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Cytotoxic cycloartane triterpenes from the roots of *Cimicifuga heracleifolia*

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ABSTRACT

Phytochemical investigation on the roots of *Cimicifuga heracleifolia* afforded nine new 9,19-cycloartane triterpenes (**1**–**9**), along with seven known constituents (**10**–**16**). The new structures were elucidated by spectroscopic and chemical methods. Biological evaluation of the compounds against human HL-60, SMMC-7721, A549, MCF-7, and SW-480 cell lines indicated that eight cimigenol-type compounds (**1**–**3**, **5**, **10**–**12**, **14**) showed different levels of cytotoxic activities, with IC_{50} values ranging from 0.83 to 23.94 μM. In addition, their structural and bioactive features enriched the structure–activity relationships we proposed before.

1. Introduction

Cimicifuga (now *Actaea* in Europe and USA),[1,2] belonging to the family Ranunculaceae, consists of 28 species and has a long history to be used as medicinal herb worldwide.[3] Previous chemical and biological studies on *Cimicifuga* species led to isolation of various bioactive constituents, such as chromones, cinnamic acid derivatives, and 9,19-cyclolanostane triterpenes.[3] In China, the roots of *C. foetida*, *C. dahurica*, and *C. heracleifolia* are important elements of Traditional Chinese Medicine (TCM), namely 'shengma' and have been officially listed in the Chinese Pharmacopoeia as cooling and detoxification agents.[4]

In the theory of TCM, tumor is defined as a kind of toxin,[5] so it is of interest to study antitumor activities of plants used as detoxification agents. Intriguingly, we chose *C. foetida* as an object to investigate potential antitumor constituents and reported a number of cytotoxic 9,19-cycloartane triterpenes both from the roots and aerial parts of this medicinal plant.[6–9] Moreover, the preliminary structure–activity relationships (SAR) of the compounds with a cimigenol-skeleton were proposed based on the analyses of related bioassay results.[9]

Although *C. heracleifolia* is a famous TCM, few works of its chemical constituents and bioactivities have been reported.[10–12] Inspired by what our group achieved studies in *C. foetida*, using the theory of tumor in TCM as a guiding principle, we further undertook phytochemical and pharmacological investigations on the roots of *C. heracleifolia*. Nine new 9,19-cycloartane triterpenes (**1**–**9**), together with seven known compounds, 7,8-didehydro-25-*O*-acetylcimigenol-3-*O*-α-L-arabinopyranoside (**10**),[13] 25-anhydrocimigenol-3-*O*-β-D-xylopyranoside (**11**),[14] 7,8-didehydro-25-anhydrocimigenol-3-*O*-β-D-xylopyranoside (**12**),[15] 24-*epi*-7,8-didehydrocimigenol (**13**),[10] acerinol (**14**),[16] 23-*epi*-26-deoxy-7,8-didehydroacteinol (**15**),[13] and acerionol (**16**),[17] were isolated and characterized (Scheme 1). The isolated compounds were tested for their cytotoxicities against human HL-60, SMMC-7721, A549, MCF-7, and SW480 cell lines, using the MTT method. Described herein are the isolation, structure elucidation, and biological activities of these compounds.

2. Results and discussion

Compound **1** was obtained as white powder and gave a molecular formula of $C_{31}H_{50}O_7$, as determined by HRTOF-ESIMS ($[M+Cl]^-$ *m*/*z* 569.3236, calcd 569.3245), requiring 7° of unsaturation. The IR spectrum showed absorptions for hydroxy groups at 3403 cm^{-1} and carbonyl groups at 1741 cm^{-1}, respectively. The ^1H NMR spectrum (Table 1) showed the presence of the characteristic cyclopropane methylene signals at δ_H 0.59 and 0.83 (1H each, d, *J*=4.0 Hz), a *sec*-methyl signal at δ_H 0.85 (d, *J*=6.5 Hz), and six *tert*-methyl groups at δ_H 1.16, 1.26, 1.37 (×2), 1.46, and 1.48, respectively. Above evidence, together with the diagnostic signals of two oxygen-bearing methine carbons at δ_C 90.7 (C-24), and 72.3 (C-23), and an oxygen-bearing quaternary carbon at δ_C 112.5 (C-16), suggesting **1** is a cimigenol-type triterpene.[6–10,18,19] Apart from a carbonyl group, the remaining degrees of unsaturation in **1** is one less than those of cimigenol (**17**).[18] Based on the evidence, we may deduce that one of the rings was opened in **1**. Comparison of the NMR data of **1** with those of **17** suggested the two compounds are structurally similar, except for

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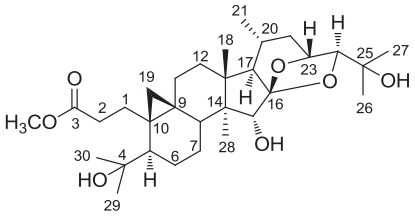

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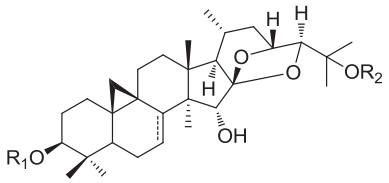
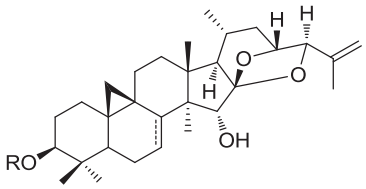
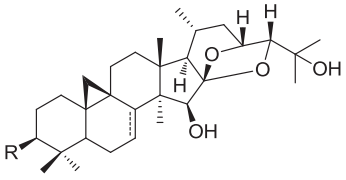


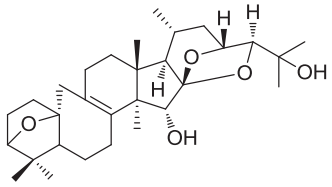
2 R_1 = 2',4'-*O*-diacetyl-*α*-L-arabinopyranosyl, R_2 = H
3 R_1 = 3',4'-*O*-diacetyl-*α*-L-arabinopyranosyl, R_2 = H
4 R_1 = 4'-*O*-acetyl-*α*-L-arabinopyranosyl, R_2 = H
10 R_1 = *α*-L-arabinopyranosyl, R_2 = Ac, Δ 7(8)
18 R_1 = 2'-*O*-acetyl-*α*-L-arabinopyranosyl, R_2 = H
17 R_1 = H, R_2 = H



