Phytochemistry Letters 18 (2016) 105–112

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Cytotoxic 9,19-cycloartane triterpenoids from the roots of *Cimicifuga foetida* L.

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ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form 10 May 2016
Accepted 6 June 2016
Available online 29 September 2016

Keywords:
Cimicifuga foetida L.
9,19-Cycloartane triterpenoids
Cytotoxicity

ABSTRACT

Eight new cycloartane triterpenoids (**1**–**8**), along with eight known analogues (**9**–**16**), were isolated from the roots of *Cimicifuga foetida* L. Their structures were elucidated by spectroscopic analysis and acidic hydrolysis. All of the new compounds were evaluated for their in vitro cytotoxicity against five human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7 and SW480), and compound **2** showed inhibitory activities with IC_{50} values raging from 2.61 to 3.32 μM.

1. Introduction

The genus *Cimicifuga* (Ranunculaceae family) or *Actaea* contains 28 species, whose roots have been used in traditional medicine since ancient times in China (Compton et al., 1998; Li and Yu, 2006). Currently, three species (*C. heracleifolia*, *C. dahurica*, *C. foetida*) of this genus are listed officially as a cooling and detoxifying remedy in the Chinese Pharmacopoeia 2010, respectively (Pharmacopoeia of the People's Republic of China, 2010). Additionally, the theory of traditional Chinese medicine defines a tumor as a kind of toxin (Tian et al., 2007). So it is interest to explore potential antitumor constituents of *Cimicifuga* based on the theory of TCM and the function of *Cimicifuga*.

Our previous phytochemical investigation led to isolation of a series of new 9,19-cycloartane triterpenes and their glycosides from *C. foetida* L., some of which showed significant cytotoxicities against different tumor cell lines (Nian et al., 2010, 2011, 2012; Lu et al., 2012). As a continuing efforts to study this source, eight new 9,19-cycloartane triterpenoids, namely cimiacerol-1(2)-en-3-one (**1**), 25-*O*-methyl-24-*O*-acetylisodahurinol-3-*O*-β-D-xylopyranoside (**2**), 12-*O*-acetyl-25-anhydrocimicigenol-3-*O*-β-D-xylopyranosyl-3′-*O*-β-D-xylopyranoside (**3**), 2′-*O*- acetylcimigenol-3-*O*-β-D-xylopyranosyl-3′-*O*-β-D-xylopyranoside (**4**), cimilactone K (**5**), 4′,23-*O*- diacetylshengmanol-3-*O*-β-D-xylopyranosyl-3′-*O*-β-D-xylopyranoside (**6**), 26-deoxyacetylacteol- 7(8)-en-3-*O*-β-D-xylopyranosyl-3′-*O*-β-D-xylopyranoside (**7**), and acteol-3-*O*-β-D-xylopyranosyl- 3′-*O*-β-D-xylopyranoside, together with eight known compounds (**9**–**16**) (Fig. 1.) were further isolated. New compounds were tested for their cytotoxicities against human tumor HL-60, SMMC-7721, A-549, MCF-7 and SW480 cell lines, using the MTT assay. We herein reported the isolation, structure elucidation, and biological activities of these compounds.

2. Results and discussion

The molecular formula of compound **1** was identified as $C_{30}H_{44}O_5$ by its HR-EIMS at *m*/*z* 484.4168 $[M]^+$. The UV (268 nm) and IR (1667, 1641 cm^{-1}) spectra of **1** indicated the presence of α,β-unsaturated carbonyl. The analysis of ^1H NMR spectrum of **1** showed the characteristic cyclopropane methylene signals at δ_H 0.62 and 1.15 (each 1H, d, J = 4.4 Hz), two olefinic protons at δ_H 6.77 and 6.16 (each 1H, d, J = 10.1 Hz), a secondary methyl at δ_H 1.24 (3H, d, J = 6.4 Hz), and five tertiary methyls at δ_H 0.83-1.79 (each 3H, s). The ^{13}C NMR and DEPT spectroscopic data (Table 2) of **1** displayed a typical cimiaceroside-type carbon resonances, corresponding to the methylene carbon of the cyclopropane ring at C-19 (δ_C 30.0), three oxymethine carbons at C-16 (δ_C 72.5), C-22 (δ_C 87.2), and C-24 (δ_C 83.6), two oxygened quaternary carbons at C-23 (δ_C 106.4) and C-25 (δ_C 84.0), as well as an α, β-unsaturated carbonyl unit at δ_C 154.3 (d), 127.2 (d), and 204.5 (s). These data were similar to

