Cytotoxic Cycloartane Triterpenes of the Traditional Chinese Medicine "Shengma" (*Cimicifuga dahurica*)

Authors Yin Nian[1,2], Hai-Yan Wang[1,2], Lin Zhou[1], Jia Su[1,2], Yan Li[1], Ming-Hua Qiu[1]

Affiliations
[1] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, PR China
[2] Graduate School of the Chinese Academy of Sciences, Beijing, PR China

Key words
- Ranunculaceae
- *Cimicifuga dahurica*
- cycloartane triterpenes
- cytotoxicity
- structure-activity relationships

Abstract

Twelve new 9,19-cycloartane triterpenes (**1**–**12**), together with fourteen known compounds (**13**–**26**), were isolated from the roots of *Cimicifuga dahurica*. Their structures were determined by application of spectroscopic analyses and chemical methods. Biological evaluation of the compounds against human HL-60, SMMC-7721, A549, MCF-7, and SW-480 cell lines indicated that cimigenol-type glycosides (**1**–**3, 19**, and **20**) showed broad-spectrum and moderate cytotoxicities, with IC_{50} values ranging from 4.2 to 14.5 µM. Meanwhile, cimigenol-type aglycones (**6**–**8, 15, 16**, and **18**) exhibited broad-spectrum and week cytotoxicities, having IC_{50} values around 20 µM. In addition, the key points of the structure-activity relationships of aglycones with a cimigenol skeleton were discussed.

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Bibliography
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Correspondence
Prof. Ming-Hua Qiu
State Key Laboratory of Phytochemistry and Plant Resources in West China
Kunming Institute of Botany
Graduate School of the Chinese Academy of Sciences
132 Lanhei Road
Kunming 650204
PR China
Phone: + 86 87 15 22 33 27
Fax: + 86 87 15 22 32 55
mhchiu@mail.kib.ac.cn

Introduction

Cancer has become one of the major causes of mortality in humans throughout the world. In 2007, 7.6 million people died from cancer. Significantly, 27 million new cancer cases and 17.5 million cancer deaths are projected to occur in the world by 2050 [1]. Natural products have been a rich source of antitumor agents, and approximately 60% of currently available drugs are natural compounds or are related to them (from 1940 to 2010) [2].

As one of the three ancient medicinal systems, traditional Chinese medicine (TCM), has gained increasing acceptance and has been recognized by pharmaceutical enterprises as a fountainhead of antitumor drugs [3]. According to the theories of TCM, cancer is caused by imbalances between endogenous physical conditions of the body and exogenous pathogenic factors, including accumulated toxins, heat, and blood stasis [3]. Therefore, the roots of *Cimicifuga foetida*, belonging to the family Ranunculaceae and officially listed in the Chinese Pharmacopoeia with the name "shengma" as a cooling and detoxicating agent [4], were chosen as the object to investigate potential antitumor constituents. As a result, a number of cytotoxic 9,19-cycloartane triterpenes were successively isolated from this herb medicine [5–7]. In addition, the preliminary structure-activity relationships (SAR) of the triterpenes with a cimigenol skeleton were proposed [8].

In the Chinese Pharmacopoeia, the roots of *C. dahurica* are another element of "shengma" [4]. Chemical studies on *C. dahurica* showed that it also mainly contains 9,19-cyclolanostane triterpenes [9–16]. Recently, other research groups paid attention to chemical and pharmaceutical properties of the aerial parts of *C. dahurica* and reported that the ethyl acetate fraction of an 80% ethanol extract and three isolated cycloartane triterpenoids displayed growth inhibitory activities against several human tumor cell lines [17, 18]. However, little is known about the cytotoxic triterpenes of the roots of *C. dahurica* [19].

Inspired by the described observations concerning *C. foetida*, we undertook phytochemical and pharmacological investigations on the roots of *C. dahurica*. Twelve new 9,19-cyclolanostane triterpenes (**1**–**12**), together with fourteen known compounds, cimigenol (**13**) [20], 7(8)-en-cimigenol (**14**) [21], 25-*O*-acetyl-7(8)-en-cimigenol (**15**) [21], cimigenol-3-one (**16**) [22], 24-*epi*-cimigenol-7(8)-en-3-one (**17**) [21], cimigenol-1(2)-en-3-one (**18**) [23], 25-*O*-acetylcimigenol-3-*O*-[2′-*O*-acetyl]-α-L-arabinopyranoside (**19**) [4], 25-anhy-

drocimigenol-3-*O*-[2′-*O*-acetyl]-*β*-D-xylopyranoside (**20**) [24], 23-*epi*-26-deoxyactein (**21**) [25], actein (**22**) [26], 24-*O*-acetyl-7 (8)-en-hydroshengmanol (**23**) [27], 24-*O*-acetylisodahurinol-3-*O*-[2′-*O*-acetyl]-*α*-L-arabinopyranoside (**24**) [25], 23-*O*-acetylshengmanol-3-*O*-*β*-D-xylopyranoside (**25**) [28], and cimiracemonol B (**26**) [29], were isolated and identified (**Fig. 1**). The isolated compounds were evaluated 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 the compounds.

Materials and Methods

General experimental procedures

Optical rotations were measured in MeOH with a Horiba SEAP-300 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded in pyridine-d_5 on Bruker DRX-500 and Avance III-600MHz spectrometers (Bruker). Unless otherwise specified, chemical shifts (δ) are expressed in ppm with respect to the solvent signals. ESIMS and HR-TOF-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 sol. 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).

Plant material

The roots of *Cimicifuga dahurica* M. (0.9 kg) were collected from Qingyuan County, Liaoning Province, China, in September 2006 and identified by Prof. Shengji Pei, Kunming Institute of Botany, Chinese Academy of Sciences. 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.

Extraction and isolation

The dried and milled roots of *Cimicifuga dahurica* (0.9 kg) were extracted with MeOH (3 × 3 L × 24 h) at room temperature to give a residue (106 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** (21.5 g), **B** (13.1 g), **C** (14.5 g), **D** (16.8 g), and **E** (16.2 g). Fraction **B** (13.1 g) was divided into five sub-fractions (**B.1**–**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** (1.5 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 to repeated semipreparative HPLC (eluted with CH_3CN-H_2O, gradient from 60 : 40 to 85 : 15) to yield **4** (3.0 mg), **5** (3.0 mg), **6** (2.0 mg), **13** (4.0 mg), **16** (4.7 mg), and **26** (3.6 mg). Compounds **7** (2.8 mg), **14** (3.2 mg), **15** (3.5 mg), **17** (2.3 mg), **18** (2.5 mg), and **23** (2.2 mg) were purified from fraction **B.4** (1.8 g) by silica gel cc (30 g, 3.5 × 40 cm) eluted with $CHCl_3$-Me_2CO (20 : 1, 3 L), followed by repeated semipreparative HPLC (eluted with CH_3CN-H_2O, gradient from 65 : 35 to 85 : 15). Fraction **B.5** (0.9 g) was applied to a silica gel column (30 g, 3.5 × 40 cm) eluted with $CHCl_3$-Me_2CO (20 : 1, 3 L), then purified over semipreparative HPLC (eluted with CH_3CN-H_2O, gradient from 65 : 35 to 90 : 10) to afford **8** (2.7 mg), **9** (1.2 mg), **10** (1.9 mg), and **11** (1.6 mg). Fraction **C** (14.5 g) was fractionated into four subfractions (**C.1**–**C.4**) by performing RP-18 cc (180 g, 5 × 25 cm) eluted with MeOH-H_2O (gradient from 60 : 40 to 90 : 10, 12 L). Fraction **C.3** (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 to repeated semipreparative HPLC (eluted with CH_3CN-H_2O, gradient from 60 : 40 to 75 : 25) to give **1** (5.5 mg), **2** (4.8 mg), **3** (4.3 mg), **12** (5.3 mg), **19** (3.3 mg), and **24** (28 mg). Fractions **C.1** and **C.2** (4.8 g) were chromatographed on silica gel cc (50 g, 4 × 40 cm), eluting with $CHCl_3$-Me_2CO (10 : 1, 8 L) to yield **20** (5.2 mg), **21** (6.0 mg), **22** (5.2 mg), and **25** (4.2 mg).