5 R = 3'-*O*-acetyl-*α*-L-arabinopyranosyl
11 R =*β*-D-xylopyranosyl
12 R =*β*-D-xylopyranosyl, Δ 7(8)
19 R = *α*-L-arabinopyranosyl



6 R = =O
13 R = OH, Δ 7(8)
20 R = OH



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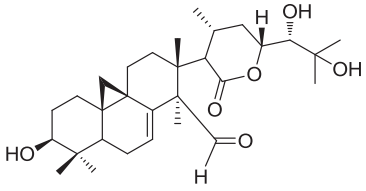

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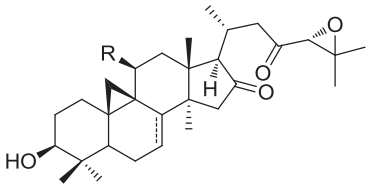
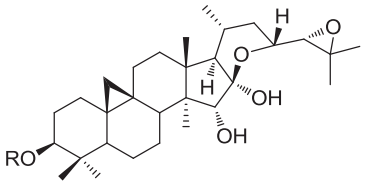
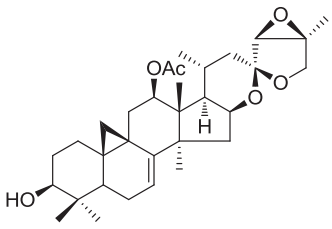


8 R = H
22= OH, Δ 7(8)



9 R = 2'-*O*-acetyl-*α*-L-arabinopyranosyl
23 R = *α*-L-arabinopyranosyl



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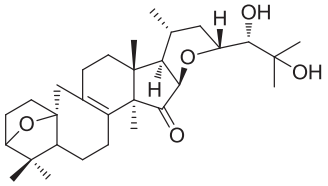

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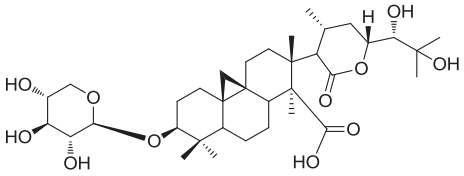

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Scheme 1. Structures of compounds isolated from the roots of *C. heracleifolia* (**1**–**16**) and referenced in the paper (**17**–**23**).

the major differences at ring A. In the ^{13}C spectrum of **1**, a hydroxymethine signal due to C-3 at δ_C 78.0 (d) was absent, showing instead an ester carbonyl group (δ_C 175.3). Besides, the signal due to C-4 exhibited a downfield shift from δ_C 41.1 to 75.6. The changes of these chemical shifts may be due to the oxidative cleavage of ring A through formation of a lactone between C-3 and C-4, and followed by hydrolysis and methyl esterification at C-3. The HMBC correlations of methoxy signal at δ_H 3.58 with the ester carbonyl group at δ_C 175.3, and CH_3-29 and CH_3-30 signals at δ_H 1.37 (×2) with the hydroxy bearing quaternary carbon at δ_C 75.6 further confirmed the deduction (Fig. 1). Significant ROESY correlations of H-5 with CH_3-28 suggested an α-orientation of the proton. In addition, the associations of H-15 with H-8 and Me-18, indicated an α-orientation of the hydroxy group at C-15 (Fig. 2). The configurations of C-23 and C-24 were assigned as *R* and *S*, respectively, by comparison of the coupling constants of H-23 (8.5 Hz) and H-24 (0 Hz) of **1** with those of known compounds.[10,19] Therefore, the structure of **1** was determined as methyl 3,4-seco-4-hydroxy-3-cimigenolate.

Compounds **2** and **3** were isolated as white powder. The spectroscopic features of compounds **2** and **3** resembled each other. The HRTOF-ESIMS of both compounds exhibited a pseudo-molecular ion at *m*/*z* 703.40 $[M-H]^-$ (**2**, *m*/*z* 703.4042; **3**, *m*/*z* 703.4066) for the same molecular formula of $C_{39}H_{60}O_{11}$ (calcd 703.4057 for $C_{39}H_{59}O_{11}$). The ^1H NMR spectrum (Table 1) of **2** indicated presence of the characteristic cyclopropane methylene signals at δ_H 0.20, and 0.46 (each 1H, d, *J*=3.5 Hz), seven methyl groups at δ_H 0.97–1.48, two acetoxymethyl groups at δ_H 1.78 and 2.11, and an anomeric proton at δ_H 4.75, suggesting **2** is a 9,19-cycloartane triterpene monoglycoside with two acetoxy groups. A correlation was observed between the anomeric proton at δ_H 4.75 (H-1′, 1H, d, *J*=7.5 Hz) and the methine signal at δ_C 88.9 (C-3) in the HMBC spectrum, suggesting that a sugar moiety was attached at the C-3. In addition, H-4′ signal of **2** was a broad singlet, the coupling constants of H1′–H2′ and H3′–H2′ were both 7.5 Hz. These evidence together with the correlations between H-1′ and H-3, H-1′ and H-3′, and H-1′ and H-4′ in the ROESY spectrum indicated that the protons at C-1′, C-2′, C-3′, and C-4′ are in the *axial*-, *equatorial*-, *axial*-, and *axial*-direction, which disclosed the hydroxy groups at C-2′, C-3′, and C-4′ are in the α-, β-, and β-positions, respectively, as found in α-L-arabinopyranose.[8,9] The sugar obtained after acid hydrolysis was confirmed as α-L-arabinopyranose by comparing its TLC and specific rotation with the standard. The NMR spectroscopic data (Tables 1

Table 1
^{1}H NMR data of compounds **1**–**5** in pyridine-d_5 at 500 MHz (**2**–**5**) and 600 MHz (**1**)

