	1.2 ± 0.4	11.2 ± 0.9	9.8 ± 0.8	13.2 ± 0.6	>40
4	3.1 ± 0.5	10.8 ± 0.8	12.5 ± 1.1	12.3 ± 1.4	23.1 ± 0.9.
5	18.2 ± 1.3	17.9 ± 0.7	23.4 ± 1.6	22.5 ± 1.3	20.3 ± 1.7
6	>40	>40	>40	>40	>40
Cisplatin	1.3 ± 0.3	6.8 ± 0.7	6.1 ± 0.6	17.6 ± 1.3	14.5 ± 1.6

[a] Cytotoxicity is the average (n = 3) of calculated IC_{50}'s; the purity of compounds 1–26 are greater than 95%, and cisplatin is greater than 99%.

to C-12, C-13 and C-3, respectively, was absent from the ^{13}C NMR spectrum of **1**. Instead, a methine carbon (δ_C 45.31), a terminal double-bond carbon (δ_C 147.80, 116.07) and a hydroxymethine (δ_C 78.16) were observed. Based on the above distinguishing differences, we deduced that for compound **1**: (1) a carbonyl carbon was replaced by a hydroxyl group at C-3; and (2) CH_3-18 was shifted from C-13 to C-12 and formed double-bond between C-12 and C-18 through dehydrogenation. These deductions were confirmed by the HMBC (Fig. 2) correlation from H-29/H-30 to C-3 (δ_C 78.16), and from the characterized proton resonance of H-19 to C-11 (δ_C 36.33), H-11/H-13 to C-12 (δ_C 147.80) and C-18 (δ_C 116.07), respectively.

The cross-peaks associated with H-16 and H-17 to CH_3-28 in the ROESY spectrum (Fig. 2) confirmed their relative orientation as α. In addition, H-13 and H-24 showed correlation with H-20 (δ_H 2.51) which indicated a β-orientation of H-13 and α-orientation of OH-24. The orientation of CH_3-27 was assigned as α by the correlation of CH_3-27 (δ_H 1.71) and CH_3-21 (δ_H 0.75). Furthermore, the configuration of C-23 was deduced as *S* by identical ROESY correlations and comparison chemical shifts of rings F and G of **1** with those of **7** (the structure of **7** was confirmed by X-ray crystallography). Thus, the structure of yunnanterpene G (**1**), was determined as (23*R*,24*R*,25*S*)-16β,23:23,26-diepoxy-18(13→12)abeo-12β -acetoxy-3β,24,25-trihydroxy-cycloartane.

Compound **2** was isolated as white powder and gave a molecular formula of $C_{31}H_6O_5$, as determined by HR-EI-MS ([M^+] *m/z* 498.3364, calcd. 498.335), requiring eight rings or sites of unsaturation. The IR spectrum exhibited absorptions for hydroxyl group at 3445.11 cm^{-1} and olefinic carbon group at 1637.11 cm^{-1}, respectively. The ^{13}C-DEPT and HSQC spectra resolved all thirty-one carbon signals as four tertiary methyls, one secondary methyl, one methoxyl, nine

