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Cycloartane triterpenoids from the aerial parts of *Cimicifuga foetida* Linnaeus

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ABSTRACT

Cycloartane triterpenoids, 2′,24-*O*-diacetylisodahurinol-3-*O*-α-L-arabinopyranoside, 24-*O*-acetylisodahurinol-3-*O*-α-L-arabinopyranoside, 12β-hydroxy-25-anhydrocimigenol, cimigenol-12-one, 12β-hydroxy-15-deoxycimigenol, 2′-*O*-acetyl-24-*epi*-cimigenol-3-*O*-α-L-arabinopyranoside, 2′-*O*-acetylcimigenol-3-*O*-β-D-xylopyranoside, 25-anhydrocimigenol-3-*O*-α-L-arabinopyranoside, 2′,23-*O*-diacetylshengmanol-3-*O*-α-L-arabinopyranoside, and 2′,24-*O*-diacetyl-25-anhydrohydroshengmanol-3-*O*-α-L-arabinopyranoside, together with eight known compounds, were isolated from aerial parts of *Cimicifuga foetida*. Their structures were determined by application of spectroscopic analyses and chemical methods. Biological evaluation of the compounds against human HL-60, SMMC-7721, A549, SK-BR-3, and PANC-1 cell lines indicated that three of these compounds exhibited broad-spectrum and moderate cytotoxic activities, with IC_{50} values ranging from 6.20 to 22.74 μM. By comparing previous cytotoxic testing data and bioassay results from this study, preliminary structure–activity relationships of compounds with a c imigenol-skeleton can be proposed.

1. Introduction

The genus *Cimicifuga* (now *Actaea* in the western world) consists of 28 species, whose roots have been used in traditional medicine worldwide (Compton et al., 1998a,b; Liske and Wustenberg, 1998). *C. foetida*, one of the most common Asiatic species, has been employed as cooling and detoxification agents by the Chinese since ancient times (Pharmacopoeia Commission of the People's Republic of China, 2005). Intrigued by the theory of Chinese traditional medicine, in which a tumor is defined as a kind of toxin, we have been studying the potential antitumor constituents of *C. foetida* and reported a series of cycloartane triterpenoids from the roots, which exhibited cytotoxicities against various tumor cell lines (Sun et al., 2007; Nian et al., 2010; Lu et al., 2010). Triterpene glycosides, such as cimicifoetisides A and B, showed significant activities against rat Ehrlich ascites carcinoma (EAC), with IC_{50} values of 0.52 and 0.19 μM, respectively. In addition, analogs like 4′-*O*-(*E*)-2-butenoyl-25-*O*-acetylcimigenol-3-*O*-β-D-xylopyranoside, 3′,25-*O*-diacetyl-cimigenol-3-*O*-β-D-xylopyranoside and 3′-*O*-acetyl-23-*epi*-26-deoxyactein exhibited potent and selective cytotoxicity against human HepG2 cells, having IC_{50} values of 1.29, 0.71 and 1.41 μM, respectively.

In the past, the aerial parts of *C. foetida* were discarded as a waste by-product in China. Recently, other research groups have paid attention to its chemical properties and a series of 9,19-cycloartane triterpene glycosides have been obtained (Pan et al., 2002, 2003, 2004, 2007, 2009; Tian et al., 2006). In addition, one group reported that the ethyl acetate fraction of an 80% ethanol extract of the tissue and several isolated cycloartane triterpenoids displayed growth inhibitory activities against human hepatoma cell lines (Tian et al., 2006, 2007). In an attempt to fully explore the chemical constituents and their cytotoxicities of this medicinal plant, we undertook phytochemical and pharmacological investigations on the aerial parts of *C. foetida*. Ten 9,19-cycloartane triterpenes (**1**–**10**), together with eight known compounds (Scheme 1), 12β-hydrocimigenol-3-one (**11**) (Nian et al., 2009), 4′,23-*O*-diacetylshengmanol-3-*O*-α-L-arabinopyranoside (**12**) (Chen et al., 2002), 23-*O*-acetylshengmanol-3-*O*-α-L-arabinopyranoside (**13**) (Kusano et al., 1999), 12β-*O*-acetylcimiracemonol (**14**) (Zhou et al., 2004), 25-*O*-acetylcimigenol-3-*O*-α-L-arabinopyranoside (**15**) (Ye et al., 1999), isodahurinol (**16**) (Kusano et al., 1976), 12β-hydrocimigenol-3-*O*-β-D-xylopyranoside (**17**) (Kusano et al., 1995), and 12β-hydrocimigenol (**18**) (Kusano et al., 1995), were isolated and identified. The isolated compounds were tested for their cytotoxicities against human HL-60, SMMC-7721, A549, SK-BR-3, and PANC-1 cell lines, using the MTT method.

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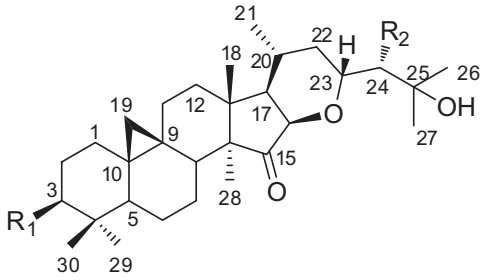
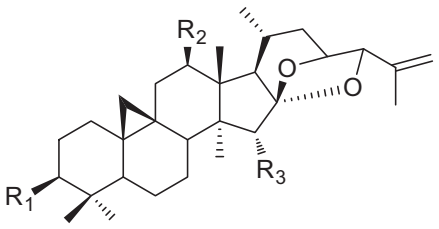
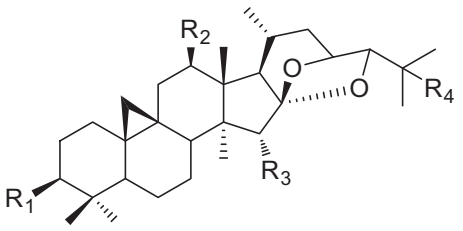


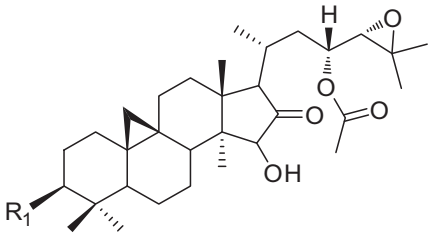
1 R_1 = -O-2'-O-acetyl-α- L-arabinose, R_2 = OAc
2 R_1 = -O-α-L-arabinose, R_2 = OAc
16 R_1 = OH, R_2 = OH



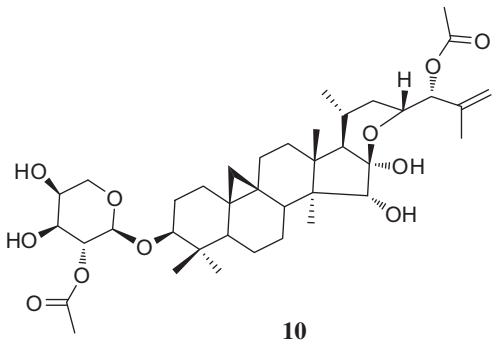
3 R_1 = OH, R_2 = OH, R_3 = OH
8 R_1 = -O-α-L-arabinose, R_2 = H, R_3 = OH



4 R_1 = OH, R_2 = =O, R_3 = OH, R_4 = OH
5 R_1 = OH, R_2 = OH, R_3 = H, R_4 = OH
6 R_1 = -O-2'-O-acetyl-α-L-arabinose, R_2 = H, R_3 = OH, R_4 = OH; 24*R*
7 R_1 = -O-2'-O-acetyl-β-D-xylose, R_2 = H, R_3 = OH, R_4 = OH
11 R_1 = =O, R_2 = OH, R_3 = OH, R_4 = OH
15 R_1 = -O-α-L-arabinose, R_2 = H, R_3 = OH, R_4 = OAc
17 R_1 = -O-β-D-xylose, R_2 = OH, R_3 = OH, R_4 = OH
18 R_1 = OH, R_2 = OH, R_3 = OH, R_4 = OH



9 R_1 = -O-2'-O-acetyl-α-L-arabinose
12 R_1 = -O-4'-O-acetyl-α-L-arabinose
13 R_1 = -O-α-L-arabinose



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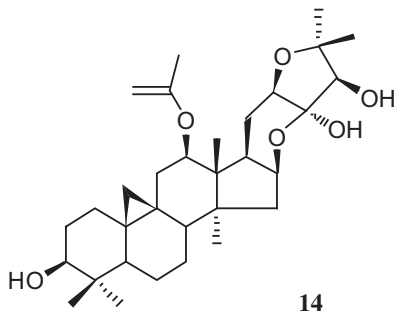

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Scheme 1. Structures of compounds isolated from the aerial parts of *C. foetida*.