Proton	**1**	**2**	**3**	**4**	**5**
	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	3.27 m	1.54 m	1.57 m	1.57 m	1.53 m
	1.65 m	1.18[a]	1.21[a]	1.20 m	1.20 m
2	3.12 m	2.25 m	2.31 m	2.34 br d (10.0)	2.27 m
	2.47 m	1.87 m	1.93 dd (12.6, 3.2)	1.95[a]	1.88 m
3		3.37 dd (11.5, 4.0)	3.47 dd (11.6, 4.0)	3.49 m	3.51 dd (11.8, 4.0)
4					
5	2.08 m	1.28 m	1.30 dd (12.2, 4.0)	1.30[a]	1.36 m
6	1.68 m	1.51[a]	1.50[a]	1.49[a]	1.54 m
	0.71 m	0.70 m	0.70 m	0.69 m	0.75 m
7	2.11 m	2.07 m	2.07[a]	2.06 m	2.19 m
	1.08 m	1.12[a]	1.14[a]	1.14[a]	1.19[a]
8	1.61 m	1.66 m	1.69 m	1.66 m	1.73 m
9					
10					
11	2.35 m	2.07 m	2.07[a]	2.06 m	2.08 m
	1.27[a]	1.03 m	1.05 m	1.04[a]	1.07 m
12	1.68 m	1.66 m	1.69 m	1.66 m	1.70 m
	1.54 m	1.55 m	1.54 m	1.53 m	1.58 m
13					
14					
15	4.30 d (8.5)	4.27 br s	4.25 br s	4.26 br s	4.34[a]
16					
17	1.51 br d (11.0)	1.49[a]	1.49[a]	1.49[a]	1.49[a]
18	1.16 s	1.14 s	1.14 s	1.13 s	1.17 s
19	0.83 d (4.0)	0.46 d (3.5)	0.50 d (4.0)	0.50 br s	0.56 br s
	0.59 d (4.0)	0.20 d (3.5)	0.26 d (4.0)	0.24 br s	0.32 d (3.0)
20	1.65 m	1.66 m	1.65 m	1.66 m	1.64 m
21	0.85 d (6.5)	0.84 d (6.5)	0.85 d (6.4)	0.84 d (5.0)	0.88 d (6.0)
22	2.26 m	2.26 m	2.26 m	2.26 m	2.22 m
	1.02 t (11.5)	1.01 m	1.01[a]	1.01[a]	0.99 m
23	4.76 d (8.5)	4.73 m	4.75 d (8.8)	4.75 d (9.0)	4.31[a]
24	3.77 s	3.77 br s	3.77 s	3.77 s	4.19 s
25					
26	1.48 s	1.48 s	1.49 s	1.48 s	5.36 s
					4.90 s
27	1.46 s	1.46 s	1.47 s	1.46 s	1.86 s
28	1.26 s	1.17 s	1.18 s	1.17 s	1.17 s
29	1.37 s	1.07 s	1.24 s	1.08 s	1.26 s
30	1.37 s	0.97 s	1.00 s	1.04 s	1.04 s
3-Ara					
1′		4.75 br d (7.5)	4.86 d (7.2)	4.78 d (7.0)	4.92 d (6.5)
2′		5.82 t (9.0)	4.43 m	4.38 br t (8.0)	4.66 br t (9.0)
3′		4.32 d (7.5)	5.54 dd (9.6, 4.0)	4.27 m	5.52 dd (8.5, 2.5)
4′		5.54 br s	5.66 br s	5.58 br s	4.60 br s
5′		4.23 br d (12.9)	4.18 dd (12.8, 2.8)	4.26 m	4.33[a]
		3.79 br d (12.0)	3.84 br d (12.0)	3.81 m	3.87 d (11.5)
3-OCH_3	3.58 s				
2′-OCOCH_3		2.11 s			
3′-OCOCH_3			2.00 s		1.99 s
4′-OCOCH_3		1.78 s	1.99 s	1.95 s	

[a] Signals overlap.

and 3) of **2** were similar to those of cimigenol-3-*O*-[2′-*O*-acetyl]-α-L-arabinopyranoside (**18**),[6] except for the sugar moiety. The molecular weight of **2** is 42 Da more than those of **18**, which may ascribe to an acetyl group. In the ^{1}H NMR spectrum of **2**, the signal due to H-4′ exhibited a downfield shift from δ_H 4.26 (m) to 5.54 (br s). In the ^{13}C and DEPT spectra, the signal due to C-4′ exhibited a downfield shift from δ_C 69.8 to 72.4, whereas the signal due to C-3′ and C-5′ showed an upfield shift from δ_C 72.5 to 70.3, and δ_C 67.3 to 64.5, respectively. Based on above evidence, we deduced that another *O*-acetyl group is attached at C-4′ of the arabinopyranose unit of **2**, which was further confirmed by the presence of the HMBC correlation

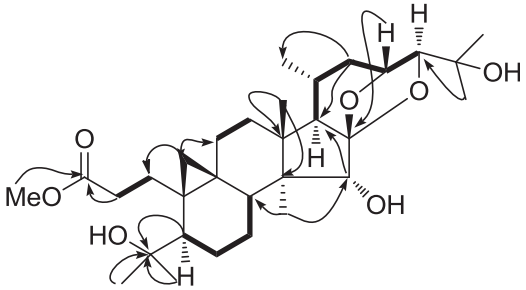

Fig. 1. Major HMBC (→) and ^{1}H–^{1}H COSY (———) correlations of compound **1**.

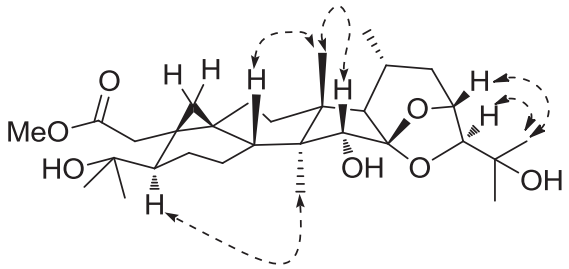

Fig. 2. Key ROESY correlations of compound **1**.