Cimigenol-3-O-[2′-O-(E)-2-butenoyl]-α-L-arabinopyranoside (**1**): A white powder; $[\alpha]_D^{24} = 18.67$ (*c* 0.10, MeOH); IR (KBr): ν_{max} = 3452, 2931, 2870, 1727, 1632, 1458, 1383, 1044, 977 cm^{-1}; ^{1}H (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) spectra, see **Tables 1** and **2**; ESIMS: *m/z* = 711 [M + Na]$^+$; HR-TOF-ESIMS: *m/z* = 711.4065 (calc. for $C_{39}H_{60}O_{10}Na$, 711.4084).

25-O-Acetylcimigenol-3-O-[4′-O-acetyl]-α-L-arabinopyranoside (**2**): A white powder; $[\alpha]_D^{24} = 6.67$ (*c* 0.06, MeOH); IR (KBr): ν_{max} = 3472, 2968, 2870, 1739, 1458, 1374, 1072, 972 cm^{-1}; ^{1}H (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectra, see **Tables 1** and **2**; ESIMS: *m/z* = 727 [M + Na]$^+$; HR-TOF-ESIMS: *m/z* = 727.4029 (calc. for $C_{39}H_{60}O_{11}Na$, 727.4033).

25-O-Acetylcimigenol-3-O-[3′-O-acetyl]-α-L-arabinopyranoside (**3**): A white powder; $[\alpha]_D^{24} = -6.80$ (*c* 0.05, MeOH); IR (KBr): ν_{max} = 3467, 2935, 2870, 1741, 1458, 1375, 1067, 972 cm^{-1}; ^{1}H (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectra, see **Tables 1** and **2**; ESIMS: *m/z* = 703 [M – H]$^-$; HR-TOF-ESIMS: *m/z* = 703.4055 (calc. for $C_{39}H_{59}O_{11}$, 703.4057).

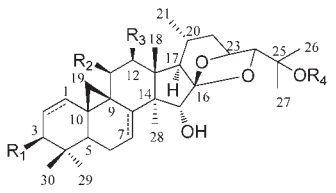
12β-Hydroxy-7(8)-en-cimigenol (**4**): A white powder; $[\alpha]_D^{24} = 3.20$ (*c* 0.10, MeOH); IR (KBr): ν_{max} = 3423, 2931, 2872, 1638, 1447, 1381, 1025, 979 cm^{-1}; ^{1}H (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectra, see **Tables 1** and **2**; ESIMS: *m/z* = 525 [M + Na]$^+$; HR-TOF-ESIMS: *m/z* = 525.3192 (calc. for $C_{30}H_{46}O_6Na$, 525.3192).

11β-Hydroxy-7(8)-en-cimigenol (**5**): A white powder; $[\alpha]_D^{24} = 0.47$ (*c* 0.10, MeOH); IR (KBr): ν_{max} 3441, 2997, 2882, 1634, 1447, 1380, 1027, 973 cm^{-1}; ^{1}H (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectra, see **Tables 1** and **2**; ESIMS: *m/z* = 525 [M + Na]$^+$; HR-TOF-ESIMS: *m/z* = 525.3199 (calc. for $C_{30}H_{46}O_6Na$, 525.3192).

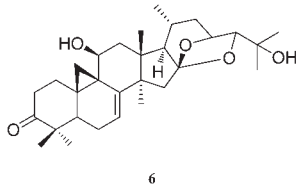
11*β*-Hydroxy-15-deoxycimigenol-7(8)-en-3-one (**6**): A white powder; $[\alpha]_D^{24} = -23.33$ (MeOH, *c* 0.05); IR (KBr): ν_{max} = 3441, 2969, 2869, 1711, 1633, 1382, 975 cm^{-1}; ^{1}H (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) spectra, see **Tables 1** and **2**; ESIMS: *m/z* = 507 [M + Na]$^+$; HR-TOF-ESIMS: *m/z* = 507.3090 (calc. for $C_{30}H_{44}O_5Na$, 507.3086).

Cimigenol-7(8)-en-3-one (**7**): A white powder; $[\alpha]_D^{24} = -21.67$ (*c* 0.10, MeOH); IR (KBr): ν_{max} = 3492, 2984, 2839, 1709, 1637, 1452, 1382, 1024, 987 cm^{-1}; ^{1}H (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectra, see **Tables 1** and **2**; ESIMS: *m/z* = 507 [M + Na]$^+$; HR-TOF-ESIMS: *m/z* = 507.3086 (calc. for $C_{30}H_{44}O_5Na$, 507.3086).

Cimigenol-1(2),7(8)-dien-3-one (**8**): A white powder; $[\alpha]_D^{24} = -60.60$ (*c* 0.10, MeOH); IR (KBr): ν_{max} = 3432, 2947, 2852, 1693, 1637, 1460, 1382, 1070, 978 cm^{-1}; ^{1}H (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectra, see **Tables 1** and **2**; ESIMS: *m/z* = 505 [M + Na]$^+$; HR-TOF-ESIMS: *m/z* = 505.2944 (calc. for $C_{30}H_{42}O_5Na$, 505.2929).



1 R_1 = *O*-2'-*O*-(*E*)-2-butenoyl-*α*-L-arabinose, R_2 = H, R_3 = H, R_4 = H, 24 *S*
2 R_1 = *O*-4'-*O*-acetyl-*α*-L-arabinose, R_2 = H, R_3 = H, R_4 = Ac, 24 *S*
3 R_1 = *O*-3'-*O*-acctyl-*α*-L-arabinose, R_2 = H, R_3 = H, R_4 = Ac, 24 *S*
4 R_1 = OH, R_2 = H, R_3 = OH, R_4 = H, Δ 7(8), 24 *S*
5 R_1 = OH, R_2 = OH, R_3 = H, R_4 = H, Δ 7(8), 24 *S*
7 R_1 = =O, R_2 = H, R_3 = H, R_4 = H, Δ 7(8), 24 *S*
8 R_1 = =O, R_2 = H, R_3 = H, R_4 = H, Δ 1(2), Δ 7(8), 24 *S*
13 R_1 = OH, R_2 = H, R_3 = H, R_4 = H, 24 *S*
14 R_1 = OH, R_2 = H, R_3 = H, R_4 = H, Δ 7(8), 24 *S*
15 R_1 = OH, R_2 = H, R_3 = H, R_4 = Ac, Δ 7(8), 24 *S*
16 R_1 = =O, R_2 = H, R_3 = H, R_4 = H, 24 *S*
17 R_1 = =O, R_2 = H, R_3 = H, R_4 = H, Δ 7(8), 24 *R*
18 R_1 = =O, R_2 = H, R_3 = H, R_4 = H, Δ 1(2), 24 *S*
19 R_1 = *O*-2'-*O*-acetyl-*α*-L-arabinose, R_2 = H, R_3 = H, R_4 = Ac, 24 *S*
27 R_1 = *O*-*α*-L-arabinose, R_2 = H, R_3 = H, R_4 = H, 24 *S*