2. Results and discussion

2.1. Characterization of the compounds

Compound **1**, isolated as a white powder, gave a pseudo-molecular ion at *m*/*z* 727 $[M+Na]^+$ in the positive ion ESIMS. The IR spectrum showed absorptions for hydroxyl (3410 cm^{-1}) and carbonyl (1741 cm^{-1}) groups. Analysis of the ^{13}C NMR and HR-ESIMS (*m*/*z* 727.4048 $[M+Na]^+$) determined its molecular formula as $C_{39}H_{60}O_{11}$. The assignment of ^1H and ^{13}C NMR spectroscopic data of **1** (Tables 1 and 2) was based on analysis of HSQC, HMBC (Fig. 1), and ^1H–^1H COSY data. The ^1H NMR spectrum (Table 1) showed signals due to the characteristic cyclopropane methylene at δ_H 0.18 and 0.41 (1H each, *d*, *J* = 4.0 Hz), six *tert*-methyl groups at δ_H 0.91, 0.97, 1.07, 1.15, 1.60 (2×), two acetoxymethyl groups at δ_H 2.11 and 2.15, a *sec*-methyl group at δ_H 0.89 (*d*, *J* = 6.0 Hz), and an anomeric proton at δ_H 4.72 (*d*, *J* = 7.6 Hz). The ^{13}C NMR and DEPT spectrum of **1** (Table 2) exhibited data consistent with a cyclopropane methylene at δ_C 31.0 (C-19), five oxygenated carbons at δ_C 88.5 (C-3), 84.2 (C-16), 79.9 (C-24), 79.0 (C-23), and 72.0 (C-25), a carbonyl carbon at δ_C 214.0, and a pentose moiety at δ_C 104.6 (C-1′), 74.4 (C-2′), 72.5 (C-3′), 69.8 (C-4′), and 67.3 (C-5′). This spectrum also indicated two acetoxy carbonyl groups at δ_C 171.1 and 170.1. This evidence suggested that **1** was a highly oxygenated

Table 1
^{1}H NMR spectroscopic data of compounds **1**–**10** in pyridine-d_5.

Proton	**1** δ_H multi. (*J* in Hz)	**2** δ_H multi. (*J* in Hz)	**3** δ_H multi. (*J* in Hz)	**4** δ_H multi. (*J* in Hz)	**5** δ_H multi. (*J* in Hz)	**6** δ_H multi. (*J* in Hz)	**7** δ_H multi. (*J* in Hz)	**8** δ_H multi. (*J* in Hz)	**9** δ_H multi. (*J* in Hz)	**10** δ_H multi. (*J* in Hz)
1	1.50 m	1.52 m	1.58 m	1.53 m	1.57 m	1.50 m	1.53 m	1.57 m	1.54 m	1.53 m
	1.12 m	1.14 m	1.24 m	1.05 m	1.24 m	1.16 m	1.16 m	1.20 m	1.19 m	1.18 m
2	2.23 m	2.36 *dd* (12.8, 3.2)	1.95 m	1.94 *dd* (13.2, 4.0)	1.95 *brd* (10.0)	2.26 *brd* (10.5)	2.24 m	2.38 m	2.28 m	2.25 m
	1.83 m	1.91 m	1.85 m	1.87 *dd* (12.2, 3.6)	1.84 m	1.87 *dd* (12.0, 2.5)	1.87 *dd* (12.5, 3.5)	1.95 *dd* (12.5, 3.5)	1.89 m	1.88 m
3	3.35 *dd* (11.8, 4.4)	3.48 *dd* (11.6, 4.0)	3.53 *dd* (11.3, 4.0)	3.52 *dd* (11.4, 4.0)	3.52 *dd* (11.8, 4.0)	3.36 *dd* (11.3, 4.0)	3.37 *dd* (12.0, 4.5)	3.50 *dd* (11.8, 4.0)	3.37 *dd* (11.5, 4.0)	3.36 *dd* (11.6, 4.4)
4										
5	1.24 m	1.27 m	1.82 *brd* (10.5)	1.90 m	1.28 m	1.28 m	1.28 m	1.33 *dd* (12.5, 4.0)	1.34 m	1.33 *dd* (12.4, 4.4)
6	1.46 *brd* (12.0)	1.46 *brd* (12.8)	1.61 *brd* (12.0)	1.55 m	1.59 m	1.55 m	1.53 m	1.53 m	1.55 m	1.55 m
	0.57 m	0.57 m	0.83 m	0.80 m	0.84 m	0.72 m	0.70 m	0.70 m	0.73 m	0.75 m
7	1.90 m	1.93 m	2.25 m	2.19 m	1.63 *brd* (4.5)	2.09 m	2.11 m	2.06 m	2.11 m	2.06 m
	1.28 m	1.26 m	1.20 m	1.08 m	1.11 m	1.12 m	1.12 m	1.18 m	1.12 m	1.05 m
8	1.66 m	1.67 m	1.31 *dd* (12.5, 4.5)	1.23 m	1.66 *brd* (5.0)	1.67 m	1.66 m	1.68 *dd* (12.5, 4.0)	1.85 m	1.79 m
9										
10										
11	2.23 m	2.23 m	2.84 *dd* (15.5, 9.0)	2.85 *d* (20.0)	2.71 *dd* (15.8, 8.5)	2.01 m	2.04 m	2.14 m	2.08 m	2.16 m
	0.99 m	1.01 m	1.48 m	2.09 *d* (20.0)	1.50 m	1.03 m	1.03 m	1.05 m	1.08 m	1.23 m
12	1.56 m	1.57 m	4.18 m		4.22 *brd* (9.0)	1.64 m	1.62 m	1.64 m	1.78 m	1.77 *dd* (12.2, 4.4)
	1.36 m	1.34 m				1.57 m	1.52 m	1.53 m	1.78 m	1.58 m
13										
14										
15			4.49 *brs*	4.48 *brs*	2.26 *d* (14.0)	4.24 *brs*	4.25 *brs*	4.30 *brs*	4.38 *brs*	4.14 *brs*
					2.09 *d* (14.0)					
16	3.79 *d* (11.6)	3.79 *dd* (3.6, 11.4)								
17	1.56 m	1.57 m	1.80 m	2.30 *brd* (11.2)	1.95 *brd* (10.0)	1.76 *brd* (11.5)	1.75 m	1.44 *d* (11.0)	2.34 *d* (6.5)	1.88 m
18	1.15 s	1.14 s	1.44 s	1.33 s	1.42 s	1.17 s	1.13 s	1.13 s	1.38 s	1.27 s
19	0.41 *d* (4.0)	0.45 *d* (4.0)	0.65 *d* (4.0)	0.69 *d* (4.0)	0.65 *d* (4.0)	0.47 *brs*	0.45 *d* (4.0)	0.51 *d* (4.0)	0.50 *d* (4.0)	0.53 *d* (4.0)
	0.18 *d* (4.0)	0.22 *d* (4.0)	0.41 *d* (4.0)	0.39 *d* (4.0)	0.35 *d* (4.0)	0.23 *d* (3.0)	0.22 *d* (4.0)	0.26 *d* (4.0)	0.24 *d* (4.0)	0.26 *d* (4.0)
20	1.79 m	1.79 m	1.80 m	1.62 m	1.86 m	1.67 m	1.66 m	1.61 *brt* (6.0)	2.11 m	1.79 m
21	0.89 *d* (6.0)	0.89 *d* (6.0)	1.39 *d* (6.0)	1.11 *d* (6.4)	1.38 *d* (6.5)	0.94 *d* (6.0)	0.82 *d* (6.5)	0.83 *d* (6.5)	1.25 *d* (6.5)	1.01 *brd* (4.4)
22	1.70 m	1.71 m	2.33 m	2.35 *dd* (6.6, 2.8)	2.38 m	2.66 *t* (12.0)	2.27 m	2.19 m	2.67 *t* (12.5)	1.82 m
	1.46 *brd* (12.0)	1.46 *brd* (12.8)	1.06 m		1.12 m	1.92 m	1.03 m	0.97 m	1.75 m	1.72 m
23	4.23 m	4.23 *t* (11.6)	4.33 *d* (9.0)	4.79 *d* (9.2)	4.80 *d* (8.5)	4.59 *brd* (10.0)	4.64 *d* (9.0)	4.28 *brd* (7.0)	5.40 *brt* (8.5)	4.11 m
24	5.31 *d* (2.0)	5.30 *d* (1.6)	4.22 *brs*	3.78 *brs*	3.77 *brs*	3.68 *d* (4.0)	4.15 *s*	4.15 *brs*	3.02 *d* (8.5)	6.02 *d* (9.2)
25										
26	1.60 *s*	1.60 *s*	5.35 *brs*	1.47 *s*	1.53 *s*	1.42 *s*	1.28 *s*	5.33 *s*	1.23 *s*	5.19 *brs*
			4.89 *brs*					4.87 *s*		5.00 *brs*
27	1.60 *s*	1.60 *s*	1.86 *s*	1.47 *s*	1.47 *s*	1.26 *s*	1.27 *s*	1.82 *s*	1.37 *s*	1.82 *s*
28	0.97 *s*	0.98 *s*	1.22 *s*	1.08 *s*	1.24 *s*	1.06 *s*	1.16 *s*	1.16 *s*	1.19 *s*	1.24 *s*
29	1.07 *s*	1.27 *s*	1.20 *s*	1.18 *s*	1.22 *s*	1.07 *s*	0.96 *s*	1.29 *s*	1.08 *s*	1.09 *s*
30	0.91 *s*	0.98 *s*	1.06 *s*	1.08 *s*	1.07 *s*	0.97 *s*	1.07 *s*	1.03 *s*	0.97 *s*	0.95 *s*
Ara (at C-3)										
1□	4.72 *d* (7.6)	4.78 *d* (7.2)				4.72 *d* (7.5)		4.80 *d* (7.0)	4.75 *d* (7.5)	4.74 *d* (7.2)
2□	5.91 *t* (8.8)	4.45 *t* (8.4)				5.91 *t* (8.5)		4.17 m	5.93 *t* (8.5)	5.84 *t* (8.4)
3□	4.18 *dd* (9.4, 3.2)	4.16 *dd* (8.8, 2.8)				4.17 *brd* (8.5)		4.46 *t* (8.0)	4.19 *dd* (9.5, 3.2)	4.16 *dd* (10.2, 4.0)
4□	4.27 m	4.31 m				4.27 m		4.30 *brs*	4.29 m	4.28 *m*
5□	4.27 m	4.28 *t* (11.6)				4.27 m		4.28 *brd* (7.0)	4.29 m	4.28 *m*
	3.75 *t* (11.2)	3.79 *dd* (11.4, 3.6)				3.75 m		3.79 *d* (11.0)	3.78 *brd* (11.0)	3.77 *d* (11.2)