Table 2
^{1}H NMR data of compounds **6**–**9** in pyridine-d_5 at 500 MHz (**6**, **7**, **9**) and 600 MHz (**8**)

Proton	**6**	**7**	**8**	**9**
	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	1.58 m	1.64[a]	1.50 dd (11.8, 4.0)	1.57 m
	1.36 m	1.24 m	1.20[a]	1.20 m
2	2.68 m	1.97 m (2H)	2.01 m	2.27 br d (11.5)
	2.33 m		1.90 m	1.89 br d (8.0)
3		3.50 m	3.54 dd (9.3, 4.0)	3.37 m
4				
5	1.59 m	1.23 m	1.30[a]	1.32[a]
6	1.41[a]	1.97 m	1.60 m	1.55 m
	0.79 m	1.57[a]	0.72 m	0.69 m
7	2.09 m	5.28 d (6.5)	2.01 m	2.11[a]
	1.13[a]		1.14 m	1.26 m
8	1.72 m		1.48 m	1.74 m
9				
10				
11	1.99 m	2.07 m	2.01 m	2.11[a]
	1.06[a]	1.31 m	1.04[a]	1.08 m
12	1.68 m	1.82 m	1.72 m	1.74 m
	1.58 m	1.64[a]	1.20[a]	1.57 m
13				
14				
15	4.25 br s	9.85 s	3.63 m 2.10 d (13.0)	4.20 br s
16				
17	1.76 m	2.73 d (4.5)	2.40 br d (8.0)	1.89 br d (8.0)
18	1.19 s	1.56 s	1.12 s	1.26 s
19	0.66 d (4.0)	0.94 d (2.5)	0.52 br s	0.49 br s
	0.48 d (4.0)	0.53 d (3.0)	0.31 d (4.0)	0.24 br s
20	1.72 m	2.26 m	2.64 br d (13.0)	1.76[a]
21	0.95 d (6.0)	0.99 d (6.5)	1.06 br s	1.03 d (6.0)
22	2.67 m	2.11 m	3.63 m	1.93 m
	1.95 m	1.90 m	2.64 br d (13.0)	1.57 m
23	4.60 ddd (9.6, 4.4, 2.0)	5.14 d (11.5)		3.87 m
24	3.68 d (4.0)	3.74 d (4.0)	3.77 s	3.56 d (8.0)
25				
26	1.42 s	1.67 s	1.38 s	1.26 s
27	1.26 s	1.72 s	1.38 s	1.30 s
28	1.04 s	1.62 s	1.06 br s	1.26 s
29	1.11 s	1.16 s	1.23 s	1.09 s
30	1.03 s	1.07 s	1.09 s	0.97 s
3-Ara				
1′				4.74 d (7.5)
2′				5.91 t (8.0)
3′				4.18 m
4′				4.29 br s
5′				4.27 m
				3.78 d (12.5)
3-OCH_3				
2′-OCOCH_3				2.11 s

[a] Signals overlapped.

between the H-4′ signal at δ_H 5.54 and the *O*-acetyl group at δ_C 170.7. Thus, the structure of **2** was assigned as cimigenol-3-*O*-[2′,4′-*O*-diacetyl]-α-L-arabinopyranoside. In the same way, two acetoxy groups were determined to be at C-3′ and C-4′ of the α-L-arabinopyranose unit for **3**, which was further confirmed by the presence of the HMBC correlations between the H-3′ and H-4′ signal at δ_H 5.54, 5.66 and the carbonyl group signal at δ_C 170.6 and 170.5, respectively. Therefore, **3** was identified as cimigenol-3-*O*-[3′,4′-*O*-diacetyl]-α-L-arabinopyranoside.

Compound **4**, isolated as white powder. The ^{13}C NMR and HRTOF-ESIMS m/z: 685.3929 $[M+Na]^+$ determined its molecular formula as $C_{37}H_{58}O_{10}$, which is identical to that of **18**.[6] The NMR data of **4** (Tables 1 and 3) resembled those of **18**, except for changes of sugar unit at C-1′ (δ_C 107.9), C-2′ (δ_C 73.5), C-3′ (δ_C 72.8), C-4′ (δ_C 72.8), and C-5′ (δ_C 64.7) due to the acetoxy group was attached to C-4′ instead of to C-2′, which confirmed by the upfield shift of H-2′ from δ_H 5.90 to 4.27, downfield shift of H-4′ from δ_H 4.26 to 5.58, as well as the correlations in the HMBC spectrum between H-4′ (δ_H 5.58) and the carbonyl carbon at δ_C 171.2. The sugar was identified as α-L-arabinopyranose after acid hydrolysis. Thus, the compound **4** was characterized as cimigenol-3-*O*-[4′-*O*-acetyl]-α-L-arabinopyranoside.

Compound **5** was isolated as white powder. The HRTOF-ESIMS established the molecular formula of **5** as $C_{37}H_{56}O_9$. The NMR spectroscopic data of **5** (Tables 1 and 3) are similar to those of 25-anhydrocimigenol-3-*O*-α-L-arabinopyranoside (**19**)[9] with the major differences at sugar unit. A significant downfield signal was observed at δ_H 5.52 (dd, J=2.5, 8.5 Hz) in the ^{1}H NMR spectrum. In addition, the proton showed correlations with the methine signal at δ_H 4.60 (H-4′) and with the methine signal at δ_H 4.66 (H-2′), which, in turn, exhibited a correlation with an anomeric proton at δ_H 4.92 in the ^{1}H–^{1}H COSY spectrum. Based on above evidence, we deduced an acetoxy group attached at C-3′ in **5**. This result was confirmed by the presence of the HMBC correlation between the H-3′ signal at δ_H 5.52 and the carbonyl group signal at δ_C 170.9. The sugar was identified as α-L-arabinopyranose by the same way as that of **4**. Therefore, **5** was elucidated as 25-anhydrocimigenol-3-*O*-[3′-*O*-acetyl]-α-L-arabinopyranoside.