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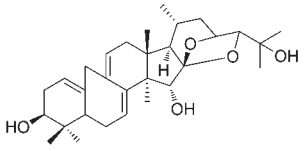

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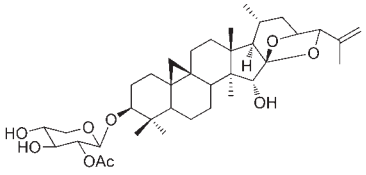

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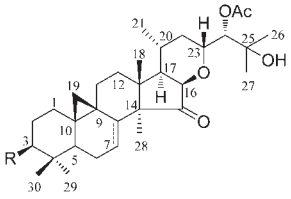
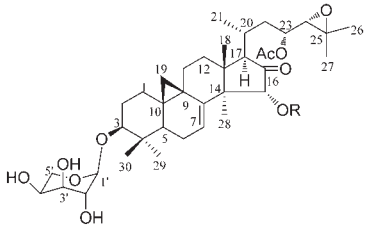
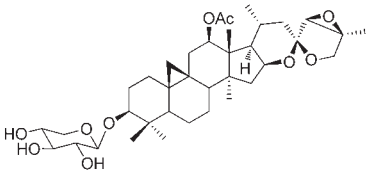


10 R = H
11 R = H, Δ 7(8)
24 R = *O*-2'-*O*-acetyl-*α*-L-arabinose
29 R = *O*-acetyl-*α*-L-arabinose



12 R = Ac
30 R = H



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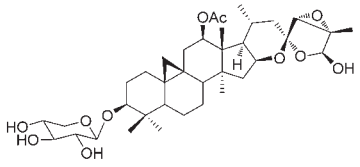

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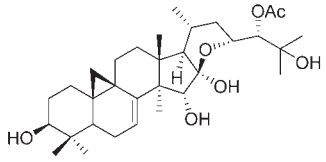

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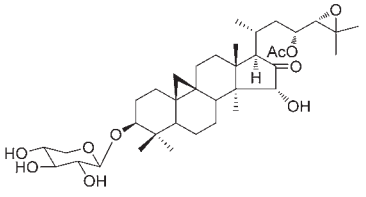

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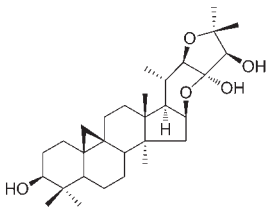

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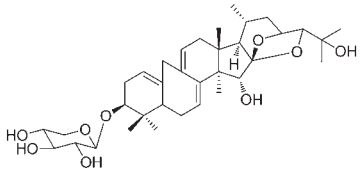

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Fig. 1 Structures of compounds **1**–**30**.

Table 1 ^{1}H NMR data of compounds **1**–**12** in pyridine-d_5 at 500 MHz.

Proton	1	2	3	4	5	6	7	8	9	10	11	12
1	1.53[a] 1.17[a]	1.54 m 1.22[a]	1.54 m 1.16[a]	1.70 m 1.35 m	2.78 m 1.71 m	2.99 m 1.88 m	1.85 m 1.54 m	6.71 d (10.0)	5.54 brs	1.53 m 1.14 m	1.68 m 1.24 m	1.64 m 1.30[a]
2	2.28 m 1.87 m	2.34 m 1.96[a]	2.34 dd (4.0, 16.0) 1.95[a]	1.92 m 1.86 brs	2.11 m 1.93 m	2.84 m 2.35 m	2.75 ddd (6.5) 2.29 m	6.16 d (10.0)	2.52 m 2.26 m	1.99 m 1.86 dd (3.2, 10.0)	1.95 m 1.89 m	2.36 m 1.95 m
3	3.39 dd (4.0, 11.5)	3.50 dd (5.0, 14.5)	3.47 dd (5.0, 14.2)	3.50 dd (3.5, 11.0)	3.57 dd (5.0, 10.5)				3.78 m	3.48 m	3.51 m	3.49 dd (3.5, 10.5)
4												
5	1.28 dd (3.0, 12.3)	1.30[a]	1.29 m	1.25 dd (5.0, 12.0)	1.31 dd (5.0, 12.0)	1.64 m	1.54 m	1.84 dd (5.0, 12.5)	2.36 m	1.25 dd (3.2, 10.0)	1.20[a]	1.27[a]
6	1.48[a] 0.66 m	1.52 m 0.70 m	1.49 m 0.69 m	1.94 m 1.68 m	1.92 m 1.77 m	1.82 m 1.64 m	1.70 m 1.54 m	1.75 m 1.60 m	2.64 m 2.36 m	1.51 m 0.65 m	1.93 m 1.56 m	1.76 m 1.43 m
7	2.06 m 1.11[a]	2.07 m 1.07[a]	2.09 m 1.04[a]	6.20 d (7.0)	6.21 d (6.5)	5.23 m	6.05 brd (7.0)	6.11 d (7.0)	5.52 brd (8.0)	2.27 m 1.95 m	6.36 brd (6.5)	5.33 d (7.0)
8	1.65[a]	1.66[a]	1.66[a]							1.72 brd (10.4)		
9												
10												
11	2.06 m 1.01 m	2.07 m 1.15[a]	2.09 m 1.15[a]	2.96 dd (9.0, 15.0) 1.54[a]	4.58 brd (8.5)	4.53 m	2.13 m 1.16[a]	2.23 m 1.40 m	5.39 brd (5.0)	2.23 m 1.05[a]	1.98 m 1.13[a]	2.11 m 1.16 m
12	1.65[a] 1.53[a]	1.66[a] 1.52 m	1.64[a] 1.52 m	4.36 t (6.5)	2.74 m 2.11 m	2.73 m 1.99 m	1.81 m 1.68 m	1.81 m 1.70 m	2.21 m 1.94 dd (6.0, 12.5)	1.58 m 1.37 m	1.65 m 1.29 m	1.89 m 1.78 m
13												
14												
15	4.26 brs	4.27 brs	4.25 brs	4.76 brs	4.62 s	2.46 d (13.0) 2.28 d (13.0)	4.55 brd (8.0)	4.54 d (9.0)	2.36 m 2.09 m			5.92 s
16										3.79 d (9.6)	3.83 d (11.2)	
17	1.48[a]	1.44 brd (14.0)	1.45 brd (14.0)	1.86 brs	1.48[a]	1.59 d (12.0)	1.50[a]	1.49[a]	1.53[a]	1.52 m	1.20 m	2.34 d (8.0)
18	1.13 s	1.13 s	1.13 s	1.51 s	1.27 s	1.26 s	1.16 s	1.10 s	0.81 brs	1.18 s	1.20 s	1.29 s
19	0.45 brs 0.21 brs	0.50 d (4.0) 0.25 d (4.0)	0.52 d (4.0) 0.28 d (4.0)	1.19[a] 0.73 d (4.0)	1.97 d (4.0) 1.07 brd (2.5)	2.11 d (4.0) 1.11 d (4.0)	1.19[a] 0.67 d (4.0)	1.52 d (4.8) 1.04 d (4.0)	3.24 d (13.5) 3.15 d (13.5)	0.52 d (3.6) 0.29 d (3.6)	1.07[a] 0.48 d (4.0)	0.99[a] 0.51 d (3.5)
20	1.65[a]	1.67[a]	1.59 m	1.86 m	1.64 brd (10.5)	1.66 brd (9.5)	1.70 m	1.68 m	1.60 m	1.80 m	1.81 m	2.10 m
21	0.84 d (7.0)	0.83 d (6.4)	0.84 d (6.4)	1.41 d (5.5)	0.82 d (6.0)	0.83 d (6.0)	0.89 d (6.5)	0.89 d (7.0)	0.81 brs	0.91 d (7.2)	0.93 d (6.5)	1.19 d (6.5)
22	2.26 m 1.01 m	2.25 m 0.97 m	2.26 m 0.95 m	2.42 m 1.16[a]	2.22 m 0.99 m	2.24 m 0.99 t (11.5)	2.30 m 1.07 m	2.28 m 1.07 m	2.21 m 0.96[a]	1.72 brd (10.4) 1.44 m	1.73 m 1.47 m	2.77 t (12.0) 1.68 m
23	4.74 d (9.0)	4.59 d (11.5)	4.59 brd (12.0)	4.80 d (9.0)	4.73 d (9.0)	4.79 d (8.5)	4.78 brd (9.0)	4.77 t (8.0)	4.77 brd (9.0)	4.25 brd (11.2)	4.27 d (11.5)	5.38 brt (9.5)
24	3.76 brs	4.11 brs	4.11 brs	3.87 s	3.77 s	3.71 s	3.80 brs	3.80 s	3.69 brs	5.31 d (1.6)	5.33 d (2.0)	3.03 d (8.0)
25												*cont.*