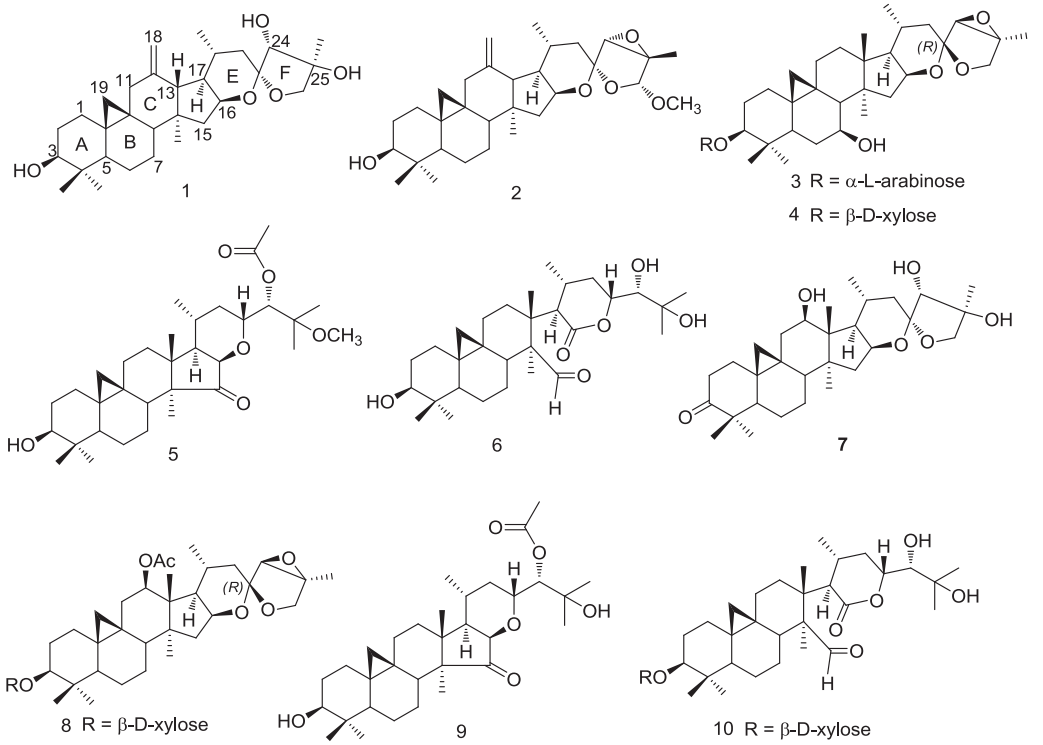

Fig. 1. Structures of compounds (**1**–**6**) isolated from the aerial parts of *C. yunnanensis* and referenced in the paper (**7**–**10**).

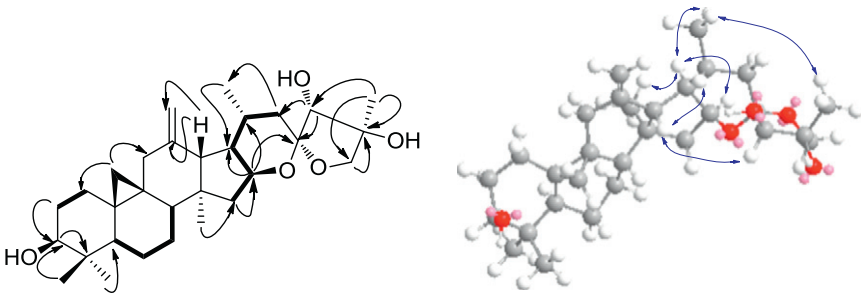

Fig. 2. Major HMBC (→), ^{1}H–^{1}H COSY (▁), and ROESY (↔) correlations of compound **1.**

methylenes, nine methines, and seven quaternary carbons. The NMR spectroscopic data for **2** resembled those of **1**, with major differences in ring F. For **2**, the resonances associated with C-24 and C-25 carbons were shifted upfield to δ_C 65.29 and δ_C 62.87 respectively, and the C-26 was shifted downfield to δ_C 105.30, which suggested that ring F contains an epoxypropane unit between C-24 and C-25, and the methoxy group was attached to C-26. These conclusions were further supported by 14 Da molecular weight more than **1** and the HMBC correlations for δ_H 3.47 ($-OCH_3$) to δ_C 105.30 (C-26).

The ROESY correlation of H-28/H-16, H-16/H-17 and H-28/H-13 inferred that protons H-13, H-16 and H-17 all possessed an α-orientation, while the H-24 and 26-methoxyl were assigned as β-orientation by the correlation of H-24, CH_3-27, H-26 and H-22β (δ_H 2.43). Accordingly, compound **2** was characterized as 26-methoxy-acteol-12(18)-en.