(*continued on next page*)

Table 1 (*continued*)

Proton	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	δ_H multi. (*J* in Hz)	δ_H multi. (*J* in Hz)	δ_H multi. (*J* in Hz)	δ_H multi. (*J* in Hz)	δ_H multi. (*J* in Hz)	δ_H multi. (*J* in Hz)	δ_H multi. (*J* in Hz)	δ_H multi. (*J* in Hz)	δ_H multi. (*J* in Hz)	δ_H multi. (*J* in Hz)
Xyl (at C-3)										
1□							4.81 *d* (8.0)			
2□							5.56 *t* (8.0)			
3□							4.19 m			
4□							4.13 m			
5□							4.30 *dd* (11.3, 5.0)			
							3.67 *t* (10.0)			
C_{23} acetyl									2.04 *s*	
C_{24} acetyl	2.15 *s*	2.15 *s*								1.93 *s*
$C_{2'}$ acetyl	2.11 *s*					2.11 *s*	2.14 *s*		2.12 *s*	2.11 *s*

9,19-cycloartane triterpene monoglycoside with two acetoxy groups. The NMR spectroscopic data (Tables 1 and 2) of **1** resembled those of 24-acetylisodahurinol-3-*O*-β-D-xylopyranoside (Shao et al., 2000) except for the presence of a sugar unit. In the ^{1}H NMR spectrum, a downfield resonance was observed at δ_H 5.91 (*t*, *J* = 8.8 Hz), which showed correlations with the methine resonance at δ_H 4.18 (H-3′) and with the anomeric proton at δ_H 4.72 in the ^{1}H–^{1}H COSY spectrum. In addition, the HMBC (Fig. 1) correlation between the *O*-acetyl group (δ_C 170.1) and the proton (δ_H 5.91, *t*, *J* = 8.8 Hz) resonances indicated the *O*-acetyl group was attached at C-2′. The sugar obtained after acid hydrolysis was identified as L-arabinose by comparing its TLC and specific rotation with a standard. The HMBC correlation between H-1′ at δ_H 4.72 (*d*, *J* = 7.6 Hz) and the methine signal at δ_C 88.5 (C-3) suggested that the sugar moiety was attached at C-3. In the ROESY spectrum (Fig. 2), H-3 showed a correlation with H-5 (biogenetically α-oriented), while H-16 showed a correlation with Me-28 (biogenetically α-oriented). Based on these observations, H-3 and H-16 were assigned in an α-orientation. The configuration of C-24 was deduced as *S* by comparison of the coupling constants of H-24 (2.4 Hz) with those of dahurinyl diacetate (9 Hz) and isodahurinyl diacetate (2 Hz) (Shao et al., 2000). Accordingly, compound **1** was characterized as 2′,24-*O*-diacetylisodahurinol-3-*O*-α-L-arabinopyranoside.

Compound **2** was isolated as a white powder. The HR-ESIMS of **2** exhibited a sodiated molecular ion at *m*/*z* 685.3939 [M+Na]$^+$ for the molecular formula of $C_{37}H_{58}O_{10}$. In the ^{1}H NMR spectrum, compound **2** showed signals for a cyclopropane methylene at δ_H 0.22 and 0.45 (1H each, *d*, *J* = 4.0 Hz), six *tert*-methyl groups at δ_H 0.98 (2×), 1.14, 1.27, 1.60 (2×), a *sec*-methyl group at δ_H 0.89 (*d*, *J* = 6.0 Hz), and an anomeric proton resonance at δ_H 4.78 (*d*, *J* = 7.2 Hz), suggesting that **2** was a 9,19-cycloartane triterpene glycoside. In the ^{13}C NMR spectrum (Table 2), compound **2** had resonances corresponding to an arabinose moiety at δ_C 107.6 (*d*), 73.0 (*d*), 74.7 (*d*), 69.6 (*d*), and 66.9 (*t*) (Shao et al., 2000). The remaining 30 carbon signals were identical with the aglycone resonances of **1**. Therefore, **2** was elucidated as 24-*O*-acetylisodahurinol-3-*O*-α-L-arabinopyranoside.