Table 1 *Continued*

Proton	1	2	3	4	5	6	7	8	9	10	11	12
26	1.48 s	1.68 s	1.68 s	1.52 s	1.47 s	1.51 s	1.48 s	1.49 s	1.52 s	1.61 s	1.62 s	1.25 s
27	1.46 s	1.66 s	1.66 s	1.51 s	1.45 s	1.46 s	1.49 s	1.48 s	1.47 s	1.61 s	1.63 s	1.39 s
28	1.16 s	1.18 s	1.18 s	1.46 s	1.36 s	1.39 s	1.38 s	1.36 s	1.28 s	1.01 s	1.13 s	1.34 s
29	1.08 s	1.31 s	1.22 s	1.17 s	1.19 s	1.16 s	1.04 s	1.15 s	1.31 s	1.19 s	1.21 s	1.31 s
30	0.95 s	1.04 s	1.00 s	1.07 s	1.14 s	1.12 s	1.07 s	0.99 s	0.98 s	1.05 s	1.07 s	1.02 s
3-Ara												
1′	4.79 d (7.5)	4.79 d (9.0)	4.88 d (8.0)									4.79 d (7.0)
2′	5.99 t (8.5)	4.38 t (10.0)	4.63 brd (6.0)									4.18 m
3′	4.23 brd (9.5)	4.24 m	5.48 dd (4.5, 11.0)									4.47 t (8.0)
4′	4.30 brs	5.50 brs	4.57 brs									4.33 brs
5′	4.28 m 3.79 brd (12.0)	4.24 m 3.81 d (16.0)	4.30 m 3.85 d (13.5)									4.31 brd (9.5) 3.79 d (12.5)
15-OCOC$\underline{H}_3$												2.26 s
23-OCOC$\underline{H}_3$												2.02 s
24-OCOC$\underline{H}_3$										2.16 s	2.18 s	
25-OCOC$\underline{H}_3$		1.96 s	1.95 s									
3′-OCOC$\underline{H}_3$			1.96 s									
4′-OCOC$\underline{H}_3$		1.96 s										
4′-OCOC$\underline{H}$=C-H-CH$_3$	6.05 d (15.5)											
4′-OCOCH=C-$\underline{H}$-CH$_3$	7.09 m											
4′-OCOCH=C-H-C$\underline{H}_3$	1.63 d (6.5)											

Chemical shifts are in δ scale with *J* values in parentheses. [a] Signals overlapped

9,10-Seco-1(10),7(8),9(11)-trien-cimigenol (**9**): A white powder; $[\alpha]_D^{24} = 5.73$ (*c* 0.05, MeOH); IR (KBr): $\nu_{max} = 3432, 2947, 2852, 1624, 1460, 1383, 1070, 978\ cm^{-1}$; ^1H (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) spectra, see **Tables 1** and **2**; ESIMS: *m/z* = 469 $[M + H]^+$; HR-TOF-ESIMS: *m/z* = 469.3311 (calc. for $C_{30}H_{45}O_4$, 469.3317).

24-O-Acetylisodahurinol (**10**): A white powder; $[\alpha]_D^{24} = 2.09$ (*c* 0.07, MeOH); IR (KBr): $\nu_{max} = 3473, 2946, 2862, 1738, 1471, 1377, 1024, 994\ cm^{-1}$; ^1H (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectra, see **Tables 1** and **2**; ESIMS: *m/z* = 553 $[M + Na]^+$; HR-TOF-ESIMS: *m/z* = 553.3494 (calc. for $C_{32}H_{50}O_6Na$, 553.3505).

24-O-Acetyl-7(8)-en-isodahurinol (**11**): A white powder; $[\alpha]_D^{24} = -4.80$ (*c* 0.05, MeOH); IR (KBr): $\nu_{max} = 3437, 2920, 2875, 1736, 1640, 1472, 1375, 1026, 996\ cm^{-1}$; ^1H (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 125 MHz) spectra, see **Tables 1** and **2**; ESIMS: *m/z* = 551 $[M + Na]^+$; HR-TOF-ESIMS: *m/z* = 551.3346 (calc. for $C_{32}H_{48}O_6Na$, 551.3348).

23-O-Diacetyl-7(8)-en-shengmanol-3-O-α-L-arabinopyranoside (**12**): A white powder; $[\alpha]_D^{24} = -7.69$ (MeOH, *c* 0.07); IR (KBr): $\nu_{max} = 3473, 2942, 2871, 1736, 1643, 1458, 1376, 1024, 972\ cm^{-1}$; ^1H (C_5D_5N, 500 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) spectra, see **Tables 1** and **2**; ESIMS: *m/z* = 725 $[M + Na]^+$; HR-TOF-ESIMS: *m/z* = 725.3895 $[M + Na]^+$ (calc. for $C_{39}H_{58}O_{11}Na$, 725.3876).

Hydrolysis and identification of the sugar moieties in compounds 1, 2, 3, and 12

Compound **1** (4.0 mg) together with **2** and **3** (3.0 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 rt 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]. Compound **12** (4.0 mg), by contrast, was directly dissolved in MeOH (5 mL) and refluxed with 0.5 N HCl (3 mL) for 4 h. Each reaction mixture was diluted with H_2O and extracted with $CHCl_3$ (3 × 10 mL). Each aqueous layer was then neutralized by Ag_2CO_3, and the formed precipitation was filtered to give a monosaccharide, which had an *Rf* (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-arabinose (Sigma-Aldrich).