Compound **6** was isolated as white powder. In the high resolution negative TOF-ESIMS, it showed a quasi-molecular ion at m/z 485.3257 $[M+Cl]^-$, for a molecular formula of $C_{30}H_{46}O_5$. The ^{13}C NMR spectrum showed 30 signals, including a carbonyl *C*-atom at δ_C 215.0. Comparison of the ^{13}C NMR data of **6** with those of 24-*epi*-cimigenol (**20**)[10,16] suggested the two compounds are structurally similar and the differences may be deduced by a methine at δ_C 78.1 (C-3) was transformed to a carbonyl carbon at δ_C 215.0. This assignment was supported by the HMBC correlation between H-2/C-3, and CH_3-29, CH_3-30/C-3, as well as the upfield shift of C-2 about 7.6 ppm in the ^{13}C spectrum. The configurations of C-23 and C-24 are proposed as *R* and *R*, respectively, by comparing the coupling constants of H-23 (9.6 Hz) and H-24 (4.0 Hz) of **6** with those of known 9,19-cyclolanostane triterpenes.[10,19] Ultimately, **6** was elucidated as 24-*epi*-cimigenol-3-one.

Compound **7** was isolated as white powder. The positive HRTOF-ESIMS showed a quasi-molecular ion at m/z 525.3206 $[M+Na]^+$, corresponding to the molecular formula of $C_{30}H_{48}O_6$. Its IR spectrum showed hydroxy, carbonyl, and double bond absorptions at 3423, 1725, and 1635 cm^{-1}, respectively. The ^{13}C NMR data of **7** were similar to the aglycone resonances of 15,16-seco-14-carboxyl-16-oxo-hydroshengmanol-3-*O*-β-D-xylopyranoside (**21**)[20] except for the presences of two olefinic carbons at δ_C 122.4 and 140.5 and a significant downfield carbonyl signal at δ_C 200.4. The correlations between H-6 at δ_H 1.97, 1.57 and olefinic proton at δ_H 5.28 in ^{1}H–^{1}H COSY spectrum indicated the double bond at C-7 and C-8. In the HSQC spectrum, the proton at δ_H 9.85 (s) correlated with the downfield carbonyl signal, showing there is a formyl group in **7**. In addition, the downfield shift of the carbonyl group by 21.8 ppm, as well as 18 Da of molecular weight less than those of aglycone part of **21** further confirmed above deduction. The correlations of CH_3-28 at δ_H 1.62 with the carbonyl signal at δ_C 200.4, and the proton of the formyl group at δ_H 9.85 with C-14 at δ_C 59.5 in the HMBC spectrum revealed the formyl group was attached at C-14. In the ROESY spectrum, CH_3-28 showed a correlation with H-5, while H-17 showed a correlation with Me-21, suggesting both CH_3-28 and H-17 in an α-orientation. In addition, the ROESY correlation between H-23 and H-20 (β-orientation) indicated the configuration of C-23 as *R*. The configuration of C-24 is proposed as *S* by comparison of the coupling constants of H-24 (4.0 Hz) of **7** with the known compounds.[20] Accordingly, compound **7** was characterizedas15,16-seco-7,8-didehydro-14-formyl-16-oxohydroshengmanol.

Compound **8** was isolated as white powder. The negative HRTOF-ESIMS showed a pseudo-molecular ion at m/z 505.3083 $[M+Cl]^-$, leading to the molecular formula $C_{30}H_{46}O_4$, which is 14 Da less than

Table 3
^{13}C NMR data of compounds **1**–**9** in pyridine-d_5 125 MHz (**2**, **3**, **5**, **6**, **7**) and 150 MHz (**1**, **4**, **8**, **9**)