Compound **3** was isolated as a white powder. In its HR-ESIMS, it showed a quasi-molecular ion at *m*/*z* 509.3256 [M+Na]$^+$ for a molecular formula of $C_{30}H_{46}O_5$. Its IR spectrum exhibited absorptions at 3423 and 1648 cm^{-1}, owing to hydroxyl groups and double bond stretches, respectively. The ^{1}H NMR spectrum (Table 1) displayed cyclopropane methylene signals at δ_H 0.41 and 0.65 (1H each, *d*, *J* = 4.0 Hz), six methyl groups at δ_H 1.06, 1.20, 1.22, 1.44, 1.86 and 1.39 (*d*, *J* = 6.0 Hz), an olefinic methylene at δ_H 4.89 and 5.35 (1H each, *brs*), respectively, suggesting **3** to be a 9,19-cyclolanostane aglycone with an olefinic methylene. The NMR spectroscopic data of **3** closely resembled those of 12β-hydroxycimigenol (Kusano et al., 1995), except for the presence of two downfield signals at δ_C 113.1 and 146.0, and the absence of a quaternary carbon and a methyl resonance due to C-25 and C-26, respectively. On the basis of these observations, it was reasonable to deduce that **3** was a 25-dehydrated derivative of 12β-hydroxycimigenol, this also being supported by the HMBC correlations of H-27 at δ_H 1.86 with C-25 (δ_C 146.0), C-26 (δ_C 113.1), and C-24 (δ_C 86.7). Significant ROESY correlations of H-3 with H-5 (biogenetically α-oriented) suggested a β-orientation of the substituent at C-3, whereas the associations of H-15 with H-8 and Me-18 (biogenetically β-oriented), indicated an α-orientation of the hydroxy group at C-15. The configurations of C-23 and C-24 are proposed as *R* and *S*, respectively, by comparing the coupling constants of H-23 and H-24 of **3** with those of known 9,19-cyclolanostane triterpene glycosides (Chen et al., 2002). Therefore, **3** was elucidated as 12β-hydroxy-25-anhydrocimigenol.

Compound **4** was isolated as a white powder. Its molecular formula $C_{30}H_{46}O_6$ was deduced from the ^{13}C NMR data and the posi-

Table 2
^{13}C NMR spectroscopic data of compounds **1**–**10** in pyridine-d_5.

Cpd/C	1	2	3	4	5	6	7	8	9	10
1	32.3 *t*	32.5 *t*	32.8 *t*	32.9 *t*	32.3 *t*	32.3 *t*	32.3 *t*	32.5 *t*	32.1 *t*	32.3 *t*
2	30.0 *t*	30.1 *t*	31.3 *t*	31.1 *t*	31.2 *t*	30.0 *t*	30.0 *t*	30.1 *t*	30.0 *t*	29.8 *t*
3	88.5 *d*	88.4 *d*	78.0 *d*	77.6 *d*	77.9 *d*	88.7 *d*	88.7 *d*	88.6 *d*	88.6 *d*	88.7 *d*
4	41.0 *s*	41.3 *s*	41.1 *s*	41.0 *s*	41.0 *s*	41.0 *s*	41.9 *s*	41.7 *s*	41.5 *s*	41.0 *s*
5	47.2 *d*	47.4 *d*	47.5 *d*	47.9 *d*	47.1 *d*	47.5 *d*	47.5 *d*	47.7 *d*	47.3 *d*	47.5 *d*
6	20.8 *t*	20.8 *t*	21.2 *t*	20.7 *t*	21.0 *t*	21.1 *t*	21.1 *t*	21.1 *t*	21.0 *t*	21.0 *t*
7	26.0 *t*	26.1 *t*	26.3 *t*	26.5 *t*	26.1 *t*	26.5 *t*	26.5 *t*	26.7 *t*	26.7 *t*	26.5 *t*
8	43.6 *d*	43.6 *d*	47.3 *d*	46.7 *d*	45.9 *d*	48.7 *d*	48.7 *d*	48.7 *d*	48.3 *d*	49.0 *d*
9	20.0 *s*	20.0 *s*	20.7 *s*	20.0 *s*	20.4 *s*	20.1 *s*	20.1 *s*	20.1 *s*	20.1 *s*	20.4 *s*
10	27.0 *s*	27.0 *s*	26.9 *s*	27.9 *s*	27.0 *s*	26.6 *s*	26.6 *s*	26.7 *s*	26.7 *s*	26.7 *s*
11	25.9 *t*	25.9 *t*	41.1 *t*	45.5 *t*	41.0 *t*	26.3 *t*	26.4 *t*	26.4 *t*	25.9 *t*	26.5 *t*
12	31.3 *t*	31.3 *t*	72.9 *d*	211.0 *s*	72.4 *d*	34.0 *t*	34.1 *t*	34.1 *t*	33.0 *t*	34.1 *t*
13	40.0 *s*	40.0 *s*	48.3 *s*	56.9 *s*	45.7 *s*	41.8 *s*	41.9 *s*	41.4 *s*	41.0 *s*	42.1 *s*
14	55.0 *s*	55.0 *s*	47.8 *s*	48.3 *s*	52.2 *s*	47.4 *s*	47.3 *s*	47.3 *s*	46.0 *s*	46.7 *s*
15	214.0 *s*	214.0 *s*	80.1 *d*	79.1 *d*	46.9 *t*	80.8 *d*	80.2 *d*	80.4 *d*	83.0 *d*	82.4 *d*
16	84.2 *d*	84.2 *d*	112.7 *s*	111.7 *s*	115.0 *s*	112.2 *s*	112.0 *s*	112.3 *s*	220.0 *s*	103.4 *s*
17	52.3 *d*	52.3 *d*	60.2 *d*	51.0 *d*	61.4 *d*	60.8 *d*	59.5 *d*	59.9 *d*	60.0 *d*	60.6 *d*
18	20.3 *q*	20.3 *q*	12.1 *q*	12.2 *q*	11.8 *q*	19.6 *q*	19.5 *q*	19.5 *q*	19.8 *q*	20.4 *q*
19	31.0 *t*	31.1 *t*	31.0 *t*	31.0 *t*	30.1 *t*	30.9 *t*	30.8 *t*	30.9 *t*	30.4 *t*	30.7 *t*
20	33.2 *d*	33.2 *d*	23.9 *d*	24.5 *d*	23.7 *d*	23.5 *d*	24.0 *d*	23.9 *d*	28.0 *d*	27.3 *d*
21	20.0 *q*	20.0 *q*	20.9 *q*	20.1 *q*	21.8 *q*	19.6 *q*	19.6 *q*	19.5 *q*	20.3 *q*	21.2 *q*
22	38.7 *t*	38.7 *t*	38.8 *t*	38.4 *t*	38.6 *t*	29.6 *t*	38.2 *t*	38.1 *t*	37.0 *t*	32.3 *t*
23	79.0 *d*	79.0 *d*	74.9 *d*	71.6 *d*	72.0 *d*	73.7 *d*	71.6 *d*	75.0 *d*	72.1 *d*	74.7 *d*
24	79.9 *d*	79.8 *d*	86.7 *d*	90.3 *d*	90.3 *d*	84.1 *d*	90.0 *d*	86.7 *d*	65.2 *d*	82.7 *d*
25	72.0 *s*	72.0 *s*	146.0 *s*	71.0 *s*	71.0 *s*	68.6 *s*	71.4 *s*	145.9 *s*	58.5 *s*	142.2 *s*
26	26.8 *q*	26.8 *q*	113.1 *t*	25.3 *q*	27.9 *q*	30.7 *q*	25.4 *q*	113.0 *t*	24.7 *q*	115.8 *t*
27	28.3 *q*	28.3 *q*	18.2 *q*	27.2 *q*	24.8 *q*	25.9 *q*	23.0 *q*	18.2 *q*	19.3 *q*	18.3 *q*
28	17.6 *q*	17.6 *q*	12.0 *q*	12.7 *q*	19.5 *q*	11.7 *q*	11.8 *q*	11.9 *q*	12.0 *q*	11.8 *q*
29	25.4 *q*	25.7 *q*	26.1 *q*	26.1 *q*	26.2 *q*	25.5 *q*	25.4 *q*	25.8 *q*	25.4 *q*	25.5 *q*
30	15.2 *q*	15.4 *q*	14.8 *q*	14.8 *q*	14.8 *q*	15.3 *q*	15.3 *q*	15.4 *q*	15.2 *q*	15.2 *q*
Ara (at C-3)										
1□	104.6 *d*	107.6 *d*				104.6 *d*		107.4 *d*	104.5 *d*	104.3 *d*
2□	74.4 *d*	73.0 *d*				74.4 *d*		72.9 *d*	74.4 *d*	74.4 *d*
3□	72.5 *d*	74.7 *d*				72.5 *d*		74.7 *d*	72.5 *d*	72.5 *d*
4□	69.8 *d*	69.6 *d*				69.8 *d*		69.5 *d*	69.8 *d*	69.7 *d*
5□	67.3 *t*	66.9 *t*				67.3 *t*		66.7 *t*	67.3 *t*	67.3 *t*
Xyl (at C-3)										
1□							104.7 *d*			
2□							75.7 *d*			
3□							76.3 *d*			
4□							71.4 *d*			
5□							67.2 *t*			
C_{23} *acetyl*										
CO									170.6 *s*	
CH_3									21.0 *q*	
C_{24} *acetyl*										
CO	171.1 *s*	171.1 *s*								170.3 *s*
CH_3	21.0 *q*	21.0 *q*								21.2 *q*
$C_{2'}$ *acetyl*										
CO	170.1 *s*					170.0 *s*	170.0 *s*		170.0 *s*	170.0 *s*
CH_3	21.4 *q*					21.3 *q*	21.3 *q*		21.3 *q*	21.4 *q*