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http://dx.doi.org/10.1016/j.phytol.2016.06.002
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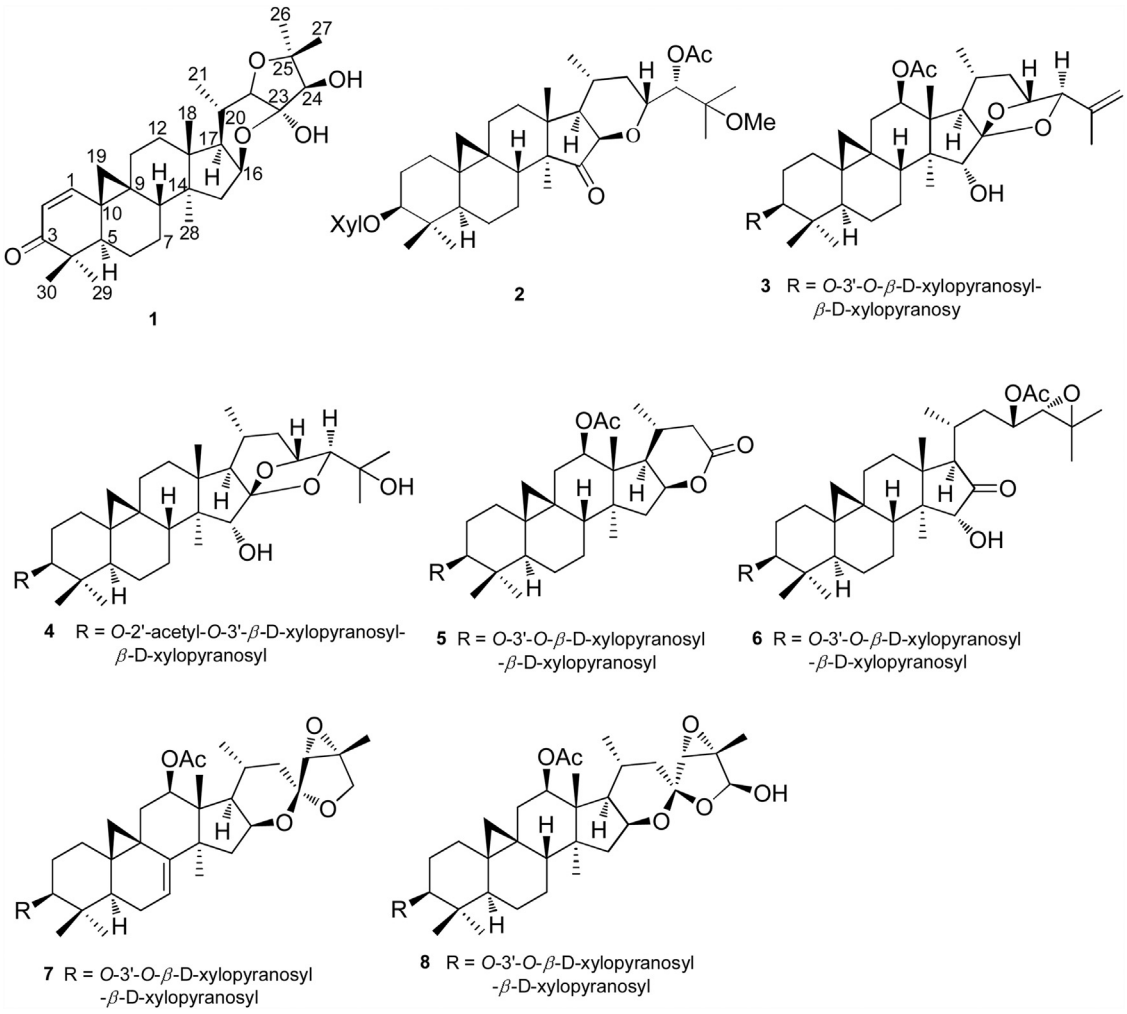

Fig. 1. The structures of compounds **1–16**.

those of **9** (Kusano et al., 1998). However, a detailed comparison of their 1D NMR spectra revealed that the α,β-unsaturated ketone moiety at δ_C 154.3, 127.2, and 204.5 of **1** replaced a saturated system at C-1 (δ_C 30.4, t), C-2 (δ_C 29.6, t), and C-3 (δ_C 88.2, d) of **9**. This deduction was further confirmed by the HMBC correlations (Fig. 2) of H-1 (δ_H 6.77) with C-3 (δ_C 204.5), C-5 (δ_C 45.1), and C-9 (δ_C 24.8), of H-2 (δ_H 6.16) with C-4 (δ_C 46.5) and C-10 (δ_C 30.6), of H_3-29 (δ_H 1.21) and H_3-30 (δ_H 0.97) with C-3, together with the ^1H–^1H COSY correlation of H-1 (δ_H 6.77, J = 10.1 Hz) and H-2 (δ_H 6.16, J = 10.1 Hz).

The cross peaks of H-5 (δ_H 2.05), H-16 (δ_H 4.99), and H-17 (δ_H 1.58) with H_3-28 (δ_H 0.97), of H_3-21 and H_3-26 with H-22 (δ_H 3.91), and of H_3-26 with H-24 in the ROESY spectrum (Fig. 2) randomly indicated H-5, H-16, H-17, H-22, H-24 and H_3-26 to be an α-directed, whereas the associations of H-8 (δ_H 1.92) and H-20 (δ_H 2.30) with H_3-18 (δ_H 1.17) suggested that the H-8 and H-20 were β-orientations. The deshielded chemical shifts of C-22 (δ_C 87.2), C-23 (δ_C 106.4), and C-24 (δ_C 83.6) indicated the 23*S*, 24*R* configurations in **1**, compared to the 23*S*, 24*R* configurations [C-22 (δ_C 86.8), C-23 (δ_C 106.1), and C-24 (δ_C 83.3)] (Kusano et al.,

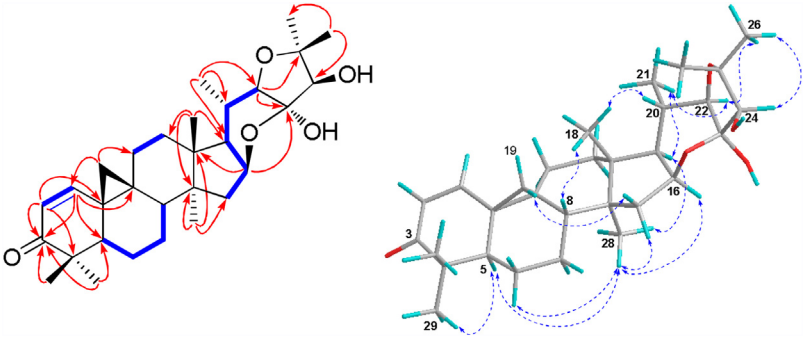

Fig. 2. Major figure HMBC (→), ^1H–^1H COSY (—), and ROESY (↔) correlations of **1**.

Table 1
^{1}H NMR data of Compounds **1–8** in pyridine-d_5 at 600 MHz (δ in ppm, J in Hz).