Carbon	1	2	3	4	5	6	7	8	9
1	31.4 t	32.3 t	32.4 t	32.8 t	32.3 t	33.9 t	31.4 t	32.7 t	32.3 t
2	32.9 t	30.0 t	30.0 t	30.4 t	30.0 t	37.6 t	31.1 t	31.7 t	30.4 t
3	175.3 s	88.9 d	89.0 d	89.2 d	88.8 d	215.0 s	77.5 d	78.3 d	89.1 d
4	75.6 s	41.0 s	41.3 s	41.7 s	41.7 s	50.3 s	40.2 s	41.6 s	41.5 s
5	46.2 d	47.5 d	47.5 d	47.9 d	47.6 d	48.5 d	40.4 d	47.8 d	47.8 d
6	25.8 t	21.1 t	21.0 t	21.3 t	21.1 t	21.5 t	22.7 t	21.7 t	21.5 t
7	26.5 t	26.3 t	26.3 t	26.8 t	26.5 t	26.3 t	122.4 d	26.6 t	26.8 t
8	49.5 d	48.6 d	48.6 d	48.9 d	48.7 d	48.5 d	140.5 s	47.7 d	49.4 d
9	23.2 s	20.1 s	20.1 s	20.3 s	20.1 s	21.1 s	19.1 s	19.7 s	20.5 s
10	28.1 s	26.6 s	26.6 s	26.9 s	26.7 s	26.4 s	28.9 s	27.0 s	27.0 s
11	27.0 t	26.5 t	26.4 t	26.7 t	26.4 t	26.5 t	24.9 t	26.7 t	26.5 t
12	34.7 t	34.1 t	34.1 t	34.4 t	34.1 t	33.7 t	30.7 t	31.7 t	34.4 t
13	41.9 s	41.9 s	41.9 s	42.2 s	41.3 s	41.7 s	43.2 s	42.8 s	42.8 s
14	47.9 s	47.3 s	47.3 s	47.6 s	47.3 s	47.4 s	59.5 s	45.8 s	46.7 s
15	81.2 d	80.3 d	80.2 d	80.5 d	80.4 d	80.8 d	200.4 s	51.5 t	82.9 d
16	112.5 s	112.0 s	112.0 s	112.3 s	112.3 s	112.1 s	173.9 s	219.8 s	103.5 s
17	60.2 d	59.5 d	59.6 d	59.9 d	59.9 d	60.8 d	55.6 d	61.2 d	61.5 d
18	20.3 q	19.5 q	19.5 q	19.9 q	19.5 q	19.6 q	22.1 q	19.4 q	20.9 q
19	32.9 t	30.8 t	30.8 t	31.2 t	30.9 t	30.4 t	28.9t	30.8 t	31.2 t
20	24.5 d	24.1 d	24.1 d	24.4 d	23.9 d	23.5 d	28.1 d	27.8 d	27.4 d
21	19.9 q	19.6 q	19.6 q	19.8 q	19.5 q	19.7 q	24.8 q	20.9 q	22.1 q
22	38.5 t	38.2 t	38.2 t	38.5 t	38.1t	29.7 t	36.4 t	47.9 t	32.7 t
23	72.3 d	71.9 d	71.9 d	72.1 d	75.0 d	73.7 d	78.2 d	206.2 s	75.9 d
24	90.7 d	90.2 d	90.2 d	90.5 d	86.7 d	84.1 d	79.9 d	66.3 d	68.2 d
25	71.5 s	70.9 s	70.9 s	71.3 s	145.9 s	68.6 s	72.4 s	61.2 s	57.8 s
26	27.7 q	27.2 q	27.2 q	27.4 q	113.0 t	30.8 q	25.9 q	18.8 q	25.8 q
27	25.9 q	25.5 q	25.4 q	25.7 q	18.2 q	26.0 q	29.2 q	25.0 q	19.6 q
28	12.5 q	11.8 q	11.8 q	12.1 q	11.9 q	11.7 q	18.8 q	20.9 q	12.4 q
29	32.5 q	25.5 q	25.5 q	25.9 q	25.8 q	22.6 q	25.8 q	26.6 q	25.9 q
30	26.9 q	15.2 q	15.2 q	15.7 q	15.4 q	20.8 q	13.3 q	15.3 q	15.7 q
3-Ara									
1′		104.4 d	106.9 d	107.9 d	106.9 d				104.9 d
2′		74.1 d	69.9 d	73.5 d	69.8 d				74.8 d
3′		70.3 d	73.9 d	72.8 d	76.9 d				72.9 d
4′		72.4 d	69.2 d	72.8 d	66.8 d				70.3 d
5′		64.5 t	63.5 t	64.7 t	66.2 t				67.7 t
3-OCH_3	51.8 q								
2′-$OCOCH_3$		170.1 s							170.6 s
2′-$OCOCH_3$		21.2 q							21.8 q
3′-$OCOCH_3$			170.6 s		170.9 s				
3′-$OCOCH_3$			20.8 q		21.2 q				
4′-$OCOCH_3$		170.7 s	170.5 s	171.2 s					
4′-$OCOCH_3$		20.8 q	20.9 q	21.4 q					

those of cimicidanol (**22**).[21] All the NMR data of **8** resembled to those of **22** and suggested the configuration at C-24 was the same as that of **22**, which was assigned as *R*. Moreover, for **8**, a methine at δ_C 63.0 (C-11) was transformed to a methylene at δ_C 26.7 and a pair of double bond at δ_C 115.6 (C-7, d) and 147.1 (C-8, s) were reduced to a methylene at δ_C 26.6 (t) and a methine at δ_C 47.7 (d). The correlations of H-12 (δ_H 1.20 and 1.72, 1H each) with C-11 (δ_C 26.7) and H-6 (δ_H 0.72 and 1.60, 1H each) with C-7 (δ_C 26.6) and C-8 (δ_C 47.7) in HMBC spectrum and H-7/H-8 and H-11/H-12 correlations in ^{1}H–^{1}H COSY spectrum confirmed above conclusion. Thus, compound **8** was characterized as 7,8-dihydro-11-dehydroxycimicidanol.

Compound **9** was isolated as white powder. In the high-resolution positive TOF-ESIMS, it showed a quasi-molecular ion at *m*/*z* 685.3930 $[M+Na]^+$, for a molecular formula of $C_{37}H_{58}O_{10}$. The ^{1}H and ^{13}C spectrum (Tables 2 and 3) exhibited signals very similar to those of shengmanol-3-*O*-α-L-arabinopyranoside (**23**)[22] except for the sugar moiety. Using the same way as that of **2**, an *O*-acetyl group was determined to be at C-2 of the sugar unit, 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. The sugar was identified as α-L-arabinopyranose after acid hydrolysis. Moreover, the ROESY correlation between H-23 and H-20 (β-orientation) assigned the configuration of C-23 as *R*. The configuration of C-24 of **9** was proposed as *S* by the same way as that of compound **1**.[19,22] Therefore, compound **9** was elucidated as shengmanol-3-*O*-[2′-*O*-acetyl]-α-L-arabinopyranoside.

All isolated compounds were evaluated against human HL-60, SMMC-7721, A549, MCF-7, and SW480 cell lines for their cytotoxicities. As summarized in Table 4, nine compounds (**1**–**3**, **5**, **7**, **10**–**12**, **14**) showed different levels of activities. Among the active triterpenes, compound **1** (did not have enough weight to test last two cell lines) showed as strong activity as positive control cisplatin against HL-60 cells with IC_{50} values of 0.83 μM, and more potent activities against SMMC-7721 and A549 cell lines, having IC_{50} values of 2.59 and 1.41 μM. While, compounds **5**, **10**, and **12** exhibited stronger cytotoxicities than cisplatin against all testing cell lines except HL-60, with IC_{50} values ranging from 5.02 to 11.40 μM. In addition, Compounds **2**, **3**, **7**, **11**, and **14** exhibited broad spectrum and weak activities with IC_{50} values around 15–25 μM. Above results further supported the theory of tumor in TCM as a guiding principle to explore potential antitumor agents from herb medicines and the roots of *C. heracleifolia* is a promising resource for anti-cancer agents.

In present study, another seven active glycosides (**2**, **3**, **5**, **10**–**12**, **14**) with a cimigenol skeleton were discovered. Either hydrophobic groups like acetoxy at the sugar unit or hydroxy group at C-25 be replaced by double bond or acetoxy group is the pharmacophore of these active compounds, which completely in accordance with the SAR we reported before.[9] Thus, the SAR we proposed before could be used for design of more potent lead compounds.