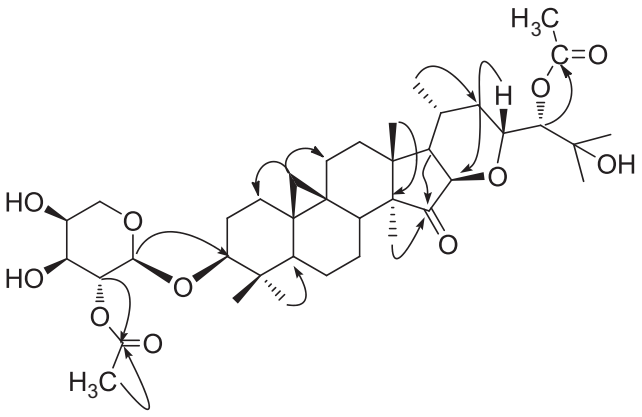

Fig. 1. Major long-distance ^{1}H–^{13}C correlations of **1**.

tive ion HR-ESIMS, which showed a molecular peak at *m*/*z* 525.3198 $[M+Na]^+$. In the IR spectrum, absorption bands at 3404 and 1709 cm^{-1} for hydroxyl and carbonyl groups were observed. The NMR spectroscopic data (Tables 1 and 2) of **4** resembled those of 12-β-hydrolcimigenol (Kusano et al., 1995) except for changes at C-11 (δ_C 45.5), C-12 (δ_C 211.0), and C-13 (δ_C 56.9) due to a hydroxyl group being replaced by a carbonyl group. The position of the carbonyl at C-12 was confirmed by correlations between H-11 (δ_H 2.09 and 2.85), Me-18 (δ_H 1.33) and C-12 (δ_C 211.0) in the HMBC spectrum. Thus compound **4** was characterized as cimigenol-12-one.

Compound **5** was isolated as a white powder. The negative ion ESIMS showed a pseudo-molecular ion at *m*/*z* 523 $[M+Cl]^-$, which in conjunction with ^{13}C NMR spectroscopic data established the molecular formula as $C_{30}H_{48}O_5$. In the ^{1}H NMR spectrum, signals for the cyclopropane methylene at δ_H 0.35 and 0.65 (1H each, *d*, *J* = 4.0 Hz), six *tert*-methyl groups at δ_H 1.07, 1.22, 1.24, 1.42, 1.47, and 1.53, and a *sec*-methyl group at δ_H 1.38 (*d*, *J* = 6.5 Hz) were observed, suggesting **5** as a 9,19-cycloartane triterpene. The

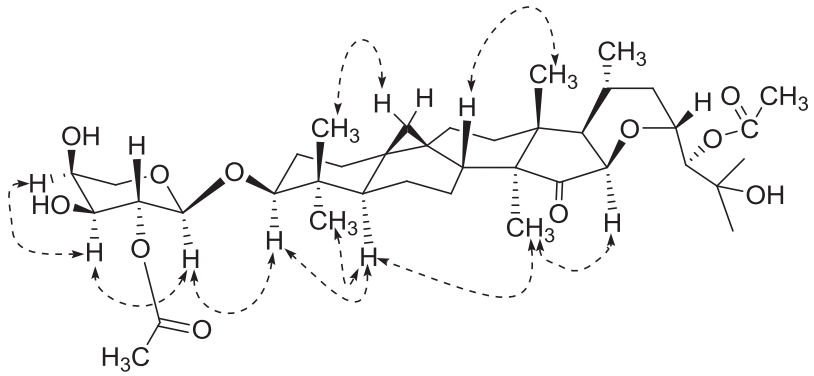

Fig. 2. Key ROESY correlations of compound **1**.

Table 3
Cytotoxicity of compounds isolated from the aerial parts of *Cimicifuga foetida* (IC_{50} values; μM).

Compounds	HL-60	SMMC-7721	A-549	SK-BR-3	PANC-1
1	>40	>40	>40	>40	>40
2	>40	>40	>40	>40	>40
3	>40	>40	>40	>40	>40
4	>40	>40	>40	>40	>40
5	>40	17.65	>40	35.14	>40
6	>40	>40	>40	>40	>40
7	15.39	14.84	9.50	19.20	17.94
8	6.20	14.14	16.69	22.74	>40
9	35.24	>40	>40	>40	>40
10	31.68	>40	>40	>40	>40
11	>40	>40	>40	>40	>40
12	>40	>40	>40	>40	>40
13	>40	>40	>40	>40	>40
14	>40	>40	>40	>40	>40
15	13.37	14.80	15.65	17.35	17.72
16	>40	>40	>40	>40	>40
17	>40	>40	>40	>40	>40
18	>40	>40	>40	>40	>40
Cisplatin	0.75	14.13	26.54	22.51	19.47

^{13}C NMR and DEPT spectroscopic data of **5** were identical with aglycone resonances of 12β-hydroxy-15-deoxycimigenol-3-*O*-β-D-xylopyranoside (Yoshimitsu et al., 2006) except for the upfield shift of the C-3 by 7.6 ppm, which could be explained by the absence of a sugar unit at C-3. Therefore, **5** was elucidated as 12β-hydroxy-15-deoxycimigenol.