No.	1	2	3	4	5	6	7	8
1	6.77 d (10.1)	1.17 m	1.09 m	1.20 m	1.08 t (13.1)	1.26 m	1.09 m	1.10 m
		1.53 m	1.56 m	1.57 m	1.48 t (10.8)	1.62 m	1.53 m	1.49 m
2	6.16 d (10.1)	2.38 m	1.87 m	1.88 dd (3.8, 12.5)	1.82 m	1.97 m	1.81 m	1.80 m
		1.94 m	2.26 m	2.24 m	2.23 m	2.34 m	2.16 m	2.20 m
3	–	3.53 dd (4.3, 11.6)	3.48 dd (4.3, 11.6)	3.39 dd (4.4, 11.7)	3.44 dd (4.1, 11.7)	3.50 dd (4.0, 11.8)	3.37 dd (3.7, 11.4)	3.40 dd (4.9, 11.6)
5	2.05 dd (12.7, 3.5)	1.30 dd (4.2, 12.5)	1.28 dd (4.1, 12.4)	1.31 dd (4.4, 12.5)	1.23 m	1.36 m	1.12 m	1.19 m
6	1.38 m	0.60 m	0.72 dd (12.4, 24.9)	0.71 dd (11.6, 24.1)	0.70 dd (12.5, 25.0)	0.76 dd (12.7, 24.9)	1.75 m	0.64 m
	1.09 m	1.46 m	1.59 m	1.53 m	1.49 m	1.61 m	2.08 m	1.38 m
7	1.34 m	0.60 dd (12.3, 24.6)	1.12 m	1.14 m	0.93 m	2.11[a]	5.08[a]	0.87 m
	1.06 m	1.31 m	2.23 m	2.11 m	1.23 m			1.15[a]
8	1.92 dd (9.6, 7.1)	1.71 dd (4.2, 12.9)	1.75 dd (4.4, 24.9)	1.68 m	1.59 dd (5.0, 11.6)	1.86 m	–	1.57 m
11	1.56 m	1.02 m	1.14 m	1.03 m	1.15 dd (3.1, 16.1)	1.16 m	1.21 m	1.16[a]
	1.10 m	2.27 m	2.95 dd (9.4, 16.1)	2.06 m	2.71 dd (9.0, 16.1)	2.14 m	2.91 dd (9.6, 16.0)	2.69 dd (9.1, 16.1)
12	1.58 m	1.35 m	5.26 dd (2.5, 9.4)	1.53 m	5.07 m	1.81 m	5.20 d (8.4)	5.07 dd (3.9, 8.6)
	1.49 m	1.60 m		1.69 m		18.1 m		
15	1.88 dd (12.0, 7.9)	–	4.45 s	4.31 m	1.82 m	4.36 br s	2.08 (2H, m)	1.72 m
	1.61 m				2.00 m			1.89 m
16	4.99 dd (12.0, 7.9)	3.79 d (11.8)	–	–	4.80 dd (7.4, 19.5)	–	4.30 m	4.6 dd (7.2, 14.6)
17	1.58 m	1.57 m	1.64 m	1.52 m	2.15 d (8.5)	2.34 d (6.4)	1.75 m	1.76 m
18	1.17 s	1.20 s	1.32 s	1.16 s	1.24 s	1.40 s	1.46 s	1.34 s
19	1.15 d (4.4)	0.26 d (4.1)	0.29 d (4.0)	0.24 d (4.0)	0.18 d (3.8)	0.29 d (3.5)	0.46 d (7.4)	0.19 d (4.0)
	0.62 d(4.4)	0.49 d (3.8)	0.58 d (3.5)	0.47 d (3.7)	0.55 d (3.2)	0.56 d (3.5)	0.82 m	0.54 d (3.6)
20	2.30 m	1.81 m	1.64 m	1.68 m	2.0 m	2.11 m	2.20 m	1.81 m
21	1.24 d (6.4)	0.92 d (6.4)	0.95 d (5.2)	0.87 d (6.5)	0.97 d (6.2)	1.25 d (6.5)	0.99 d (6.4)	0.94 d (6.3)
22	3.91 d (10.7)	1.42 m	1.00 m	1.05 m	2.27 t (13.8)	1.75 m	1.41 d (9.4)	1.66 m
		1.67 m	2.24 m	2.30 m	2.47 dd (3.0, 14.7)	2.67 t (12.8)	1.57 m	2.20 m
23	–	4.03 m	4.31 d (9.4)	4.79 d (10.4)	–	5.39 br t (8.4)	–	–
24	4.21 br s	5.30 d (2.2)	4.19 br s	3.80 br s		3.02 d (8.4)	3.66 br s	3.93 br s
26	1.79 s	1.43 s	4.90 s	1.49 s	–	1.34 s	3.60 d (10.4)	5.74 br s
			5.38 s				4.04 m	
27	1.71 s	1.46 s	1.86 s	1.52 s	–	1.36 s	1.45 s	1.77 s
28	0.83 s	1.46 s	1.22 s	1.20 s	0.84 s	1.22 s	1.02 s	0.77 s
29	1.21 s	1.33 s	1.32 s	1.10 s	1.32 s	1.24 s	1.29 s	1.28 s
30	0.97 s	1.04 s	1.04 s	0.98 s	1.01 s	1.08 s	0.97 s	0.98 s
CH_3CO-12			2.13 s		2.14 s		2.16 s	2.13 s
CH_3CO-23						2.04 s		
CH_3CO-24		2.17 s						
CH_3O-25		3.23 s						
sugar								
1′		4.88 d (7.6)	4.82 d (7.6)	4.80 d (8.1)	4.82 d (7.2)	4.83 d (7.6)	4.77 d (7.2)	4.79 d (7.5)
2′		4.07 t (8.0)	4.07 dd (7.4, 15.1)	5.54 t (8.1)	4.07 m	4.08 m	4.03 m	4.03 m
3′		4.19 t (8.8)	4.16 m	4.09 t (9.1)	4.16 m	4.08 m	4.12 m	4.13 m
4′		4.26 m	4.12 m	4.19 m	4.13 m	4.15 m	4.10 m	4.18 m
5′		3.75 d (10.6)	3.67 t (10.8)	3.65 t (11.3)	3.70 m	3.72 m	3.59 m	3.68 m
		4.39 dd (5.2, 11.3)	4.34 m	4.19 m	4.34 m	4.36 m	4.03 m	
1″			5.33 d (7.7)	4.92 d (7.8)	5.33 d (7.7)	5.31 d (7.4)	5.30 d (7.3)	5.29 d (7.6)
2″			4.31 m	3.91 m	4.07 dd (7.4, 15.1)	4.09 m	4.31 m	4.03 m
3″			4.18 m	4.13 t (8.8)	4.18 m	4.18 m	4.15 m	4.15 m
4″			4.20 m	4.04 m	4.20 m	4.21 m	4.17 m	4.16 m
5″			3.72 t (13.6)	3.76 t (10.7)	3.70 m	3.72 m	3.59 m	3.68 m
			4.34 m	4.32 m	4.34 m	4.36 m	4.03 m	4.31 m
CH_3CO −2′				2.36 s				

[a] Signals overlapped.

