New Cytotoxic Cycloartane Triterpenes from the Aerial Parts of *Actaea heracleifolia* (syn. *Cimicifuga heracleifolia*)

Authors
Qiang-Qiang Shi[1,2,3*], Wei-Hua Wang[1,2,3*], Jing Lu[1,2,3], Da-Shan Li[1,3], Lin Zhou[1,3], Ming-Hua Qiu[1,3]

Affiliations
1 State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Science, Kunming, China
2 University of the Chinese Academy of Science, Beijing, China
3 Yunnan Key Laboratory of Natural Medicinal Chemistry Chinese Academy of Sciences, Kunming, China

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Correspondence
Prof. Ming-Hua Qiu
State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Science
132 Lanhei Road, Kunming 650201, China
Phone: + 86 8 71 65 22 33 25, Fax: + 86 8 71 65 22 33 27
mhchiu@mail.kib.ac.cn

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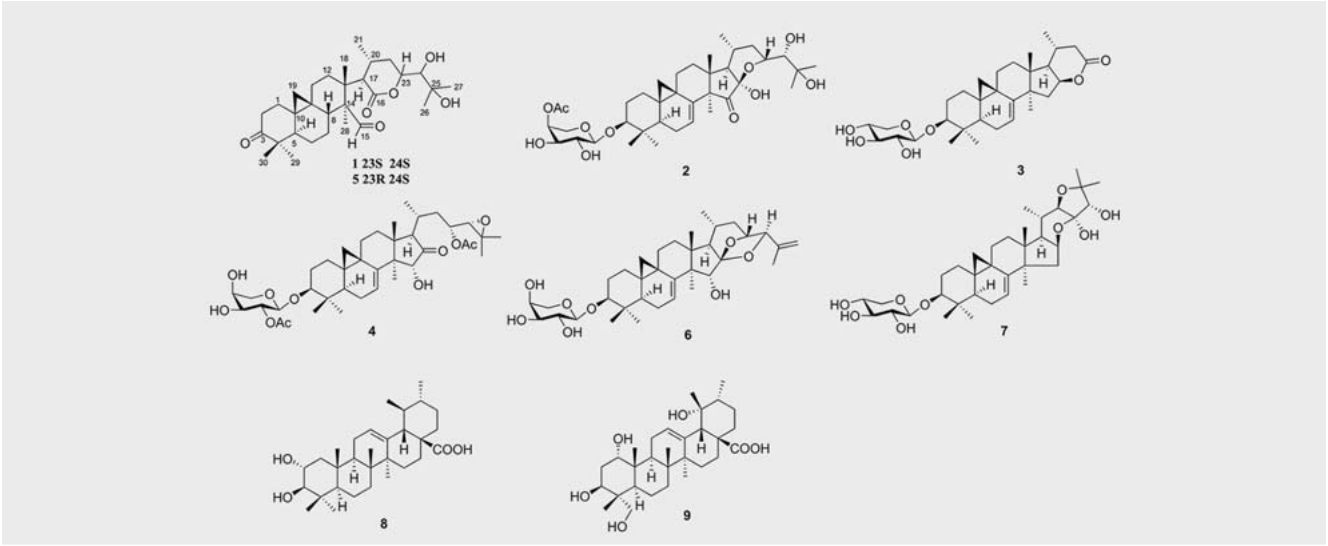
ABSTRACT

One new 15,16-seco-cycloartane triterpene (**1**), three new cycloartane triterpene glycosides (**2**–**4**), and five known compounds (**5**–**9**) were isolated from the aerial parts of *Actaea heracleifolia*. The chemical structures of these compounds were determined on the basis of NMR analysis, HRTOF-ESIMS data, and other spectroscopic methods. Selected compounds were evaluated for their cytotoxicity against human tumor cell lines (HL-60, SMMC-7721, A549, MCF-7, and SW480) *in vitro*. Compounds **3** and **4** showed weak activity against the HL-60, A-549, and MCF-7 cell lines with IC_{50} values ranging from 21.34 to 36.98 μM.