Compound **6** was isolated as a white powder, which exhibited a pseudo-molecular ion at *m*/*z* 685 $[M+Na]^+$ in the positive ion ESIMS. Analysis of its ^{13}C NMR and HR-ESIMS *m*/*z*: 685.3915 (calc. 685.3927) helped confirm the molecular formula as $C_{37}H_{58}O_{10}$. The ^{13}C and DEPT NMR spectrum of **6** (Table 2) showed signals ascribable to the methylene carbon of the cyclopropane ring at δ_C 30.9 (C-19), four oxygen-bearing methine carbons at δ_C 88.7 (C-3), 84.1 (C-24), 80.8 (C-15), and 73.7 (C-23), two oxygen-bearing quaternary carbons at δ_C 112.2 (C-16) and 68.8 (C-25), and a carbonyl group at δ_C 170.0. The ^{13}C NMR spectroscopic data of **6** showed a close resemblance with those of 24-*epi*-cimigenol-3-*O*-β-D-xylopyranoside, except for the sugar unit (Li et al., 1993). The pentose moiety signals at δ_C 104.6 (*d*), 74.4 (*d*), 72.5 (*d*), 69.8 (*d*), and 67.3 (*t*) were identical with those of **1**, indicating that the sugar was 2′-*O*-acetyl-3-*O*-α-L-arabinose. This conclusion was confirmed by the presence of the HMBC correlation between the H-2′ signal at δ_H 5.91 and the carbonyl group signal at δ_C 170.0. The configurations of C-23 and C-24 were considered to be *R* and *R*, respectively, by comparing the coupling constants of the C-23 and C-24 proton signals of **6** with those of known 9,19-cyclolanostane triterpene glycosides (Li et al., 1993). Ultimately, **6** was elucidated as 2′-*O*-acetyl-24-*epi*-cimigenol-3-*O*-α-L-arabinopyranoside.

Compound **7**, a white powder, gave a pseudo-molecular ion at *m*/*z* 685.3944 $[M+Na]^+$ in the positive ion HR-ESIMS, corresponding to the molecular formula $C_{37}H_{58}O_{10}$, which is 42 Da less than those of 2′,25-*O*-diacetylcimigenol-3-*O*-β-D-xylopyranoside (Zhou et al., 2004). When its spectroscopic data (Tables 1 and 2) were compared with those of 2′,25-*O*-diacetylcimigenol-3-*O*-β-D-xylopyranoside, the resonances of an *O*-acetyl group were absent in **7**, showing instead a hydroxyl functionality at C-25. This deduction was confirmed by the upfield shift of C-25 by 11.8 ppm and correlations of δ_H 1.16 (H-27) and 1.27 (H-26) with δ_C 71.4 (C-25) in the HMBC spectrum. The relative configuration of **7** was proposed in a similar manner to that of **6**. Accordingly, compound **7** was characterized as 2′-*O*-acetylcimigenol-3-*O*-β-D-xylopyranoside.

Compound **8** was isolated as a white powder. The positive ion HR-ESIMS showed a pseudo-molecular ion at *m*/*z* 625.3707 $[M+Na]^+$, leading to the molecular formula $C_{35}H_{54}O_8$, which is 58 Da less than 12-*O*-acetyl-25-anhydrocimigenol-3-*O*-α-L-arabinopyranoside (Chen et al., 2002). The DEPT spectrum of **8** differed from 12β-*O*-acetyl-25-anhydrocimigenol-3-*O*-α-L-arabinopyranoside in that a methine at δ_C 77.3 (C-12) were transformed to a methylene at δ_C 34.1. This deduction was further supported by the HMBC correlation (Fig. 1) between H-11/C-12, and H-12/C-13, as well as by the upfield shift of C-11 and C-13 about 12.1 and 7.3 ppm in the ^{13}C spectrum, respectively. Thus, compound **8** was characterized as 25-anhydrocimigenol-3-*O*-α-L-arabinopyranoside.

Compound **9**, isolated as a white powder, showed a pseudo-molecular ion at *m*/*z* 727 $[M+Na]^+$ in the positive ion ESIMS. The ^{13}C NMR and HRTOF-ESIMS (*m*/*z*: 727.4043 $[M+Na]^+$) data determined its molecular formula as $C_{39}H_{60}O_{11}$. The ^{1}H and ^{13}C NMR spectra of **9** resembled those of **12** except for the signals of a sugar moiety. In the ^{1}H NMR spectrum, the resonance for H-2′ in **9** at δ_H 5.93 (*t*, *J* = 8.5 Hz) was shifted upfield to δ_H 4.29 (*t*, *J* = 8.6 Hz) in **12**, meanwhile the signal for H-4′ was shifted downfield from δ_H 4.29 in **9** to δ_H 5.41 in **12**, which indicated that an acetoxy group is attached at C-2′ in **9** instead of at C-4′ in **12**. This conclusion was also supported by an analysis of the HMBC spectrum, which showed a correlation between H-2′ at δ_H 5.93 and the acetoxy carbon at δ_C 170.0. The configurations of C-23 and C-24 were assigned as *R* and *S*, respectively, by comparing the coupling constants of H-23 and H-24 of **9** with those of known 9,19-cyclolanostane triterpene glycosides (Kusano et al., 1999). Therefore, compound **9** was identified as 2′,23-*O*-diacetylshengmanol-3-*O*-α-L-arabinopyranoside.

Compound **10** was isolated as a white powder. In the high-resolution positive ESIMS, it showed a quasi-molecular ion at *m*/*z* 727.4017 $[M+Na]^+$ for a molecular formula of $C_{39}H_{60}O_{11}$. In the ^{13}C and DEPT spectrum (Table 2), **10** exhibited signals very similar

to those of 24-*O*-acetyl-25-anhydrohydroshengmanol-3-*O*-β-D-xylopyranoside (Nian et al., 2009) except for the sugar moiety. As for **9**, an acetyl group was determined to be at C-2′ for **10**, which was further confirmed by the presence of the HMBC correlation between H-2′ (δ_H 5.84) and the carbonyl carbon at δ_C 170.3. Compound **10** was treated with 4% K_2CO_3, followed by 5% HOAc (Nian et al., 2009) to afford compound **8**. This conversion suggested the stereochemistry of the 23*R* and 24*S* configurations in **10**. Therefore, compound **10** was elucidated as 2′,24-di-*O*-*acetyl*-25-anhydrohydroshengmanol-3-*O*-α-L-arabinopyranoside (**10**).

2.2. Cytotoxic activity

The cytotoxicities of these eighteen 9,19-cycloartane triterpenes (**1**–**18**) were evaluated against human HL-60, SMMC-7721, A549, SK-BR-3, and PANC-l cell lines. Among them, **7**, **8**, and **15** exhibited broad-spectrum and moderate cytotoxicities with IC_{50} values ranging from 6.20 to 22.74 μM. Compound **5** exhibited moderate cytotoxicity against SMMC-7721 cell line and weak cytotoxicity against SK-BR-3 cell line, having IC_{50} values of 17.65 and 35.14 μM, respectively. Compounds **9** and **10** showed weak inhibition activities against HL-60 cell line, with IC_{50} values of 35.24 and 31.68 μM, respectively. Other compounds showed no cytotoxic activities up to a highest concentration of 40.00 μM, in any of the cell lines tested (Table 3).

2.3. Concluding remarks

Previously, we reported that a series of active compounds, isolated from the roots of *C. foetida* exhibited selective cytotoxicity against the human HepG2 cell line rather than against the MCF7, HT29 and MKN28 cell lines (Nian et al., 2010). With the purpose of finding cytotoxic 9,19-cycloartane triterpenoids with new targets and completely studying their active properties, we used five different human tumor cell lines to evaluate cytotoxicities of the isolated compounds in this study. As a result, compounds, such as **7**, **8**, and **15**, in contrast, exhibited moderate and broad-spectrum cytotoxicities against the testing cell lines.