Table 2
^{13}C DEPT data of compounds **1**–**8** in pyridine-d_5 at 150 Hz (δ in ppm).

No.	1	2	3	4	5	6	7	8
1	154.3 d	32.9 t	32.8 t	32.3 t	31.9 t	32.5 t	30.5 t	31.9 t
2	127.2 d	30.6 t	30.5 t	30.3 t	29.9 t	30.4 t	29.8 t	29.9 t
3	204.5 s	88.3 d	88.9 d	89.4 d	88.3 d	88.9 d	88.4 d	88.3 d
4	46.5 s	41.8 s	41.8 s	41.4 s	41.2 s	41.8 s	40.7 s	41.2 s
5	45.1 d	47.8 d	47.6 d	47.5 d	46.9 d	47.7 d	42.7 d	46.9 d
6	24.4 t	21.2 t	21.2 t	21.1 t	20.4 t	21.3 t	22.1 t	20.4 t
7	20.0 t	26.5 t	26.5 t	26.5 t	25.6 t	27.0 t	114.4 d	25.6 t
8	44.0 d	44.1 d	47.7 d	49.0 d	45.9 d	48.6 d	147.7 s	45.6 d
9	24.8 s	20.4 s	20.6 s	20.3 s	20.7 s	20.4 s	21.6 s	20.1 s
10	30.6 s	27.5 s	27.2 s	26.8 s	26.8 s	27.0 s	28.5 s	26.7 s
11	27.9 t	26.3 t	38.0 t	26.8 t	36.4 t	26.3 t	36.9 t	36.7 t
12	33.3 t	31.8 t	77.8 d	34.3 t	76.6 d	33.3 t	77.1 d	77.1 d
13	47.6 s	40.3 s	46.5 s	42.2 s	48.3 s	41.7 s	50.8 s	48.7 s
14	45.6 s	55.4 s	48.8 s	47.6 s	48.6 s	46.4 s	48.4 s	48.0 s
15	42.8 t	214.5 s	79.8 d	80.4 d	43.8 t	83.2 d	43.4 t	43.6 t
16	72.5 d	84.7 d	112.7 s	112.3 4	80.4 d	220.1 s	74.9 d	73.0 d
17	52.5 d	52.3 d	60.0 d	59.9 d	53.6 d	60.2 d	56.9 d	56.4 d
18	20.1 q	20.7 q	13.2 q	19.8 q	13.3 q	19.7 q	15.2 q	13.6 q
19	30.0 t	31.6 t	31.4 t	31.0 t	29.6 t	30.8 t	29.1 t	29.5 t
20	35.1 d	33.6 d	24.4 d	24.4 d	26.8 d	28.2 d	23.5 d	26.0 d
21	17.9 q	20.4 q	20.2 q	19.8 q	22.2 q	20.6 q	21.7 q	21.7 q
22	87.2 d	39.0 t	39.0 t	38.4 t	38.6 t	37.3 t	37.5 t	37.6 t
23	106.4 s	78.9 d	75.0 d	72.3 d	173.7 s	72.4 d	106.3 s	105.9 s
24	83.6 d	77.3 d	87.0 d	90.7 d		65.5 d	62.7 d	63.5 d
25	84.0 s	77.5 s	146.3 s	71.3 s		58.9 s	62.8 s	65.6 s
26	28.2 q	21.9 q	113.7 t	25.6 q		25.8 q	68.5 t	98.4 d
27	25.2 q	23.8 q	18.6 q	27.5 q		19.7 q	14.6 q	13.1 q
28	19.2 q	18.0 q	12.5 q	12.3 q	19.9 q	12.3 q	27.2 q	19.5 q
29	22.2 q	26.1 q	26.0 q	25.6 q	15.5 q	25.0 q	25.9 q	25.5 q
30	19.7 q	15.9 q	15.9 q	15.9 q	25.6 q	15.8 q	14.6 q	15.3 q
$CH_3\underline{C}O$-12			171.0 s		170.7 s		170.8 s	170.6 s
$\underline{CH_3}CO$-12			22.2 q		21.5 q		21.4 q	21.7 q
$\underline{C}H_3CO$-23						170.7 s		
$\underline{CH_3}CO$-23						21.0 q		
$\underline{C}H_3CO$-24		171.3 s						
$\underline{CH_3}CO$-24		21.4 q						
$\underline{C}H_3O$-25		49.70 q						
sugar								
1′		108.1 d	107.6 d	105.0 d	107.2 d	107.5 d	107.4 d	107.2 d
2′		76.1 d	75.9 d	73.8 d	74.1 d	75.7 d	75.8 d	74.6 d
3′		79.2 d	87.8 d	85.4 d	87.4 d	87.7 d	87.4 d	87.4 d
4′		71.7 d	69.8 d	71.3 d	69.3 d	69.6 d	69.7 d	71.0 d
5′		67.6 t	67.1 t	66.9 t	66.6 t	66.9 t	67.0 t	66.6 t
1″			106.7 d	106.8 d	106.3 d	106.5 d	106.6 d	106.3 d
2″			75.0 d	74.7 d	75.4 d	74.8 d	74.9 d	75.4 d
3″			78.8 d	79.0 d	78.3 d	78.6 d	78.7 d	78.3 d
4″			71.5 d	69.8 d	71.0 d	71.3 d	71.4 d	69.4 d
5″			67.9 t	67.8 t	67.5 t	67.7 t	67.8 t	67.5 t
$CH_3\underline{C}O$-2′				170.4 s				
$\underline{CH_3}CO$-2′				21.8 q				

1998), and 23*S*, 24*S* configurations [C-22 (δ_C 84.9), C-23 (δ_C 103.0), and C-24 (δ_C 81.8)] (Chen et al., 2014). Thus, the structure of **1** was elucidated as cimiacerol-1(2)-en-3-one.