Table 2 ^{13}C NMR data of compounds **1–12** in pyridine-d_5 at 125 MHz (**2–5, 7, 8, 10, 11**) and 150 MHz (**1, 6, 9, 12**).

C	1	2	3	4	5	6	7	8	9	10	11	12
1	32.8 t	32.4 t	32.4 t	30.8 t	27.7 t	29.5 t	31.7 t	152.6 d	121.0 d	32.7 t	31.3 t	30.6 t
2	30.4 t	30.0 t	29.9 t	30.7 t	31.1 t	36.9 t	36.9 t	127.2 d	33.7 t	31.3 t	30.7 t	29.9 t
3	89.2 d	88.9 d	88.7 d	77.8 d	78.1 d	216.1 s	214.9 s	203.8 s	74.2 d	77.9 d	77.6 d	88.5 d
4	41.6 s	41.3 s	41.3 s	40.2 s	40.5 s	49.5 s	48.9 s	45.1 s	39.6 s	41.1 s	40.2 s	40.9 s
5	47.9 d	47.6 d	47.6 d	42.4 d	43.6 d	45.9 d	44.2 d	40.4 d	51.7 d	47.3 d	41.9 d	42.8 d
6	21.5 t	21.1 t	21.1 t	22.0 t	22.2 t	22.7 t	21.9 t	21.8 t	38.5 t	21.0 t	21.9 t	22.2 t
7	26.9 t	26.3 t	26.4 t	114.5 d	115.2 d	114.5 d	114.2 d	115.5 d	125.1 d	26.2 t	115.0 d	115.6 d
8	49.1 d	48.6 d	48.6 d	147.4 s	147.3 s	149.1 s	147.9 s	147.4	144.8 s	43.7 d	141.9	146.6 s
9	20.5 s	20.0 s	20.1 s	21.9 s	28.0 s	28.9 s	22.1 s	26.3 s	138.3 s	20.0 s	21.0 s	21.8 s
10	27.0 s	26.5 s	26.7 s	28.3 s	29.4 s	29.4 s	28.2 s	32.8 s	139.8 s	27.4 s	29.3 s	29.1 s
11	26.8 t	26.4 t	26.5 t	40.3 t	63.5 d	63.7 d	25.6 t	26.5 t	122.1 d	26.0 t	25.3 t	25.3 t
12	34.5 t	34.0 t	34.1 t	72.5 d	49.4 t	48.7 t	33.9 t	33.8 t	38.6 t	31.4 t	30.6 t	33.4 t
13	42.3 s	41.8 s	41.9 s	47.1 s	41.6 s	46.0 s	41.2 s	41.3 s	45.2 s	40.0 s	40.2 s	41.9 s
14	47.7 s	47.2 s	47.3 s	51.3 s	50.7 s	48.6 s	50.6 s	50.9 s	48.3 s	55.1 s	56.4 s	48.9 s
15	80.7 d	80.2 d	80.2 d	78.1 d	78.2 d	45.8 t	78.1 d	77.9 d	44.3 t	213.9 s	211.2 s	82.1 d
16	112.5 s	112.4 s	112.5 s	112.5 s	112.4 s	114.9 s	112.2 s	112.1 s	114.5 s	84.3 d	84.3 d	214.2 s
17	60.0 d	59.4 d	59.4 d	59.8 d	58.9 d	61.6 d	59.4 d	59.4 s	60.4 d	52.4 d	52.3 d	60.3 d
18	20.0 q	19.5 q	19.5 q	13.2 q	21.0 q	21.4 q	21.7 q	21.5 q	17.8 q	20.3 q	25.4 q	22.3 q
19	31.2 t	30.9 t	30.9 t	28.8 t	18.7 t	18.7 t	27.7 t	30.3 t	44.3 t	31.3 t	28.5 t	30.4 t
20	24.5 d	23.9 d	23.9 d	23.8 d	24.0 d	24.3 d	24.0 d	24.0 d	24.4 d	33.3 d	33.0 d	28.9 d
21	20.1 q	19.5 q	19.5 q	21.3 q	19.6 q	20.2 q	19.7 q	19.7 q	20.5 q	20.0 q	20.0 q	20.3 q
22	38.6 t	37.9 t	37.9 t	38.5 t	38.0 t	38.5 t	38.0 t	38.6 t	25.9 t	38.8 t	38.7 t	37.5 t
23	72.3 d	71.7 d	71.7 d	72.0 d	72.0 d	72.4 d	72.1 d	72.1 d	72.3 d	79.1 d	79.0 d	72.3 d
24	90.7 d	86.8 d	86.8 d	90.1 d	90.3 d	91.1 d	90.3 d	90.3 d	91.0 d	79.8 d	79.8 d	65.6 d
25	71.4 s	83.1 s	83.1 s	71.0 s	71.9 s	71.4 s	70.9 s	70.9 s	71.5 s	72.1 s	72.0 s	59.1 s
26	27.6 q	23.4 q	23.4 q	27.0 q	27.1 q	28.4 q	27.1 q	27.1 q	28.4 q	26.8 q	26.8 q	25.2 q
27	25.9 q	21.5 q	21.5 q	25.6 q	25.4 q	25.2 q	25.4 q	25.4 q	25.1 q	28.4 q	28.4 q	19.8 q
28	12.3 q	11.8 q	11.8 q	18.3 q	19.5 q	27.9 q	18.4 q	18.2 q	25.3 q	17.6 q	21.6 q	20.0 q
29	26.0 q	25.7 q	25.7 q	26.1 q	26.3 q	23.2 q	22.6 q	21.7 q	25.7 q	26.1 q	26.1 q	26.3 q
30	15.7 q	15.4 q	15.4 q	13.6 q	13.9 q	20.8 q	20.2 q	18.9 q	15.1 q	14.9 q	13.6 q	14.7 q
3-Ara												
1′	105.0 d	107.3 d	106.8 d									108.1 d
2′	74.5 d	73.2 d	69.7 d									73.4 d
3′	73.0 d	72.5 d	76.9 d									75.2 d
4′	70.3 d	72.3 d	66.7 d									70.2 d
5′	67.6 t	63.2 t	66.2 t									66.5 t
15-O$\underline{C}$OCH$_3$												170.9 s
15-OCO$\underline{C}$H$_3$												21.4 q
23-O$\underline{C}$OCH$_3$												171.2 s
23-OCO$\underline{C}$H$_3$												21.5 q
24-O$\underline{C}$OCH$_3$										171.1 s	171.1 s	
24-OCO$\underline{C}$H$_3$										21.0 s	20.9 s	
25-O$\underline{C}$OCH$_3$		170.2 s	170.2 s									
25-OCO$\underline{C}$H$_3$		21.2 q	22.3 q									
3′-O$\underline{C}$OCH$_3$			170.8 s									
3′-OCO$\underline{C}$H$_3$			21.6 q									
4′-O$\underline{C}$OCH$_3$		170.8 s										
4′-OCO$\underline{C}$H$_3$		22.2 q										
4′-O$\underline{C}$OCH=CH--CH$_3$	166.3 s											
4′-OCO$\underline{C}$H=CH--CH$_3$	122.3 d											
4′-OCOCH=$\underline{C}$H--CH$_3$	145.2 d											
4′-OCOCH=CH-$\underline{C}$H$_3$	18.2 q											

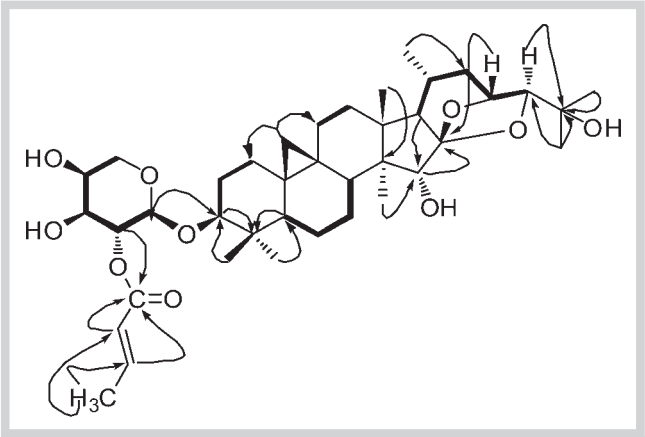

Fig. 2 Major HMBC (→) and ^{1}H-^{1}H COSY (—) correlations of compound **1**.