Although a number of 9,19-cycloartane triterpene glycosides were isolated from the aerial parts of *C. foetida* (Pan et al., 2002, 2003, 2004, 2007, 2009; Tian et al., 2006), its chemical components are not completely known. In the present study, another 18 analogs, including seven aglycones, were isolated and identified for the first time. Based on the above results, we suggest that the main chemical constituents of the aerial parts of *C. foetida* are 9,19-cycloartane triterpenes and that the tissue may be a potential resource for promising antitumor agents.

Almost 50% of 9,19-cycloartane triterpenes from *Cimicifuga* species possess a cimigenol-skeleton (Li and Yu, 2006). Chemical constituents possessing the cimigenol-skeleton, such as 25-methoxycimigenol-3-*O*-α-L-arabinopyranoside (Watanbe et al., 2002), cimifugoside (Einbond et al., 2004), 25-*O*-anhydrocimigenol-3-*O*-β-D-xylopyranoside (Tian et al., 2006), cimicifoetisides A and B (Sun et al., 2007), 3′,25-*O*-diacetylcimigenol-3-*O*-β-D-xylopyranoside (Nian et al., 2010), and **5**, **7**, **8**, and **15** in the present study, are the main cytotoxic constituents from *Cimicifuga* species. Based on the analysis of reported bioassay results, we may propose preliminary structure–activity relationships for active compounds with cimigenol-skeleton: (**1**) compounds with groups like acetyl or butenoyl attached to the sugar unit are more potent than those without such substituent; (**2**) the configurations of C-23 and C-24 should be *R* and *S* for activity; (**3**) hydrophobic groups, like acetoxy and methoxyl, instead of a hydroxyl group at C-25, or dehydration between C-25 and C-26 increase inhibition activities of these compounds.

3. Experimental

3.1. General experimental procedures

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 AM-400 and DRX-500 spectrometers (Bruker, Zűrich, Switzerland), using TMS as internal standard. ESIMS and HRTOF-ESIMS data were obtained using a VG Autospec-3000 spectrometer. Infrared spectra were recorded on a Shimadzu IR-450 instrument with KBr pellets. Thin-layer chromatography was performed on precoated TLC plates (Silica gel 60 F_{254}, Qingdao Marine Chemical, Inc.) and spots were visualized by heating after spraying with 10% H_2SO_4. Silica gel (mesh 200–300, Qingdao Marine Chemical, Inc.), Lichroprep RP-18 (40–63 μm, Merck), Amberlite IR-35 (10 ml) column and Sephadex LH-20 (Pharmacia) were used for column chromatography (cc).

3.2. Plant material

Aerial parts of *Cimicifuga foetida* L. (10 kg) were collected from Shangrila County, Yunnan Province, China, in September 2007 and identified by Prof. Baogui Li, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Science. A voucher specimen (KUN No. 200709004) 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 dried and milled aerial parts of *C. foetida* (10 kg) were extracted with MeOH (3 × 20 L, 24 h each) at room temperature to give a residue (1243 g) after evaporating in vacuum at 50°. This residue was suspended in H_2O (2.5 L) and then extracted successively with petroleum ether (bp 40–60 °C), EtOAc and *n*-BuOH (3 × 2.5 L each) to give petroleum ether-soluble (135 g), EtOAc-soluble (313 g), and *n*-BuOH-soluble (480 g) extracts. The EtOAc extract (313 g) was subjected to silica gel cc (2 kg, 15 × 150 cm) eluted with $CHCl_3$–MeOH [100:0 (2.5 L), 50:1 (4 L), 20:1 (8 L), 10:1 (10 L)] to afford fractions **1** (43 g), **2** (23 g), **3** (78 g) and **4** (91 g). Fraction **2** (23 g) was divided into four sub-fractions (**2.1**–**2.4**) after performing silica gel cc (200 g, 8 × 60 cm), eluted with $CHCl_3$–MeOH (gradient from 60:1 to 40:1, 4 L). Compounds **3** (13 mg), **4** (51 mg), **5** (29 mg), **11** (21 mg), **14** (9 mg), **16** (11 mg), and **18** (20 mg) were purified from fraction **2.2** (8.1 g) by conducting cc (5 × 25 cm) on RP-18 (180 g), eluted with MeOH–H_2O (7:3, 4 L) and then Sephadex LH-20 (150 g, 2.5 × 200 cm), eluted with MeOH (0.8 L each). Fraction **3** (78 g) was fractionated into three sub-fractions (**3.1**–**3.3**) by performing silica gel cc (400 g, 10 × 80 cm), eluted with $CHCl_3$–MeOH (gradient from 40:1 to 20:1, 8 L). Fraction **3.2** (16.6 g) was subjected to silica gel cc (200 g, 6 × 60 cm) eluted with $CHCl_3$–Me_2CO (4:1, 4 L), then RP-18 cc (180 g, 5 × 25 cm), eluting with MeOH–H_2O (3:2, 3 L) to yield **1** (78 mg), **2** (59 mg), **6** (12 mg), **7** (11 mg), **8** (81 mg), **9** (25 mg) and **10** (26 mg), **12** (45 mg), **13** (21 mg). Fraction **3.3** (38 g) was applied to a silica gel (250 g) column (8 × 60 cm) eluted with $CHCl_3$–Me_2CO (gradient from 3:1 to 2:1, 8 L) to afford fractions **3.3.1**–**3.3.4**. Fraction **3.3.1** (9.5 g) was subjected to a RP-18 cc (180 g, 5 × 25 cm), eluting with MeOH–H_2O (3:2, 4 L), then purified on Sephadex LH-20 (2.5 × 200 cm, 150 g), eluting with MeOH (1 L) to afford **15** (320 mg). Compound **17** (280 mg) was crystallized in MeOH from Fraction **3.3.4** (7.8 g).

3.4. Acid hydrolysis

Compounds **1**, **9**, and **10** (15 mg of each), and **6** and **7** (10 mg of each) were individually dissolved in MeOH (15 mL), then 4% K_2CO_3 (15 mL) was added and each solution was stirred at room temperature overnight. Each solution was neutralized by 10% HOAc, and extracted with EtOAc (3 × 30 mL). Each EtOAc extract, after removal of solvent, was dissolved in MeOH (10 mL) and refluxed with 0.5 N HCl (3 mL) for 4 h (Nian et al., 2010). Compounds **2** and **8** (15 mg of each), by contrast, were directly individually dissolved in MeOH (10 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 × 20 mL). Each aqueous layer was then applied to an Amberlite IR-35 (10 mL) column and each resulting fraction was concentrated *in vacuum* to give a monosaccharide, which had an *Rf* (EtOAc–$CHCl_3$–MeOH–H_2O, 3:2:2:1) and specific rotation $[\alpha]_D^{20}$ +84.06 (*c* 0.09, H_2O) corresponding to those of L-arabinose (Sigma–Aldrich); compound **7** was treated in same way as **1**, then identified the sugar with a *Rf* (EtOAc–$CHCl_3$–MeOH–H_2O, 3:2:2:1) and specific rotation $[\alpha]_D^{20}$ +38.09 (*c* 0.07, H_2O) corresponding to those of D-xylose (Sigma–Aldrich).

3.5. Conversion of **10** to **8**

Compound **10** (10 mg) was dissolved in MeOH (10 mL), then 4% K_2CO_3 (10 mL) was added and the solution was stirred at room temperature overnight. The solution was neutralized with 5% HOAc, and then extracted with EtOAc (3 × 25 mL). The EtOAc part, after removal of the solvent, was dissolved in tetrahydrofuran (5 mL) and 5% HOAc (5 mL), then heated on a boiling water bath for 3 h. After evaporation of the solvent *in vacuo*, the products were subjected to silica gel cc, eluted with $CHCl_3$–MeCO 8:1 to afford **8** (7.6 mg).