Compound **2** gave the molecular formula of $C_{38}H_{60}O_{10}$, as determined by HR-ESIMS ($[M+Na]^+$ at m/z 699.4074; calcd for $C_{38}H_{60}O_{10}Na$, 699.4084). Apart from the signals for an acetyl group, a methoxy group, and a xylose unit, 30 signals of the aglycone were assigned as 7 methyls, 8 methylenes, 8 methines, and 7 quaternary carbons (Table 2) based on its 1D NMR and HSQC spectra. The 1D NMR data of **2** (Tables 1 and 2) displayed the structural resemblance with those of 24-*O*-acetylisodahurinol-3-*O*-β-D-xylopyranoside (Shao et al., 2000), except for the occurrence of

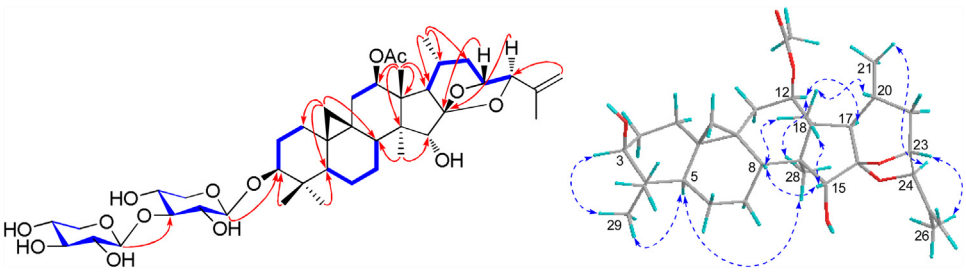

Fig. 3. Major HMBC (→), ^{1}H–^{1}H COSY (—), and ROESY (⇢) correlations of **3**.

a methoxy group in **2**. The substituent methoxy group was attached at C-25 by the observation of HMBC correlation of the methoxyl proton (d_H 3.23) with C-25 (d_C 77.5). Furthermore, the absolute configuration of C-24 was determined to be *S* by comparison of the coupling constants of H-24 (J = 2.2 Hz) of **2** with those of dahurinyl diacetate (J = 9.0 Hz) and isodahurinyl deacetate (J = 2.0 Hz) (Kusano et al., 1976). Therefore, the structure of **2** was elucidated as 25-*O*-methyl-24- *O*-acetylisodahurinol-3-*O*-β-D-xylopyranoside.

Compound **3** possessed a molecular formula of $C_{42}H_{64}O_{14}$ as determined by the HR-ESIMS at *m*/*z* 815.4180 [M+Na]$^+$. The 1D NMR data (Tables 1 and 2) of **3** showed that **3** resembled 25-anhydrocimicigenol-3-*O*-[2′-*O*-acetyl]-β-D-xylopyranoside (Zhou et al., 2006) with the major differences of the sugar unit and an additional acetoxyl group of the aglycone. Among them, the acetoxyl [d_C 171.0 (s), 22.2 (q); d_H 2.13 (3H, s)] was located at C-12, which was confirmed by the HMBC correlation (Fig. 3) of H-12 (δ_H 5.26) with the acetyl carbonyl carbon (δ_C 171.0), together with the spin system of H-12/H_2-11 (δ_H 1.14, 2.95) deduced from a COSY excrement (Fig. 3). Meanwhile, its relative configuration was determined as β-orientation through the significant ROESY correlations (Fig. 3) of H-12 with H-17 (d_H 1.64) and H_3-28 (d_H 1.22).

Moreover, the ^{1}H NMR spectrum displayed two anomeric protons at d_H 4.82 (d, J = 7.6 Hz) and 5.33 (d, J = 7.7 Hz), in accordance with two glycosidic moieties [d_C 107.7 (C-1′), 75.9 (C-2′), 87.8 (C-3′), 69.8 (C-4′), 67.1 (C-5′); 106.7 (C-1″), 75.0 (C-2″), 78.8 (C-3″), 71.5 (C-4″), 67.9 (C-5″)] were observed in the ^{13}C NMR spectrum. The sugar obtained after acid hydrolysis was unambiguously identified as D-xylose by comparing its TLC and specific rotation with a standard. HMBC correlations of H-1′ with C-3 and H-1″ with C-3′ implied that the disaccharide unit attached to C-3 of the aglycone and the terminal xylosyl to C-3′ of the inner xylosyl, respectively. Furthermore, the relative configuration of H-1′ and H-1″ were determined to be β orientations on the basis of the coupling constants (J = 7.6, 7.7 Hz) of the anomeric proton signals. Thus, the structure of **3** was elucidated as 12-*O*-acetyl-25-anhydrocimicigenol-3-*O*-β-D-xylopyranosyl-3′-*O*- β-D-xylopyranoside.

Compound **4**, a white powder, gave a pseudo-molecular ion at *m*/*z* 817.4339. [M + Na]$^+$ in HR-ESIMS, corresponding to the molecular formula of $C_{42}H_{66}O_{14}$. The 1D NMR spectroscopic data (Table 2) of **4** showed that the aglycone of **4** were identical with that of cimigenol-3-*O*-[2′-*O*-acetyl]-β-D-xylopyranoside (Nian et al., 2011). Meanwhile, the presence of 2′-*O*-acetyl-β-D-xylopyranose fraction was deduced by comparing the 1D NMR data between **4** and cimigenol-3-*O*- [2′-*O*-acetyl]-β-D-xylopyranoside. However, another pentosyl signals [δ_C 106.8 (C-1″), 74.7 (C-2″), 79.0 (C-3″), 69.8 (C-4″), 67.8 (C-5″)] were observed in its 1D NMR spectra of **4**. Moreover, this sugar moiety was connected to C-3′ via an ether bond based on the HMBC correlations (Fig. 4) of H-3″ with C-3′ (δ_C 105.0). Similarly, this sugar was identified as D-xylose by using above method. Moreover, the configurations of the two xylosyl moieties were both determined as β on the basis of the coupling constants (J = 7.2, 7.7 Hz) of the anomeric protons. Hence, the structure of compound **4** was determined to be 2′-*O*-acetylcimigenol-3-*O*-β-D-xylopyranosyl-3′-*O*-β-D-xylopyranoside.