Up to now, there are more cytotoxic triterpene glycosides with stronger activities than aglycones obtained from *Cimicifuga* spp.[6–9,23–27] However, compound **1**, an aglycone, showed the more

Table 4
IC_{50} values (μM) of compounds **1**–**16** for human tumor cell lines (n=3)

Compounds	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	**0.83**	**2.59**	**1.41**	—	—
2	**8.40**	**13.83**	**15.73**	**16.25**	**12.92**
3	**5.99**	**15.73**	**15.98**	**23.94**	**20.64**
4	>40	>40	>40	>40	>40
5	**5.99**	**7.26**	**8.13**	**9.58**	**7.81**
6	>40	>40	**40.00**	>40	>40
7	**11.96**	>40	**28.07**	**23.73**	**24.28**
8	>40	>40	>40	>40	>40
9	>40	>40	>40	>40	>40
10	**6.39**	**9.25**	**8.40**	**6.86**	**5.02**
11	**13.83**	**14.61**	**13.14**	**14.99**	**18.68**
12	**11.40**	**11.04**	**10.02**	**10.05**	**10.67**
13	>40	>40	>40	>40	>40
14	**11.96**	>40	**19.70**	**20.13**	**13.78**
15	>40	>40	>40	>40	>40
16	>40	>40	>40	>40	>40
Cisplatin	**0.52**	**13.39**	**12.35**	**15.06**	**14.43**

potent activities than glycoside in present study. The main structural features of **1** being that C-3 and C-4 were cleaved and a methyl ester group was formed at C-3 through a flexible carbon chain (C-1 and C-2). The carbonyl group at C-3 showed different dimensional character as that of none ring opened active aglycon, actrin-3-one, which also contained a carbonyl group at C-3.[7] Based on above analyses, we may suggest that the dimensional position of the carbonyl group at C-3 is critical to the activities of cimigenol-type triterpenes and afford a new perspective for chemical modification.

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured in MeOH with a Horiba SEAP-300 polarimeter. ^{1}H and ^{13}C NMR spectrum were recorded in pyridine-d_5 on Bruker DRX-500 and Avance III-600 MHz spectrometer (Bruker, Zűrich, Switzerland). Unless otherwise specified, chemical shifts (δ) are expressed in ppm with respect to the solvent signals, 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 (200—250 μm thickness, Silica gel 60 F_{254}, Qingdao Marine Chemical, Inc.) and spots were visualized by heating after spraying with 10% aq H_2SO_4 soln. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a YMC-Pack Pro C18 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).

3.2. Plant material

The roots of *C. heracleifolia* Kom. (1.0 kg) were collected from Qingyuan County, Liaoning Province, China, in September 2006 and identified by Prof. Zongyu Wang, Kunming Institute of Botany, Chinese Academy of Science. A voucher specimen (KUN No. 200609004) 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 roots of *C. heracleifolia* (1.0 kg) were extracted with MeOH (3×3 L×24 h) at room temperature to give a residue (116 g) after evaporating in vacuum at 50 °C. The extract was subjected to silica gel cc (2 kg, 10×150 cm) and eluted with $CHCl_3$/MeOH [100:0 (2 L), 50:1 (4 L), 20:1 (5 L), 10:1 (4 L), 0:100 (3 L)] to afford fractions **A** (24.5 g), **B** (11.1 g), **C** (12.8 g), **D** (17.1 g), and **E** (20.8). Fraction **B** (11.1 g) was divided into five sub-fractions (**B-1** to **B-5**) after performing RP-18 cc (180 g, 5×25 cm), eluting with MeOH/H_2O (gradient from 60:40 to 100:0, 10 L). Fraction **B-3** (1.3 g) was subjected to repeated silica gel cc (40 g, 4×40 cm), eluted with $CHCl_3$/Me_2CO (gradient from 20:1 to 10:1, 4 L) and then semipreparative HPLC (eluted with CH_3CN/H_2O, gradient from 60:40 to 85:15) to yield **6** (4.0 mg), **13** (4.0 mg), **14** (5.1 mg). Compounds **8** (0.5 mg), **15** (2.3 mg), and **16** (1.5 mg) were purified from fraction **2.4** (1.3 g) by conducting silica gel cc (30 g, 3.5×40 cm), eluting with $CHCl_3$/Me_2CO (20:1, 3 L), followed by semipreparative HPLC (eluted with CH_3CN/H_2O, gradient from 65:35 to 85:15). Fraction **B-5** (1.1 g) was applied to a silica gel (30 g) column (3×40 cm), eluted with $CHCl_3$/Me_2CO (20:1, 3 L), then purified on semipreparative HPLC (eluted with CH_3CN/H_2O, gradient from 65:35 to 90:10) to afford **1** (0.7 mg), and **7** (1.5 mg). Fraction **C** (12.8 g) was fractionated into three sub-fractions (**C-1** to **C-3**) by performing RP-18 cc (180 g, 5×25 cm), eluting with MeOH/H_2O (gradient from 50:40 to 90:10, 12 L). Fraction **C-1** (2.8 g) was subjected to silica gel cc (50 g, 4×40 cm) eluted with $CHCl_3$/Me_2CO (gradient from 10:1 to 5:1, 4 L), then semipreparative HPLC (eluted with CH_3CN/H_2O, gradient from 60:40 to 75:25) to obtain **10** (3.3 mg), **11** (3.7 mg), and **12** (4.2 mg). Fraction **C-2** (3.4 g) was chromatographed on a silica gel cc (50 g, 4×40 cm), eluting with $CHCl_3$/Me_2CO (10:1, 8 L), then repeated chromatography over semipreparative HPLC (eluted with CH_3CN/H_2O, gradient from 60:40 to 80:20) to yield **2** (6.2 mg), **3** (6.0 mg), **4** (5.0 mg), **5** (4.8 mg), and **9** (5.2 mg).