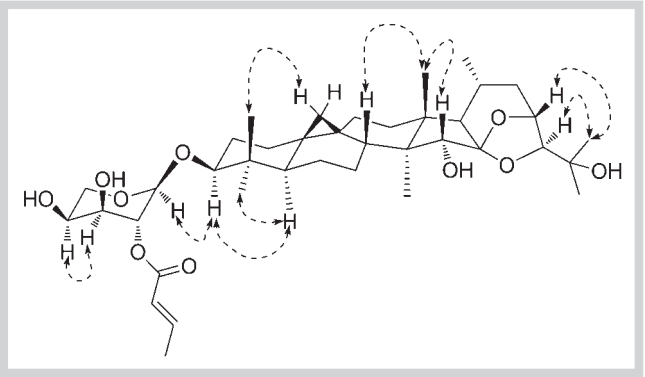

Fig. 3 Key NOESY correlations of compound **1**.

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) supplemented with 10% fetal bovine serum (Hyclone), 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 [32, 33]. Briefly, 100 µL of adherent cells were 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 an initial density of 1×10^5 cells/mL. Each tumor cell line was exposed to the test compounds (dissolved in DMSO, then diluted by DMEM medium) at concentrations of 0.064, 0.32, 1.6, 8, and 40 µM in triplicate for 48 h, with cisplatin (Sigma) and culture solution as positive and negative controls, respectively. 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 [34].

Supporting information

The NMR spectra of compounds **1**–**12**, as well as ESI and HR-TOF-ESI data of **1**–**12** are available as Supporting Information.

Results and Discussion

Compound **1** was obtained as white powder. Its molecular formula ($C_{39}H_{60}O_{10}$) was deduced from the analysis of ^{13}C NMR and HR-TOF-ESIMS data {*m/z*: 711.4065 [M + Na]$^+$ (calc. for $C_{39}H_{60}O_{10}Na$, 711.4084)}. The IR spectrum showed absorptions for hydroxy groups at 3452 cm^{-1}, carbonyl groups at 1727 cm^{-1}, and double bonds at 1632 cm^{-1}, respectively. The ^{1}H-NMR spectrum (**Table 1**) showed the presence of the characteristic cyclopropane methylene signals at δ_H 0.21 and 0.45 (each 1H, brs), an anomeric proton at δ_H 4.79 (d, J = 7.5 Hz), two olefinic protons at δ_H 6.05 (1H, d, J = 15.5 Hz) and 7.09 (1H, m), two secondary methyl signals at δ_H 0.84 (d, J = 7.0 Hz) and 1.63 (d, J = 6.5 Hz), and six tertiary methyl groups at δ_H 0.95–1.48. In the ^{13}C and DEPT NMR spectra of **1** (**Table 2**), the signals ascribable to an *α,β*-unsaturated ketone moiety at δ_C 166.3, 122.3, and 145.2 were observed. A comparison of the spectroscopic data of **1** with those of cimigenol-3-*O*-*α*-L-arabinopyranoside (**27**) [15] showed that, structurally, **1** closely resembles **27**, with the main differences of the sugar unit and the presence of another tetra-carbon unit, including the *α,β*-unsaturated ketone resonances. In the ^{1}H-^{1}H COSY spectrum (**Fig. 2**), a correlation was observed between the secondary methyl signal at δ_H 1.63 (d, J = 6.5 Hz) and the olefinic proton at δ_H 7.09 (m), which indicated the tetra-carbon unit to be a 2-butenoyl. In addition, the coupling constant (J = 15.6 Hz) of the two olefinic protons at δ_H 6.05 and 7.09 confirmed the *E*-geometry of a double bond in the 2-butenoyl moiety. In the HMBC spectrum (**Fig. 2**), a correlation was observed between the anomeric proton at δ_H 4.79 (H-1′, 1H, d, J = 7.5 Hz) and the methine signal at δ_C 89.2 (C-3), suggesting that a sugar unit was attached at the C-3. The sugar obtained after acid hydrolysis was identified as *α*-L-arabinose by comparing its TLC and specific rotation with the standard. In the ^{1}H NMR spectrum, a downfield resonance was observed at δ_H 5.99 (t, J = 8.5 Hz), which showed correlations with the methine resonance at δ_H 4.30 (H-3′) and the anomeric proton at δ_H 4.79 in the ^{1}H-^{1}H COSY spectrum (**Fig. 2**). Furthermore, the HMBC (**Fig. 2**) correlation between the carbonyl group (δ_C 166.3) and the proton resonance (δ_H 5.99, t, J = 8.5 Hz) indicated the (*E*)-2-butenoyl unit was attached at C-2′. In the ROESY spectrum (**Fig. 3**), H-3 showed a correlation with H-5 suggesting an *α*-orientation of the H-3, while H-15 showed a correlation with Me-18, indicating an *α*-orientation of the hydroxyl group at C-15. The configuration of C-23 and C-24 was deduced as *R* and *S*, respectively, by comparison of the coupling constant of H-23 and H-24 with those of cimigenol-type compounds [6, 22, 26]. Therefore, the structure of **1** was determined as cimigenol-3-*O*-[2′-*O*-(*E*)-2-butenoyl]-*α*-L-arabinopyranoside.

Compounds **2** and **3** were determined to have the same molecular formula $C_{39}H_{60}O_{11}$ by HR-TOF-ESIMS (*m/z* 727.4029 [M + Na]$^+$ and 703.4055 [M – H], respectively). The NMR spectroscopic data (**Tables 1** and **2**) of **2** and **3** were similar to those of 25-*O*-acetylcimigenol-3-*O*-[2′-*O*-acetyl]-*α*-L-arabinopyranoside (**19**), except for the sugar moiety. In the ^{1}H-NMR spectrum (**Table 1**) of **2**, the signal due to H-4′ showed a downfield shift from δ_H 4.16 to 5.50. Meanwhile, the signal of H-2′ was shifted from δ_H 5.89 to δ_H 4.38. In addition, the signal due to C-4′ exhibited a downfield shift from δ_C 69.8 to 72.3 in the ^{13}C NMR spectrum (**Table 2**). The changes of these chemical shifts may be explained by the *O*-acetyl group being attached to C-4 of the sugar unit, which was further confirmed by the presence of the HMBC correlation between the H-4′ signal at δ_H 5.50 and the carbonyl group signal at δ_C 170.8. The sugar obtained after acid hydrolysis was confirmed

as α-L-arabinopyranose by comparing its TLC and specific rotation with the standard. The configurations of C-23 and C-24 are proposed as *R* and *S*, respectively, by the same way as that of **1**. Thus, the structure of **2** was assigned as 25-*O*-acetylcimigenol-3-*O*-[4′-*O*-acetyl]-α-L-arabinopyranoside. In the same way, an acetoxy group was determined to be at C-3′ for **3**, which was further confirmed by the presence of the HMBC correlation between the H-3′ signal at δ_H 5.48 and the carbonyl group signal at δ_C 170.8. Therefore, **3** was identified as 25-*O*-acetylcimigenol-3-*O*-[3′-*O*-acetyl]-α-L-arabinopyranoside.