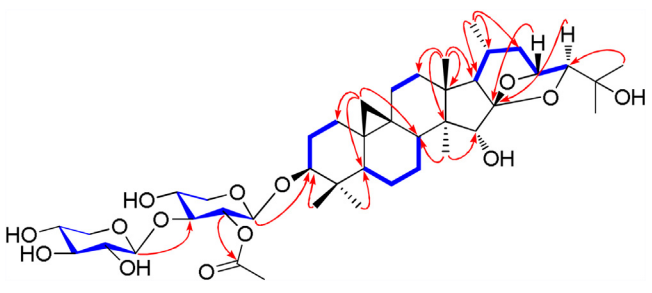

Fig. 4. Major HMBC (→) and ^{1}H–^{1}H COSY (—) correlations of **4**.

The molecular formula of compound **5** was assigned as $C_{38}H_{58}O_{13}$ by the HR-ESIMS at *m*/*z* 745.3765 [M+Na]$^+$. Comparison of the 1D NMR spectroscopic data (Tables 1 and 2) of **5** with those of cimilactone A (Liu et al., 2002) revealed that they had the similar structures, and the only difference was the sugar moieties. Moreover, the sugar moieties was determined to be 3-*O*-β-D-xylopyranosyl-3′-*O*-β-D-xylopyranoside unit by comparing to the 1D NMR data of **3** (Tables 1 and 2) and analysis of their 2D NMR correlations. Accordingly, the structure of **5** was finally elucidated as shown and named cimilactone K.

Compound **6** was obtained as a white powder. Its molecular formula was determined to be $C_{42}H_{66}O_{14}$ (817.4342 [M+Na]$^+$, calcd 817.4350) by its HR-ESIMS. Detailed comparison the 1D NMR data of **6** (Tables 1 and 2) with those of 4′,23-*O*-diacetylshengmanol-3-*O*-β-D-xylopyranoside (Chen et al., 2002a,b) showed that they were similar in structure except for signal of the sugar moieties. The NMR data of the sugar residues of **6** agreed well with those of **3**. Moreover, the correlations of an anomeric proton H-1′ (δ_H 4.83) with C-3 (δ_C 88.9), and of another anomeric proton H-1″ (δ_H 5.31) with C-3′ (δ_C 87.7) in the HMBC spectrum, indicated that the inner xylosyl attached to C-3 of the aglycone and the terminal xylosyl to C-3′ of the inner xylosyl. The configuration of C-23 and C-24 were determined as *R* and *S*, respectively, by comparing the known 9,19-cycloartane triterpene glycosides (Kusano et al., 1999). Thus, the structure of **6** was characterized as 4′, 23-*O*-diacetylshengmanol-3-*O*-β-D-xylopyranosyl-3′-*O*-β-D-xylopyranoside.

The HR-ESIMS (813.4029 [M+Na]$^+$, calcd 813.4037) of compound **7** suggested a molecular formula of $C_{42}H_{62}O_{14}$. The 1D NMR data (Tables 1 and 2) of **7** were similar to those of 26-deoxyactein (Chen et al., 2002a,b), except for the presence of an additional double bond and an monosaccharide unit of the sugar moieties. In the ^{13}C NMR and DEPT spectra, two olefinic carbon signals at δ_C 114.4 (d) and 147.7 (s) were observed in the aglycone of **7**, instead of a methylene and a methine resonances at C-7 (δ_C 25.7) and C-8 (δ_C 45.6) in 26-deoxyactein, respectively. Thus, the aglycone of **7** was a 7,8-dehydro derivative of the corresponding analogue, which were supported by HMBC correlations of H-6 (δ_H 1.75) with C-7 (δ_C 114.4), C-8 (δ_C 147.7) and of H_3-28 (δ_H 1.02) and H_2-11 (δ_H 1.21, 2.91) with C-8. Similarly, HMBC correlation between H-1″ (δ_H 5.30) and δ_C C-3′ (87.4) indicated the additional xylosyl residue was connected to C-3′. Furthermore, the disaccharide unit was located at C-3 by the HMBC correlation of H-1′ (δ_H 4.77) with C-3 (δ_C 88.4). And the configurations of the sugar units were both determined to be β by the coupling constants (J = 7.2, 7.3 Hz), respectively. In addition, the configuration of C-23 was assigned as *S* by detail comparison the known compound 26-deoxyactein (Chen et al., 2002a,b) which was secured by X-ray spectrum analysis. Hence, the structure of **7** was elucidated as 26-deoxyacetylacteol-7(8)-en-3-*O*-β-D-xylopyranosyl-3′-*O*-β-D-xylopyranoside.

Compound **8** was purified as a white powder, and the molecular formula of $C_{42}H_{64}O_{15}$ was deduced from the HR-ESIMS at *m*/*z* 831.4140 [M+Na]$^+$. Analysis of the NMR spectroscopic data (Tables 1 and 2) revealed that the structure of **8** closely resembled those of 2′-*O*-acetylactein (Zhu et al., 2001), except for the sugar moieties. A 3-*O*-β-D-xylopyranosyl-3′-*O*-β-D-xylopyranoside unit was observed by comparison of the 1D and 2D NMR data with those of **7**. Its location was determined by the HMBC correlation between H-1′ (δ_H 4.79, d, J = 7.5 Hz) and C-3 (δ_C 88.3). Additionally, the configuration of C-23 was further determined as *R* by comparison the known compound 26-deoxyactein (Chen et al., 2002a,b). Thus,

Table 3
Cytotoxicity compounds **1–8** (IC_{50} values, μM).