*3.3.1. Methyl 3,4-seco-4-hydroxy-3-cimigenolate (**1**).* White powder; $[\alpha]_D^{20}$ +20.00 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3403, 2965, 2828, 1741, 1456, 1380, 1023, 978 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negative ESIMS *m*/*z* 569 $[M+Cl]^-$; HRTOF-ESIMS *m*/*z* 569.3236 $[M+Cl]^-$ (calcd for $C_{31}H_{50}O_7Cl$, 569.3245).

*3.3.2. Cimigenol-3-O-[2′,4′-O-diacetyl]-α-L-arabinopyranoside (**2**).* White powder; $[\alpha]_D^{20}$ +86.58 (*c* 0.17, MeOH); IR (KBr) ν_{max} 3435, 2943, 2871, 1738, 1457, 1381, 1033, 987 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negative ESIMS *m*/*z* 703 $[M-H]^-$; HRTOF-ESIMS *m*/*z* 703.4042 $[M-H]^-$ (calcd for $C_{39}H_{59}O_{11}$, 703.4057).

*3.3.3. Cimigenol-3-O-[3′,4′-O-diacetyl]-α-L-arabinopyranoside (**3**).* White powder; $[\alpha]_D^{20}$ +91.11 (*c* 0.18, MeOH); IR (KBr) ν_{max} 3473, 2962, 2871, 1740, 1380, 1053, 987 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negativ ESIMS *m*/*z* 703 $[M-H]^-$; HRTOF-ESIMS *m*/*z* 703.4066 $[M-H]^-$ (calcd for $C_{39}H_{59}O_{11}$, 703.4057).

*3.3.4. Cimigenol-3-O-[4′-O-acetyl]-α-L-arabinopyranoside (**4**).* White powder; $[\alpha]_D^{20}$ +26.52 (*c* 0.09, MeOH); IR (KBr) ν_{max} 3449, 2932, 2867, 1735, 1459, 1382, 1030, 982 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m*/*z* 685 $[M+Na]^+$; HRTOF-ESIMS *m*/*z* 685.3929 $[M+Na]^+$ (calcd for $C_{37}H_{58}O_{10}Na$, 685.3927).

*3.3.5. 25-Anhydrocimigenol-3-O-[3′-O-acetyl]-α-L-arabinopyranoside (**5**).* White powder; $[\alpha]_D^{20}$ +20.73 (*c* 0.18, MeOH); IR (KBr) ν_{max} 3472, 2954, 2872, 1739, 1648, 1457, 1019, 901 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negative ESIMS *m*/*z* 679 $[M+Cl]^-$; HRTOF-ESIMS *m*/*z* 679.3613 $[M+Cl]^-$ (calcd for $C_{37}H_{56}O_9Cl$, 679.3612).

*3.3.6. 24-epi-Cimigenol-3-one (**6**).* \White powder; $[\alpha]_D^{20}$ −2.11 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3430, 2962, 2868, 1715, 1452, 1383, 1027, 977 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; negative ESIMS

m/z 485 $[M-H]^-$; HRTOF-ESIMS *m/z* 485.3257 $[M-H]^-$ (calcd for $C_{30}H_{45}O_5$, 485.3267).

*3.3.7. 15,16-Seco-7,8-didehydro-15-formyl-16-oxohydroshengmanol (**7**).* White powder; $[\alpha]_D^{20}$ −15.55 (*c* 0.08, MeOH); IR (KBr) ν_{max} 3423, 2929, 2871, 1742, 1725, 1635, 1461, 1380, 1027, 984 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m/z* 525 $[M+Na]^+$; HRTOF-ESIMS *m/z* 525.3206 $[M+Na]^+$ (calcd for $C_{30}H_{46}O_6Na$, 525.3192).

*3.3.8. 7,8-Dihydro-11-dehydroxycimicidanol (**8**).* White powder; $[\alpha]_D^{20}$ −14.31 (*c* 0.07, MeOH); IR (KBr) ν_{max} 3489, 2938, 2872, 1718, 1700, 1640, 1458, 1382, 1045, 970 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; negative ESIMS 505 $[M+Cl]^-$; HRTOF-ESIMS *m/z* 505.3083 $[M+Cl]^-$ (calcd for $C_{30}H_{46}O_4Cl$, 505.3084).

*3.3.9. Shengmanol-3-O-[2′-O-acetyl]-α-L-arabinopyranoside (**9**).* White powder; $[\alpha]_D^{20}$ +53.07 (*c* 0.18, MeOH); IR (KBr) ν_{max} 3456, 2964, 2871, 1741, 1456, 1378, 1070, 987 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive ESIMS *m/z* 685 $[M+Na]^+$; HRTOF-ESIMS *m/z* 685.3930 $[M+Na]^+$ (calcd for $C_{37}H_{58}O_{10}Na$, 685.3927).

3.4. Hydrolysis and identification of the sugar moieties in compounds 2–5 and 9

Compounds **2** and **3** (5 mg of each), together with **4**, **5**, and **9** (4 mg of each) were individually dissolved in MeOH (5 mL), then 4% K_2CO_3 (5 mL) was added and each solution was stirred at room temperature overnight. Each solution was neutralized by 10% HOAc, and extracted with EtOAc (3×15 mL). Each EtOAc extract, after removal of solvent, was dissolved in MeOH (5 mL) and refluxed with 0.5 N HCl (3 mL) for 4 h.[7] Each reaction mixture was diluted with H_2O and extracted with $CHCl_3$ (3×10 mL). Each aqueous layer was then neutralized by Ag_2CO_3 and filtered the precipitate to give a monosaccharide, which had an R_f (EtOAc/$CHCl_3$/MeOH/H_2O, 3:2:2:1) and specific rotation $[\alpha]_D^{20}$ +82.78 (*c* 0.05, MeOH) corresponding to those of L-arabinopyranose (Sigma–Aldrich).

3.5. Cytotoxicity bioassay

Five human cancer cell lines, 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 performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates.[28,29] Briefly, 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 1×10^5 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.[30]

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2012.05.083. These data include MOL file and InChiKey of the most important compound described in this article.

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