Introduction

The *Actaea* species, belonging to the family Ranunculaceae, has a long history of uses as a medicinal herb worldwide. In China, the rhizomes of *Actaea heracleifolia* Kom., *Actaea dahurica* (Turcz.) Maxim., and *Actaea foetida* L., officially listed in the Chinese Pharmacopoeia with the name "shengma", are used as cooling and detoxifying agents for the treatment of headache, sore throat, and toothache [1]. In Europe and the United States, *Actaea racemosa* (L.) Nutt., commonly called black cohosh, is also used as a dietary supplement for women's health during climacteric periods [2, 3]. Triterpene glycosides have been considered the main active components of *Actaea* species, and phytochemical investigations of plant species led to the isolation of a series of 9,19-cycloartane triterpenes characterized with unique structural features [4], including ring A opened cycloartane triterpenes, such as 3,4-seco-4-hydroxy-3-cimigenolate [5]; ring A expanded cimigenol-type triterpenes, such as cimiheraclein A [6]; 15,16-seco-cycloartane triterpenes [5–10]; ring B opened triterpenes [6, 11, 12]; and cycloartane triterpenes with new skeletons, such as cimicifugadine [13], cimicifine B [14], and cimyunnins A–D [15]. In a further investigation of the aerial parts of *A. heracleifolia*, we found four new cycloartane triterpenes (**1**–**4**) and five known ones: cimiheraclein D (**5**) [6], 25-anhydrocimigenol-3-*O*-*α*-L-arabinopyranoside (**6**) [16], cimiaceroside A (**7**) [17], 2*α*-hydroxyursolic acid (**8**) [18], and 1*α*,3*β*,19*α*,23-tetrahydroxyurs-12-en-28-oic acid-28-*O*-*β*-D-xylopyranoside (**9**) [19] (▶ **Fig. 1**). Compound **1** was a D ring-cleaved 15,16-seco-cycloartane triterpene. Compounds **2**–**4** were 9,19-cycloartane triterpenoid saponins with a fused monosaccharide moiety. Herein, we reported the structure determination by 1D/2D NMR analysis of the new compounds and the evaluation of the cytotoxic activity of selected compounds against the HL-60, SMMC-7721, A549, MCF-7, and SW480 cell lines.

* These authors contributed equally to the work reported in this article.



▶ **Fig. 1** Chemical structures of compounds **1**–**9**.

Results and Discussion

Compound **1** was obtained as white powder. The HRTOF-ESIMS ion signal at *m/z* 525.3180 $[M + Na]^+$ (calcd for 525.3192) indicated the molecular formula $C_{30}H_{46}O_6$ with eight degrees of unsaturation. Its IR spectra showed the presence of hydroxy (3438 cm^{-1}) and carbonyl functional groups (1707 cm^{-1}). The ^1H and ^{13}C NMR data for **1** (▶ **Table 1**) were similar to those of cimiheraclein D (**5**) [6], and the slight difference indicated that compound **1** was a configurational isomer of **5**, which was confirmed by its 2D NMR spectra (HSQC, ^1H–^1H COSY, and HMBC) (▶ **Fig. 2**). The ROESY correlations of H-23 to H-20 and of H-20 to Me-18 suggested a 23*R* configuration in compound **5**, while the correlations of H-23 to H-17 and of H-17 to Me-28 suggested a 23*S* configuration in compound **1** (▶ **Fig. 3**). In addition, the configuration of C-24 in compound **5** was proposed to be *S* by comparison of the chemical shifts and coupling constants of H-23 (5.07, d, J = 11.2) and H-24 (3.76, s) of **5** with 23*R*, 24*S* configuration analogue [H-23 (5.01, d, J = 11.1 Hz), H-24 (3.70, s)] [8]. The configuration at C-24 in **1** was finally confirmed by molecular modeling, in which θ = 73.8° in 23*R* 24*S* configuration, θ = 177.7° in 23*R* 24*R* configuration, θ = 168.9° in 23*S* 24*R* configuration, and θ = 61.1° in 23*S* 24*S* configuration (**Fig. 10S**, Supporting Information), the coupling constants of H-23 and H-24 were 10.9 Hz and 0 Hz, so the configuration of C-24 in **1** was proposed as *S* based on the function of dihedral angle and $^3J_{H\text{-}C\text{-}C\text{-}H}$. Also, this was consistent with other 15,16-seco-cycloartane derivatives reported previously [5–10]. Therefore, the structure of **1** was established as 15,16-seco-14-formyl-(23*S*, 24*S*)-16-oxohydroshengmanol-3-one (▶ **Fig. 1**) and given the name cimiheraclein E.