No.	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	>40	>40	>40	>40	>40
2	3.32	3.17	2.61	2.93	3.11
3	13.70	30.29	29.62	14.80	17.24
4	8.00	8.93	11.40	8.66	15.09
5	32.12	15.63	33.22	18.27	20.68
6	>40	>40	>40	>40	>40
7	16.57	29.86	18.40	16.16	15.72
8	27.80	28.52	>40	24.93	>40
Cisplatin[a]	1.01	5.29	6.13	13.62	14.03

[a] Cisplatin were used as positive control.

the structure of **8** was determined as acteol-3-*O*-β-D-xylopyranosyl-3′-*O*-β-D-xylopyranoside.

The known compounds were identified as cimiacerogenin B (**9**) (Kusano et al., 1998), 12-*O*-acetyl-25-anhydrocimigenol-3-*O*-β-D-xylopyranoside (**10**) (Chen et al., 2002a,b), cimigenol-3-*O*-β-D-xylopyranoside (**11**) (Li et al., 1994), 25-*O*-acetylcimigenol-3-*O*-β-D-xylopyranoside (**12**) (Kadota et al., 1995), 12β-hydroxylcimigenol-3-*O*-β-D-xylopyranoside (**13**) (Yoshimitsu et al., 2006), 12β-hydroxycimigenol-3-*O*-β-D-xylopyranosyl-3′-*O*-β-D-xylopyranoside (**14**) (Kusano et al., 1976), 26-deoxyactein (**15**) (Chen et al., 2002a,b), and 26-deoxyacetylacteol-3-*O*-β-D-xylopyra- nosyl-3′-*O*-β-D-xylopyranoside (**16**) (Sun et al., 2008), by comparing their physical and spectroscopic data with those reported values.

Eight new compounds isolated in the present study were evaluated for their cytotoxicity against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7 and SW480) using MTT method.Compound **2** showed promissing cytotoxic activities against SMMC-7221, A-549, MCF-7 and SW480 cell lines with IC_{50} values of 3.17, 2.61, 2.93, and 3.11 μM (Table 3), respectively, compared to positive control (cisplatin, IC_{50} 5.29, 6.13, 13.62, 14.03). Meanwhile, compounds **3–5** and **7** had moderate to weak inhibitory effects against five tested human cancer cell lines, with IC_{50} values range from 8.0 to 33.2 μM (Table 3).These data suggest that some of the chemical constituents from this species might be valuable antitumor promoters and show supportive evidence for the theory of Chinese Medicine about cancer.

3. Experimental

3.1. General

Optical rotations were measured in MeOH with a Horiba SEAP-300 polarimeter. ^1H and ^{13}C NMR spectra were recorded in pyridine-d_5 on Bruker DRX-500 and Avance III–600 MHz spectrometers (Bruker, Zürich, Switzerland), using TMS as internal standard for chemical shifts. Chemical shifts (δ) were expressed in *ppm* with reference to the TMS resonance. ESIMS, HRTOF-ESIMS and EIMS, HREIMS data were obtained using a VG Autospec-3000 and API QSTAR TOF spectrometer, respectively. Infrared spectra were recorded on a Shimadzu IR-450 instrument with KBr pellets. Thin-layer chromatography was performed on precoated TLC plates (Silica gel GF254, Qingdao Marine Chemical, Inc.) and spots were visualized by heating after spraying with 10% H_2SO_4 in EtOH. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a YMC-Pack Pro C18 RS 10 mm × 250 mm column. Silica gel (mesh 200–300, Qingdao Marine Chemical, Inc.), Lichroprep RP-18 (40–63 lm, Merck), Amberlite IR-35 (10 ml) column and Sephadex LH-20 (Pharmacia) were used for column chromatography.

3.2. Plant materials

The roots of *Cimicifuga foetida* L. (82 kg) were collected from Yulong County, Yunnan Province, in September 2010 and identified by Professor Shengji Pei, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KUN No. 20100906) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, PR China.

3.3. Extraction and isolation

The air-dried roots of *C. foetida* (82 kg) were crushed with a blender and refluxed with 95% EtOH for three times (5 h, each). The residue yielded by removal of the solvent was dissolved in water to form a suspension. The aqueous suspension was successively partitioned with EtOAc and *n*-BuOH. The EtOAc (5.6 kg) fraction was absorbed on 12 kg silica gel and chromatographed on a prepacked (120 kg) silica gel column, eluting stepwise with $CHCl_3$-MeOH (1:0, 100:1, 50:1, 20:1, 5:1) to give five fractions (A-E). Fraction C (230 g) was subjected to column chromatograph (CC) on silica gel (1.5 kg) and eluted with PE-Me_2CO (5:1, 2:1, 0:1) to obtain C1, C2, C3 and C4. Fraction C2 (40 g) was purified using an ODS silica gel column with MeOH—H_2O, followed by purification using semi-preparative HPLC eluted with CH_3CN—H_2O (60:40), furnished **1** (3.0 mg) and **9** (5.3 mg). Fraction D (200 g) was separated on silica gel eluted with $CHCl_3$-Me_2CO (15:1 to 5:1) to give ten sub-fractions (D1-D10). Fraction D1 (10 g) was separated by CC (ODS silica gel) with MeOH-H_2O (60:40 to 90:10) and purified by HPLC eluting with CH_3CN—H_2O (45:55) to obtain **2** (5.9 mg) and **10** (10.0 mg). Fraction D3 (5 g) was separated over CC (Sephadex LH-20, MeOH), and further treated by CC (ODS silica gel) with MeOH—H_2O (60:40 to 90:10), followed by purification using semi-preparative HPLC with CH_3CN—H_2O (50:50) to obtain **13** (20.5 mg). Fraction D6 was subjected to silica gel (CH_3Cl_3–MeOH, gradient from 25:1 to 15:1) and then purified by an ODS silica gel column to obtain **11** (15.3 mg) and **12** (10.6 mg). Fraction E (20 g) was also divided into five fractions (E1-E5), after performing ODS column (MeOH—H_2O, gradient from 40:60 to 70:30). Repeated CC of fraction E2 using silica gel $CHCl_3$–MeOH (15:1 to 8:1) and semi-preparative HPLC (CH_3CN—H_2O, 32:68) to afford **3** (13.2 mg), **5** (8.4 mg), **7** (7.8 mg) and **8** (23.4 mg). Similarly, compound **4** (10.6 mg), **6** (36.8 mg), **14** (8.5 mg), **15** (11.6 mg), and **16** (20.5 mg) were isolated from E4 and E5 by repeated CC, respectively.