Compound **4** gave a molecular formula of $C_{30}H_{46}O_6$ as established by HR-TOF-ESIMS (*m/z* 525.3192 $[M + Na]^+$), which is 16 Da more than those of 7(8)-en-cimigenol (**14**). The ^1H NMR spectrum (**Table 1**) displayed downfield cyclopropane methylene signals at δ_H 0.73 (1H, d, *J* = 4.0 Hz) and 1.19 (overlapped), seven methyl groups at δ_H 1.07–1.52, and an olefinic proton at δ_H 6.20 (1H, d, *J* = 7.0 Hz), respectively, suggesting **4** to be a 9,19-cyclolanostane aglycone with a pair of olefinic carbons close to the cyclopropane methylene at C-19 [20]. The NMR data of **4** showed a close resemblance with those of **14**, except that the methylene signal at δ_C 33.7 (C-12) was absent, showing instead a hydroxymethine at δ_C 72.5. On the basis of the above observations, it was reasonable to deduce that **4** was a 12-hydroxy derivative of **14**, which was also supported by the HMBC correlations of H-12 at δ_H 4.36 with C-11 (δ_C 40.3), C-13 (δ_C 47.1), and CH_3-18 (δ_C 13.2), as well as by the downfield shift of C-11 about 14.9 ppm in the ^{13}C spectrum (**Table 2**). Significant ROESY correlations of H-12 with H-5, and CH_3-28 suggested a β-orientation of the substituent at C-12. Therefore, **4** was elucidated as 12β-hydroxy-7(8)-en-cimigenol.

Compound **5** was assigned as $C_{30}H_{46}O_6$, as deduced from the HR-TOF-ESIMS (*m/z* 525.3199 $[M + Na]^+$), which is identical to that of compound **4**. The NMR data of **5** were similar to that of **4** with the major difference being that a hydroxyl group was shifted from C-12 to C-11. In the ^{13}C NMR spectrum (**Table 2**) of **5**, the signal due to C-12 exhibited an upfield shift from δ_C 72.5 to 49.4, while C-11 showed a downfield shift from δ_C 40.3 to 63.5, further confirming the deduction. The relative configuration of the hydroxyl group at C-11 was proposed as β-orientated by analyses of the ROESY spectrum. Accordingly, compound **5** was characterized as 11β-hydroxy-7(8)-en-cimigenol.

Compound **6** had the molecular formula $C_{30}H_{44}O_5$ from its positive mode HR-TOF-ESIMS (507.3090 $[M + Na]^+$). In the ^{13}C and DEPT spectra (**Table 2**), **6** exhibited signals very similar to those of **5** except that hydroxy methine signals due to C-3 and C-15 were absent, whereas a carbonyl carbon at δ_C 216.1 and a downfield methylene at δ_C 45.8 were observed. In addition, HMBC correlations of H-2, CH_3-29, and CH_3-30 with the carbonyl group at δ_C 216.1 and protons (δ_H 2.46 and 2.28, each 1H) due to the methylene at δ_C 45.8 with C-14, C-16, and CH_3-28 indicated that a carbonyl carbon replaced a hydroxyl group at C-3, and the methine at C-15 was transformed to a methylene in **6**. Ultimately, **6** was elucidated as 11β-hydroxy-15-deoxycimigenol-7(8)-en-3-one.

The HR-TOF-ESIMS (*m/z* 507.3086 $[M + Na]^+$) of compound **7** determined its molecular formula as $C_{30}H_{44}O_5$, which is identical with 24-*epi*-cimigenol-7(8)-en-3-one (**17**). The NMR data (**Tables 1** and **2**) of **7** resembled those of **17** with major differences at C-22 (δ_C 38.0), C-23 (δ_C 72.1), C-24 (δ_C 90.3), and C-25 (δ_C 70.9), which are similar to the key structural differences between cimigenol-type and 24-*epi*-cimigenol-type triterpenes [21]. By comparing the coupling constant of H-24 (0 Hz) with those of cimigenol-type compounds (0 Hz) and 24-*epi*-cimigenol-type constituents (4 Hz), the configuration of C-24 of **7** was determined to be *S* [6,21,25]. Thus, **7** was elucidated as cimigenol-7(8)-en-3-one.

The molecular formula of compound **8** was determined as $C_{30}H_{42}O_5$ from the HR-TOF-ESIMS (*m/z* 505.2944 $[M + Na]^+$). Its NMR data (**Tables 1** and **2**) were similar to those of **7** except for the signals of ring A. Unsaturated carbon signals at δ_C 152.6 and 127.2 were observed in **8**, whereas signals of two methylenes due to C-1 and C-2 were absent. Significant HMBC correlations were observed between the carbonyl C-atom at δ_C 203.8 and the olefinic protons at δ_H 6.16 and 6.71 (each 1H, d, *J* = 10.0 Hz). The above evidence suggested that compound **8** is transformed from **7** through dehydrogenation between C-1 and C-2. Therefore, compound **8** was characterized as cimigenol-1(2),7(8)-dien-3-one.

Compound **9** was assigned a molecular formula of $C_{30}H_{46}O_4$ from its HR-TOF-ESIMS (*m/z* 469.3311 $[M + H]^+$). In the ^1H NMR spectrum, signals for the significant downfield cyclopropane methylene at δ_H 3.15 and 3.24 (1H each, d, *J* = 14.0 Hz), seven methyl groups at δ_H 0.81–1.52, and three olefinic protons at δ_H 5.39, 5.52, and 5.54 were observed, suggesting **9** is a 9,10-seco-9,19-cycloartane triterpene [30]. The ^{13}C NMR and DEPT spectroscopic data of **9** were identical with the aglycone resonances of cimicinol (**28**) [31], except for the upfield shift of the C-3 by 9.7 ppm, which could be explained by the absence of a sugar unit at C-3. Therefore, **9** was elucidated as 9,10-*seco*-1(10),7(8),9(11)-trien-cimigenol.