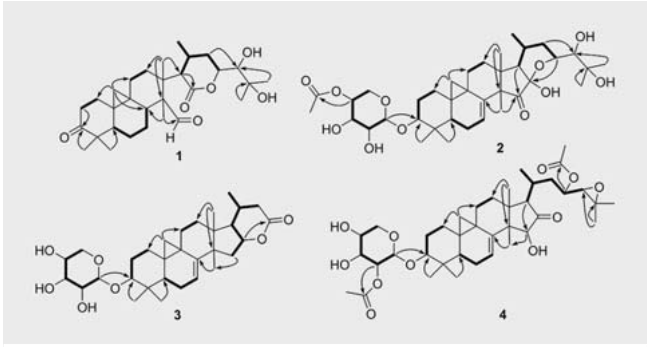
Compound **2** possessed a molecular formula of $C_{37}H_{56}O_{11}$ based on its HRTOF-ESIMS ion signal at *m/z* 699.3715 $[M + Na]^+$ (calcd for 699.3720). Its ^1H NMR spectrum displayed resonances for cyclopropane methylene protons at δ_H 0.50 (1H, d, J = 4.0 Hz) and 1.03 (1H, d, J = 4.0 Hz) for CH_2-19, six tertiary methyl singlets at δ_H 1.06, 1.27, 1.35, 1.61, 1.62, and 1.64 (each 3H, s), and one secondary methyl at δ_H 1.14 (3H, d, J = 6.3 Hz) as well as an anomeric proton signal at δ_H 4.86 (1H, d, J = 7.6 Hz), which is characteristic of a 9,19-cyclolanostane-type triterpene glycoside. The sugar obtained after acid hydrolysis was identified as L-arabinose by comparing its TLC mobility and specific rotation with those of a standard. Detailed analysis of its NMR data (▶ **Table 2**) indicated that **2** is a 16α-hydroxyl dahurinol-type triterpene glycoside and is similar to cimidahuside C [20]. The major difference was the acetyl substituent. The observed ^1H–^1H COSY correlation of δ_H 4.28 (1H, m, H-5′) to δ_H 5.26 (1H, brs, H-4′) and the HMBC correlation between δ_H 5.26 (1H, brs, H-4′) and δ_H 171.2 (-OAc) indicated the acetyl group was located at C-4′ of the sugar moiety (▶ **Fig. 2**). The significant ROESY associations (▶ **Fig. 3**) of H-3/H-5 and H-20/H-17 suggested a 3*S*, 23*R* configuration. The hydroxyl group at C-24 was confirmed as *S* by comparison of the chemical shifts and coupling constant of **2** with those of cimidahuside C [20]. Accordingly, the structure of **2** was determined as 16α-hydroxyl-7(8)-en-dahurinol-3-*O*-[4′-*O*-acetyl]-α-L-arabinopyranoside (▶ **Fig. 1**) and named as cimiheraclein F.

Compound **3** was isolated as a white powder and found to have the molecular formula $C_{31}H_{46}O_7$ on the basis of the HRTOF-ESIMS ion peak at *m/z* 553.3138 $[M + Na]^+$ (calcd for 553.3141). The NMR data (▶ **Table 2**) of **3** were very similar to those of 3β,11β-dihydroxy-24,25,26,27-tetranor-cycloart-7-en-23,16β-olide 3-*O*-β-D-xylopyranoside [21] except for the absence of the hydroxy group at C-11. The stereochemistry of **3** was determined from its ROESY spectrum (▶ **Fig. 3**). The crosspeaks of H-3 (δ_H 3.50, 1H, dd, J = 11.7, 4.1 Hz) with H-5 (δ_H 1.26, 1H, dd, J = 12.5, 5.1 Hz) and H-16 (δ_H 4.88, 1H, m) with H-17 (δ_H 1.92, 1H) and CH_3-28 (δ_H 1.06, 3H, s) indicated the β-orientation of the substituents at C-3 and C-16. Therefore, **3** was elucidated as 3β-hydroxy-24, 25, 26, 27-tetranor-cycloart-7(8)-en-23,16β-olide 3-*O*-β-D-xylopyranoside (▶ **Fig. 1**) and given the name cimiheraclein G.

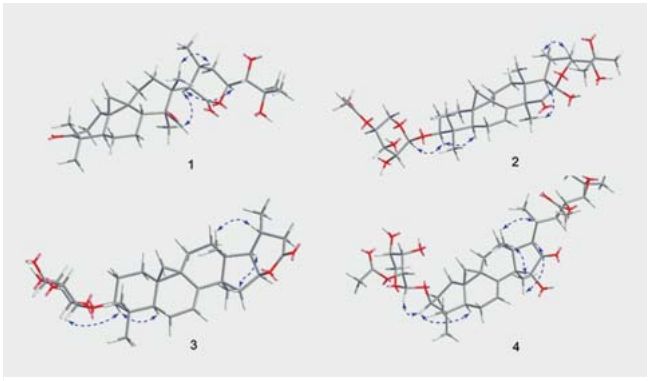
▶ **Table 1** The NMR data of compounds **1** and **5** (δ in ppm).