3.3.1. Cimiacerol-1(2)-en-3-one

White powder; $[\alpha]_D^{20}$ −138.6 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3441, 1667, 1641, 1459, 1375, 1237, 1240, 1167, 1072, 1046, 980 cm^{-1}; ^1H and ^{13}C NMR data see Tables 1 and 2; HREIMS *m*/*z* 484.3168 $[M]^+$ (calcd for 484.3189).

3.3.2. 25-O-methyl-24-O-acetylisodahurinol-3-O-β-D-xylopyranoside (2)

White powder; $[\alpha]_D^{20}$ + 55.89 (*c* 0.03, MeOH); IR (KBr) ν_{max} 3433, 2938, 2872, 1743, 1631, 1457, 1373, 1237, 1161, 1047, 971 cm^{-1}; ^1H (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) data see Tables 1 and 2; ESIMS *m*/*z* 699 $[M + Na]^+$; HRESIMS *m*/*z* 699.4074 (calcd for 699.4084).

3.3.3. 12-O-acetyl-25-anhydrocimicigenol-3-O-β-D-xylopyranosyl-3′-O-β-D-xylopyranoside (3)

White powder; $[\alpha]_D^{20}$ − 51.76 (*c* 0.01, MeOH); IR (KBr) ν_{max} 3441, 2931, 2871, 1735, 1632, 1456, 1383, 1240, 1169, 1043, 985 cm^{-1}; ^1H

(C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) data see Tables 1, 2; HRESIMS *m/z* 815.4180 $[M+Na]^+$ (calcd for 815.4194).

*3.3.4. 2′-O-acetylcimigenol-3-O-β-D-xylopyranosyl-3′-O-β-D-xylopyranoside (**4**)*

White powder; $[\alpha]_D^{20}$ +1.21 (*c* 0.02, MeOH); IR (KBr) ν_{max} 3441, 2966, 2935, 2870, 1739, 1632, 1459, 1383, 1242, 1171, 1042, 979 cm^{-1}; ^1H (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) data see Tables 1 and 2; ESIMS *m/z* 817 $[M+Na]^+$; HRESIMS *m/z* 817.4339 (calcd for 817.4350).

*3.3.5. Cimilactone K (**5**)*

White powder; $[\alpha]_D^{20}$ −87.44 (*c* 0.01, MeOH); IR (KBr) ν_{max} 3440, 2934, 2885, 1719, 1632, 1461, 1383, 1366, 1247, 1082, 1041, 989 cm^{-1}; ^1H (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 600 MHz) data see Tables 1 and 2; ESIMS *m/z* 745 $[M+Na]^+$; HRESIMS *m/z* 745.3765 (calcd for 745.3775).

*3.3.6. 23-O-acetylshengmanol-3-O-β-D-xylopyranosyl-3′-O-β-D-xylopyranoside (**6**)*

White powder; $[\alpha]_D^{20}$ − 46.97 (*c* 0.02, MeOH); IR (KBr) ν_{max} 3440, 2936, 2871, 1737, 1632, 1459, 1382, 1241, 1170, 1043, 993 cm^{-1}; ^1H (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) data see Tables 1 and 2; ESIMS *m/z* 817 $[M+Na]^+$; HRESIMS *m/z* 817.4342 (calcd for 817.4350).

*3.3.7. 26-Deoxyacetylacteol-7(8)-en-3-O-β-D-xylopyranosyl-3′-O-β-D-xylopyranoside (**7**)*

White powder; $[\alpha]_D^{20}$ − 101.3 (*c* 0.01, MeOH); IR (KBr) ν_{maxmax} 3442, 2932, 2877, 1731, 1632, 1456, 1384, 1246, 1164, 1044, 987 cm^{-1}; ^1H (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) data see Tables 1 and 2; ESIMS *m/z* 813 $[M+Na]^+$; HRESIMS *m/z* 813.4029 (calcd for 813.4037).

*3.3.8. Acteol-3-O-β-D-xylopyranosyl-3′-O-β-D-xylopyranoside (**8**)*

White powder; $[\alpha]_D^{20}$ − 69.54 (*c* 0.01, MeOH); IR (KBr) ν_{max} 3442, 2934, 2875, 1732, 1634, 1454, 1383, 1244, 1169, 1131, 1046, 988 cm^{-1}; ^1H (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) data see Tables 1 and 2; ESIMS *m/z* 831 $[M+Na]^+$; HRESIMS *m/z* 831.4140 (calcd for 831.4143).

*3.4. Hydrolysis and identification of sugar residue in the new compounds **2–8***

The new compounds **2–8** (4 mg of each) were 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). The water layer was then neutralized by Ag_2CO_3, and the precipitate filtered to give a monosaccharide. Each monosaccharide of those compounds had an R_f (EtOAc/$CHCl_3$/MeOH/H_2O, 3:2:2:1) and specific rotation $[\alpha]_D^{20}$ +24.3 (*c* 0.10, H_2O) corresponding to those of D-xylose (Sigma–Aldrich).

3.5. Cytotoxicity bioassay

Five 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 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-diphenyltetrazolium bromide] method in 96-well microplates (Mosmman, 1983; Alley et al., 1938). Exactly 100 μl of adherent cells was seeded into each well of 96-well cell culture plates and allowed to adhere for twelve hours before addition of test compounds, while suspended cells were seeded just before drug addition with initial density of 1×10^5 cells/mL. Each cancer cell line was exposed to the compounds dissolved in DMSO at five different considerations in triplicate for 48 h, with cisplatin as positive controls. 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 (Reed and Muench, 1938).

Ackonwledgements

This research work was supported by Program for National Natural Science Foundation of China (No. U1132604), The Major Program of CAS (No. KSZDEW-Z-004-03), as well as Foundation of State Key Laboratory of Phytochemistry and Plant Resources in West China (P2015-ZZ01). Authors also were particularly grateful to Research Group of Prof. & Dr. Yan Li for bioassay tests of cytotoxic activity.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phytol.2016.06.002.

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