The molecular formula of compound **10** was established as $C_{32}H_{50}O_6$ on the basis of HR-TOF-ESIMS (*m/z* 553.3494 $[M + Na]^+$). In the IR spectrum, absorption bands at 3473 and 1733 cm^{-1} for hydroxyl and carbonyl groups were observed. In the ^{13}C and DEPT NMR spectra, the signals ascribable to the methylene carbon of the cyclopropane ring at δ_C 31.3 (C-19), four oxygen-bearing methine carbons at δ_C 84.3 (C-16), 79.8 (C-24), 79.1 (C-23), and 77.9 (C-3), as well as two carbonyl carbons at δ_C 213.9 (C-15) and 171.1 (C_{24}-acetoxy) were observed, suggesting that **10** was a highly oxygenated 9,19-cycloartane triterpene aglycon with an *O*-acetyl group. By comparison of NMR spectroscopic data, **10** was determined to be the aglycon of 24-*O*-acetylisodahurinol-3-*O*-α-L-arabinopyranoside (**29**) [7]. The configuration of C-24 was deduced as *S* by comparison of the coupling constants of H-24 (1.6 Hz) with those of dahurinyl diacetate (9 Hz) and isodahurinyl diacetate (2 Hz) [29]. Accordingly, compound **10** was characterized as 24-*O*-acetylisodahurinol.

Compound **11** gave a molecular formula of $C_{32}H_{48}O_6$ by HR-TOF-ESIMS at *m/z* 551.3346 $[M + Na]^+$. The NMR spectroscopic data (**Tables 1** and **2**) of **11** resembled those of **10** except for major differences at C-7 (δ_C 115.0) and C-8 (δ_C 141.9) due to dehydrogenation at these positions. This deduction was confirmed by the correlations of δ_H 6.36 (H-7) with δ_H 1.56 and 1.93 (H-6) in the ^1H-^1H COSY spectrum. Accordingly, compound **11** was characterized as 24-*O*-acetyl-7(8)-en-isodahurinol.

Compound **12** exhibited the molecular formula $C_{39}H_{58}O_{11}$, as established by HR-TOF-ESIMS at *m/z* 725.3895 $[M + Na]^+$. The molecular weight of **12** is 42 Da more than that of 23-*O*-acetyl-7(8)-en-shengmanol-3-*O*-α-L-arabinopyranoside (**30**) [27], which may be due to an acetyl group. When its spectroscopic data (**Tables 1** and **2**) were compared with those of **30** [27], an additional *O*-acetyl group was assigned to C-15 on the basis of the upfield shift of the carbonyl carbon (C-16) from δ_C 220.3 to 214.2, the downfield shift of H-15 from δ_H 4.56 to 5.92, as well as the HMBC correlation of H-15 and the carbonyl group signal at δ_C 170.9. Significant ROESY correlations of H-15 with CH_3-18 sug-

Table 3 Cytotoxicity[a] (IC_{50}, μM ± SD) of compounds isolated from the roots of *C. dahurica*.

Compounds	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	4.2 ± 0.4	13.4 ± 1.1	13.8 ± 0.8	14.2 ± 0.7	11.8 ± 1.3
2	8.1 ± 0.5	12.2 ± 1.2	14.5 ± 0.7	12.7 ± 1.4	13.1 ± 0.9
3	5.8 ± 0.2	8.7 ± 0.3	10.3 ± 1.1	12.6 ± 0.5	11.3 ± 1.2
4	> 40	> 40	> 40	> 40	> 40
5	> 40	> 40	> 40	> 40	> 40
6	18.9 ± 1.6	19.5 ± 0.7	21.1 ± 1.8	23.7 ± 1.5	20.3 ± 1.7
7	21.1 ± 1.5	18.3 ± 1.7	20.5 ± 1.0	19.2 ± 0.9	> 40
8	21.9 ± 2.2	17.4 ± 1.8	19.5 ± 0.8	17.5 ± 1.3	22.5 ± 1.2
9	> 40	> 40	> 40	> 40	> 40
10	> 40	> 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	10.5 ± 0.9	22.5 ± 1.3	19.1 ± 2.1	20.6 ± 1.4	13.8 ± 0.8
16	20.2 ± 0.7	22.9 ± 1.5	20.4 ± 1.7	21.1 ± 1.2	21.3 ± 1.4
17	> 0	> 40	> 40	> 40	> 0
18	20.2 ± 0.8	22.9 ± 1.7	20.4 ± 1.3	15.1 ± 1.2	> 40
19	5.8 ± 0.7	8.7 ± 0.8	10.3 ± 2.1	12.6 ± 1.7	11.3 ± 0.7
20	8.1 ± 0.6	12.2 ± 1.4	14.5 ± 1.2	12.7 ± 2.3	13.1 ± 1.5
21	> 40	> 40	> 40	> 40	> 40
22	> 40	> 40	> 40	> 40	> 40
23	> 40	> 40	> 40	> 40	> 40
24	> 40	> 40	> 40	> 40	> 40
25	> 40	> 40	> 40	> 40	> 40
26	> 40	> 40	> 40	> 40	> 40
Cisplatin	**0.52 ± 0.05**	**13.4 ± 0.6**	**12.4 ± 0.7**	**15.0 ± 1.4**	**14.4 ± 1.1**

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

gested an α-orientation of the substituent at C-15. The configurations of C-23 and C-24 were considered to be *R* and *S*, respectively, by comparing coupling constants of H-23 and H-24 with those of known 9,19-cyclolanostane triterpene glycosides [25]. In the ^{13}C NMR spectrum (**Table 2**), compound **12** showed resonances corresponding to an α-L-arabinose moiety at δ_C 108.1 (d), 73.4 (d), 75.2 (d), 70.2 (d), and 66.5 (t) [8,29], which was further confirmed by comparing its TLC and specific rotation with the standard after hydrolysis. Ultimately, **12** was elucidated as 15,23-*O*-diacetyl-7(8)-en-shengmanol-3-*O*-α-L-arabinopyranoside.

All isolated compounds were screened for their *in vitro* antitumor activities. As summarized in **Table 3**, the new compounds **1**–**3** and the known compounds **19** and **20** showed broad-spectrum and moderate cytotoxicities against human HL-60, SMMC-7721, A549, MCF-7, and SW480 cell lines, with IC_{50} values ranging from 4.2 to 14.5 μM. In addition, the new compounds **6**–**8** and the known compounds **15, 16**, and **18** exhibited broad-spectrum and week cytotoxicities, having IC_{50} values around 20 μM. Based on the above results, we suggest that the roots of *C. dahurica* may be another potential resource for promising antitumor agents.

In the present study, structural and bioactive properties of five cimigenol-type glycosides (**1**–**3, 19, 20**) are completely in accordance with the SAR we proposed before. Thus, the SAR proposed in our previous studies may be used for the design of more potent lead compounds. Furthermore, six cimigenol-type aglycons (**6**–**8, 15, 16, 18**) exhibited broad-spectrum and week cytotoxicities. The main structural characters of these compounds are: (1) the configurations of C-23 and C-24 are *R* and *S*, respectively; (2) carbonyl and acetoxy groups instead of a hydroxyl group at C-3 or C-25. Previously, we reported that cimigenol-type aglycone actrin-3-one has potent and moderate activities against human HepG-2 and HT 29 cell lines, respectively. Meanwhile, 25-*O*-acetylcimigenol exhibited moderate activity against the human HepG-2 cell line [7]. Based on the analyses of these data, we may propose that for cimigenol-type aglycones, hydrophobic groups, such as carbonyl and acetoxy, instead of a hydroxyl group at C-3 or C-25 are essential for cytotoxicity.

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Conflict of Interest

All authors declare that there are no conflicts of interest. The isolation and structural elucidations were accomplished by Yin Nian, Hai-Yan Wang, Lin Zhou, and Ming-Hua Qiu, and the cell culture and cytotoxicity assay were performed by Jia Su and Yan Li. All the authors knew about this manuscript and had no objection to submitting it.

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