Position	1	5	Position	1	5
	δ_H[b]	δ_H[b]		δ_C[c]	δ_C[c]
1	1.81[a] 1.51[a]	1.82[a] 1.53[a]	1	32.2 t	32.2 t
2	2.73 m 2.37 brd	2.72 m 2.38 brd	2	37.4 t	37.4 t
3			3	215.2 s	215.1 s
4			4	50.3 s	50.2 s
5	1.82[a]	1.81[a]	5	45.4 d	45.4 d
6	1.36[a] 1.01[a]	1.38[a] 1.01[a]	6	19.2 t	19.2 t
7	1.42[a] 1.16 m	1.39[a] 1.16 m	7	22.0 t	22.0 t
8	2.47 m	2.44 dd (5.2 7.4)	8	39.2 d	38.9 d
9			9	22.1 s	22.0 s
10			10	25.7 s	25.7 s
11	1.64[a] 1.54[a]	1.64[a] 1.54[a]	11	27.2 t	27.1 t
12	1.62[a] 1.41[a]	1.62[a] 1.41 m	12	32.7 t	32.7 t
13			13	47.9 s	47.5 s
14			14	55.2 s	55.3 s
15	9.97 s	9.94 s	15	208.0 s	207.6 s
16			16	175.5 s	175.3 s
17	2.76 d (5.5)	2.74 d (5.6)	17	55.8 d	55.5 d
18	1.56 s	1.56 s	18	18.4 q	18.7 q
19	0.80 d (4.7) 0.24 d (4.7)	0.80 d (4.7) 0.24 d (4.7)	19	21.9 t	21.9 t
20	2.05[a]	2.06[a]	20	28.8 d	28.8 d
21	1.02 d (6.4)	0.99 d (6.2)	21	25.7 q	25.1 q
22	2.57 m 1.82[a]	2.07[a] 1.90 m	22	35.0 t	37.0 t
23	5.02 d (10.9)	5.07 d (11.2)	23	80.9 d	78.9 d
24	4.00 s	3.76 s	24	79.1 d	80.2 d
25			25	72.2 s	72.7 s
26	1.59 s	1.67 s	26	27.7 q	26.2 q
27	1.62 s	1.73 s	27	28.0 q	29.5 q
28	1.53 s	1.51 s	28	14.8 q	14.6 q
29	1.01 s	1.01 s	29	20.6 q	20.6 q
30	1.08 s	1.08 s	30	22.6 q	22.5 q

[a] Signals overlapped. [b] Recorded at 600 MHz in Pyridine-d_5. [c] Recorded at 150 MHz in Pyridine-d_5.



▶ **Fig. 2** Major HMBC (→) and ^{1}H–^{1}H COSY (–) correlations of compounds **1**–**4**.



▶ **Fig. 3** Key ROESY correlations of compounds **1**–**4**.

Compound **4** was also obtained as a white powder, and its molecular formula was $C_{39}H_{58}O_{11}$ based on its HRTOF-ESIMS ion signal at *m/z* 725.3877 $[M + Na]^+$ (calcd for 725.3877), which corresponds to 11 degrees of unsaturation. The NMR spectrum of **4** clearly displayed the signals characteristic of a 9,19-cycloartane-type triterpene. Direct analysis of its NMR data (▶ **Table 2**) indicated that compound **4** resembles a 23-*O*-acetyl-7,8-didehydroshengmanol-3-*O*-α-L-arabinopyranoside [22] except for the presence of one additional acetyl group. The location of the acetyl group at C-2′ (▶ **Fig. 2**) was identified by the HMBC correlation between H-2′ (δ_H 5.58, 1H, t, *J* = 8.3 Hz) and the carbonyl signal at δ_C 170.4. The similarity between the chemical shifts and coupling constants of C-23 and C-24 in compound **4** with those of 23-*O*-diacetyl-7,8-didehydroshengmanol-3-*O*-α-L-arabinopyranoside indicated the configurations of C-23 and C-24 were *R* and *S*, respectively. Finally, the structure of **4** was confirmed as 23-*O*-acetyl-7(8)-en-shengmanol-3-*O*-[2′-*O*-acetyl]-α-L-arabinopyranoside, as shown (▶ **Fig. 1**).