3.6. 2′,24-Di-O-acetylisodahurinol-3-O-α-L-arabinopyranoside (**1**)

White powder; $[\alpha]_D^{20}$ 46.86 (MeOH, *c* 0.10); for ^1H and ^{13}C (C_5D_5N) NMR spectroscopic data, see Tables 1 and 2; IR (KBr) ν_{max} 3410, 2938, 1741, 1632, 1383, 1243, 1085, 1041 cm^{-1}; ESIMS (positive ion mode) *m*/*z* 727 $[M+Na]^+$; HRTOF-ESIMS (positive ion mode) *m*/*z* 727.4048 $[M+Na]^+$ (calc. for $C_{39}H_{60}O_{11}Na$, 727.4033).

3.7. 24-O-Acetylisodahurinol-3-O-α-L-arabinopyranoside (**2**)

White powder; $[\alpha]_D^{20}$ 42.03 (MeOH, *c* 0.10); for ^1H and ^{13}C (C_5D_5N) NMR spectroscopic data, see Tables 1 and 2; IR (KBr) ν_{max} 3432, 2970, 2873, 1721, 1632, 1466, 1377, 1249, 1066, 981 cm^{-1}; ESIMS (positive ion mode) *m*/*z* 685 $[M+Na]^+$; HRTOF-ESIMS (positive ion mode) *m*/*z* 685.3939 $[M+Na]^+$ (calc. for $C_{37}H_{58}O_{10}Na$, 685.3927).

3.8. 12β-Hydroxy-25-anhydrocimigenol (**3**)

White powder; $[\alpha]_D^{20}$ 4.69 (MeOH, *c* 0.10); for ^1H and ^{13}C (C_5D_5N) NMR spectroscopic data, see Tables 1 and 2; IR (KBr) ν_{max} 3423, 2938, 2870, 1726, 1648, 1459, 1382, 1148, 1066, 981 cm^{-1}; ESIMS (positive ion mode) *m*/*z* 509 $[M+Na]^+$; HRTOF-ESIMS (positive ion mode) *m*/*z* 509.3256 $[M+Na]^+$ (calc. for $C_{30}H_{46}O_5Na$, 509.3242).

3.9. Cimigenol-12-one (**4**)

White powder; $[\alpha]_D^{20}$ 0.00 (MeOH, *c* 0.06); for ^1H and ^{13}C (C_5D_5N) NMR spectroscopic data, see Tables 1 and 2; IR (KBr) ν_{max} 3431, 2933, 2870, 1709, 1451, 1383, 1168, 1024, 981 cm^{-1}; ESIMS (positive ion mode) *m*/*z* 525 $[M+Na]^+$; HRTOF-ESIMS (positive ion mode) *m*/*z* 525.3198 $[M+Na]^+$ (calc. for $C_{30}H_{46}O_6Na$, 525.3192).

3.10. 12β-Hydroxy-15-deoxycimigenol (**5**)

White powder; $[\alpha]_D^{20}$ 28.11 (MeOH, *c* 0.08); For ^1H and ^{13}C (C_5D_5N) NMR spectroscopic data, see Tables 1 and 2; IR (KBr) ν_{max} 3462, 2927, 2857, 1462, 1034 cm^{-1}; ESIMS (negative ion mode) *m*/*z* 523 $[M-Cl]^-$ HRTOF-ESIMS (negative ion mode) *m*/*z* 523.3198 $[M+Cl]^-$ (calc. for $C_{30}H_{48}OCl$, 523.3190).

3.11. 2′-O-Acetyl-24-epi-cimigenol-3-O-α-L-arabinopyranoside (**6**)

White powder; $[\alpha]_D^{20}$ 5.92 (MeOH, *c* 0.09); For ^1H and ^{13}C (C_5D_5N) NMR spectroscopic data, see Tables 1 and 2; IR (KBr) ν_{max} 3462, 2959, 2870, 1733, 1457, 1253, 1167, 1072, 972 cm^{-1}; ESIMS (positive ion mode) *m*/*z* 685 $[M+Na]^+$; HRTOF-ESIMS (positive ion mode) *m*/*z* 685.3915 $[M+Na]^+$ (calc. for $C_{37}H_{58}O_{10}Na$, 685.3927).

3.12. 2′-O-Acetylcimigenol-3-O-β-D-xylopyranoside (**7**)

White powder; $[\alpha]_D^{20}$ 16.48 (MeOH, *c* 0.09); For ^1H and ^{13}C (C_5D_5N) NMR spectroscopic data, see Tables 1 and 2; IR (KBr) ν_{max} 3483, 2940, 2874, 1731, 1457, 1256, 1167, 1081 cm^{-1}; ESIMS (positive ion mode) *m*/*z* 685 $[M+Na]^+$; HRTOF-ESIMS (positive ion mode) *m*/*z* 685.3944 $[M+Na]^+$ (calc. for $C_{37}H_{58}O_{10}Na$, 685.3927).

3.13. 25-Anhydrocimigenol-3-O-α-L-arabinopyranoside (**8**)

White powder; $[\alpha]_D^{20}$ 10.58 (MeOH, *c* 0.06); For ^1H and ^{13}C (C_5D_5N) NMR spectroscopic data, see Tables 1 and 2; IR (KBr) ν_{max} 3463, 2960, 2869, 1631, 1430, 1256, 1073, 981 cm^{-1}; ESIMS (positive ion mode) *m*/*z* 625 $[M+Na]^+$; HRTOF-ESIMS (positive ion mode) *m*/*z* 625.3707 $[M+Na]^+$ (calc. for $C_{35}H_{54}O_8Na$, 625.3716).

3.14. 2′,23-Di-O-acetylshengmanol-3-O-α-L-arabinopyranoside (**9**)

White powder; $[\alpha]_D^{20}$ −10.17 (MeOH, *c* 0.08); For ^1H and ^{13}C (C_5D_5N) NMR spectroscopic data, see Tables 1 and 2; IR (KBr) ν_{max} 3467, 2927, 2855, 1737, 1459, 1378, 1241, 1082, 992 cm^{-1}; ESIMS (positive ion mode) *m*/*z* 727 $[M+Na]^+$; HRTOF-ESIMS (positive ion mode) *m*/*z* 727.4043 $[M+Na]^+$ (calc. for $C_{39}H_{60}O_{11}Na$, 727.4033).

3.15. 2′,24-Di-O-acetyl-25-anhydrohydroshengmanol-3-O-α-L-arabinopyranoside (**10**)

White powder; $[\alpha]_D^{20}$ −11.26 (MeOH, *c* 0.07); for ^1H and ^{13}C (C_5D_5N) NMR spectroscopic data, see Tables 1 and 2; IR (KBr) ν_{max} 3435, 2933, 2871, 2017, 1756, 1636, 1375, 1247, 1052, 973 cm^{-1}; ESIMS (positive ion mode) *m*/*z* 727 $[M+Na]^+$; HRTOF-ESIMS (positive ion mode) *m*/*z* 727.4017 $[M+Na]^+$ (calc. for $C_{39}H_{60}O_{11}Na$, 727.4033).

3.16. Cytotoxicity experiments

Cytotoxicity evaluations were performed using the MTT method for the human tumor HL-60, SMMC-7721, A549, SK-BR-3, and PANC-l cells and cisplatin was used as a positive control (Zhou et al., 1993). Briefly, 2 × 10^4/mL cells were added to each well (100 μL/well), and incubated with various concentrations of drugs (100, 30, 10, 3, 1, 0.3 μg/mL) in three replicates for 48 h at 37 °C in a humidified atmosphere of 5% CO_2. After 48 h, 20 μL of methylthiazolyldiphenyl tetrazalium bromide (MTT) solution (5 mg/mL) were added to each well, which were incubated for another 4 h. Then 10% SDS–5% iB_4O_4–0.012 M HCl was added to each well (100 μL/well). After 12 h at room temperature, the OD value of each well was recorded on a Model680 (Bio-Rad) reader at 595 nm.

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