Compound**s 3** and **4** had the same molecular formula of $C_{35}H_{54}O_9$, which was deduced from the HR-EI-MS {*m/z* 618.3757 $[M]^+$ (calcd. 618.3768) for **3** and 618.3751 $[M]^+$ (calcd. 618.3768) for **4**, respectively}. The ^{1}H NMR spectrum of **3** revealed the typical cyclopropane methylene proton resonances at δ_H 0.67 and 0.20 (1H each, d, $J = 3.9$ Hz), together with five tertiary methyls between δ_H 1.00 and 1.42, one secondary methyl at δ_H 0.98 (d, $J = 7.0$ Hz), and an anomeric proton resonance at δ_H 3.48 (dd, $J = 11.6$, 3.9 Hz), suggesting that **3** was a 9,19-cycloartane-type triterpene glycoside. The location of the sugar moiety at C-3 was inferred from the HMBC correlation between the anomeric proton at δ_H 3.48 (dd, $J = 11.6$, 3.9 H z) and the methine signal at δ_C 88.25 (C-3). In addition, the sugar obtained following acid hydrolysis was identified as L-arabinose by comparison of its TLC and specific rotation with that of a standard. The NMR data (Tables 1 and 2) of the aglycone part of **3** resembled those of 23-*epi*-26-deoxyactein (**8**) [17], except for the absence of the acetyl group at C-12, and an additional hydroxyl group was attached to C-7. The correlations of H-6 (δ_H 1.87 and 1.19) and H-8 (δ_H 1.87) with the hydroxymethine proton (δ_H 3.72), and H-11 (δ_H 1.95 and 1.15) with the methylene protons (δ_H 1.58 and 1.49) in the ^{1}H–^{1}H COSY spectrum further confirmed the above deduction. The ROESY spectrum showed correlations of H-3/H-5 and CH_3-28/H-7 suggesting a β-orientation for the substituents at C-3 and C-7. Thus, **3** was determined to be 7β-hydroxy-23-*epi*-acteol-3-*O*-α-L-arabinopyranoside. Furthermore, Compound **4** shared the same skeleton with **3** by careful analysis of the ^{1}H and ^{13}C NMR spectra, with the major differences being associated with the sugar moiety. The sugar was identified as D-xylose by comparison of its TLC and specific rotation with that of a standard following acid hydrolysis. Thus, compound **4** was determined to be 7β-hydroxy-23-*epi*-acteol-3-*O*-β-D-xylosepyranoside.

Compound **5** was obtained as white powder. The molecular formula $C_{33}H_{52}O_6$ was deduced from HR-EI-MS ($[M]^+$ m/z 544.3766, calcd. 544.3764), indicating eight rings or sites of unsaturation. The IR absorptions at 3741.71, and 1745.71 and 1724.16 cm^{-1} suggested the presence of hydroxyl and carbonyl groups, respectively. The NMR data of **5** resembled those of 24-*O*-acetylisodahurinol (**9**) [8] (Tables 1 and 2), except for an additional methoxyl at C-25, which was supported by 14 Da molecular weight more than **9** and the HMBC correlation between methoxyl protons and C-25. The configuration of C-24 was deduced as *S* by comparison of the coupling constants of H-24 ($J_{H\text{-}24/H\text{-}23} = 2.4$ Hz) with those of dahurinyl diacetate (9.0 Hz) and isodahurinyl diacetate (2.4 Hz) [18]. Accordingly, compound **5** was assigned as 25-methoxy-24-*O*-acetylisohurinol.

Compound **6** was purified as white powder, with the molecular formula $C_{30}H_{48}O_6$, given by the HR-EI-MS ($[M]^+$ m/z 504.353, calcd. 504.351). The IR spectrum showed the presence of hydroxyl (3436.34 cm^{-1}) and carbonyl (1716.21 cm^{-1}) groups. The NMR data for compound **6** (Tables 1 and 2) resembles those of 15,16-seco-cimiterpenes B (**10**) [7]. However, the major difference was with the absence of a sugar moiety, suggesting that **6** is the aglycone of compound **10**. Furthermore, correlations between H-20 and H-23, and H-21 and H-17 in ROESY spectrum, indicated that H-17 has the α-orientation, and H-20 and H-23 both had the β-orientation, sharing the same relative configuration with the aglycone of 15,16-seco-cimiterpenes B (**10**) [7]. Accordingly, compound **6** was elucidated as 15,16-*seco*-shengmanol C.

Cimicifuga spp. is a natural resource for 9,19-cycloartane triterpenoids. At present, different types of 9,19-cycloartane triterpenes, such as cimigenol-type, acteol-type, shengmanol-type, hydroxyshengmanol-type, dahurinol-type, cimiacerol-type, cimilactone-type, and foetidonol-type were identified. In the present study, compounds **1** and **2**, characterized by CH_3-18 shifting from C-13 to C-12, were isolated from the genus for the first time, which further enriched structural diversity of 9,19-cycloartane triterpene in *Cimicifuga* spp.

All isolated compounds were evaluated for their cytotoxicities against human HL-60, SMMC-7721, A-549, MCF-7 and SW480 cell lines. As summarized in Table 3, compound **1** exhibited broad-spectrum and moderate to potent cytotoxicities with IC_{50} values of 3.8, 6.7, 8.3, 8.6, and 10.3 μm, respectively. Compounds **3** and **4** exhibited more selective

activities against HL-60 cells, having IC_{50} values of 1.2 and 3.1 μm, respectively. Compound **5** exhibited weak cytotoxicities to all cell lines, with IC_{50} values ranging from 17.9 to 23.4 μm, while, compounds **2** and **6** were inactive.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2014.05.019.

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