The new compounds (**1**–**4**) were evaluated for their cytotoxicities against HL-60, SMMC-7721, A549, MCF-7, and SW480 cell lines (▶ **Table 3**). Compounds **1** and **2** did not show cytotoxic activity with IC_{50} value > 40 μM. Compound **3** showed weak activity against A549 and MCF-7 cell lines with IC_{50} value 27.75 and 22.45 μM, respectively. Compound **4** also showed antitumor activity against the HL-60, A549, and MCF-7 cell lines with IC_{50} value 26.54, 36.98, and 21.34 μM, respectively.

The NMR, IR, UV, and HRTOF-ESIMS spectra of compounds **1**–**5**, as well as the dose-response curves of cytotoxic activity, are available as Supporting Information.

▶ **Table 2** The NMR data of compounds **2–4** (δ in ppm, J in Hz).

Position	2		3		4	
	δ_H[b]	δ_C[c]	δ_H[b]	δ_C[c]	δ_H[b]	δ_C[c]
1	1.78 m 1.23 m	30.7 t	1.66 m 1.33 m	30.7 t	1.51 m 1.37 m	32.4 t
2	2.33 m 1.98[a]	29.8 t	2.35 m 1.97 m	29.7 t	2.27 m 1.91 m	29.7 t
3	3.50 dd (11.6 4.0)	88.8 d	3.50 dd (11.7 4.1)	88.5 d	3.39 dd (11.4 4.1)	88.6 d
4		40.7 s		40.7 s		40.4 s
5	1.27 m	42.2 d	1.26 dd (12.5,5.1)	43.0 d	1.31[a]	42.8 d
6	2.00[a] 1.64[a]	22.1 t	1.87 m 1.55[a]	22.2 t	1.97 m 1.62 m	22.0 t
7	6.70 d (6.3)	117.3 d	5.10 d (6.8)	114.2 d	6.14 d (7.2)	115.3 d
8		142.6 s		149.2 s		147.6 s
9		21.8 s		21.3 s		21.8 s
10		28.8 s		28.7 s		28.7 s
11	2.12 m 1.04 m	25.1 t	2.06 m 1.15 m	25.3 t	2.20 m 1.76 m	25.5 t
12	1.91 m 1.45 m	31.6 t	1.63 m 1.55[a]	32.2 t	1.80 m	33.8 t
13		40.7 s		44.4 s		41.2 s
14		54.2 s		50.5 s		49.8 s
15		212.5 s	2.23 m 1.93 m	42.9 t	4.61 s	81.2 d
16		96.6 s	4.88[a]	81.1 d		220.8 s
17	2.17 m	57.8 d	1.92[a]	54.9 d	2.33 d (7.8)	60.4 d
18	1.27 s	23.3 q	1.07 s	23.1 q	1.44 s	19.7 q
19	1.03[a] 0.50 d (4.0)	28.1 t	1.07[a] 0.47 d (3.9)	28.7 t	0.86 d (4.0) 0.50 d (4.0)	28.1 t
20	1.97 m	26.7 d	1.93[a]	27.7 d	2.17 m	28.8 d
21	1.14 d (7.1)	23.4 q	0.99 d (5.9)	21.5 q	1.25 d (6.5)	20.1 q
22	2.45 m 1.69 m	33.0 t	2.46 dd (14.7 2.5) 2.28 dd (14.7 12.9)	39.1 t	2.91 m 1.74 m	37.8 t
23	4.66 dd (11.7 5.6)	77.0 d		174.3 s	5.45 m	72.3 d
24	3.62 brs	78.9 d			3.08 d (8.4)	65.6 d
25		73.3 s				58.9 s
26	1.61 s	26.8 q			1.28 s	25.1 q
27	1.62 s	29.0 q			1.45 s	19.2 q
28	1.64 s	25.0 q	1.06 s	27.1 q	1.30 s	22.1 q
29	1.35 s	26.0 q	1.37 s	26.1 q	1.13 s	25.8 q
30	1.06 s	14.5 q	1.08 s	14.6 q	1.02 s	14.4 q
1′	4.81 d (7.3)	107.9 d	4.88[a]	107.9 d	4.85 d (7.9)	104.9 d
2′	4.42 t (9.0)	73.6 d	4.08 m	75.9 d	5.58 t (8.3)	75.9 d
3′	4.29 m	72.7	4.19 t (8.8)	79.0 d	4.22[a]	76.6 d
4′	5.62 brs	72.7	4.27 m	71.6 d	4.23[a]	71.7 d
5′	4.28[a] 3.85 m	64.8 t	4.40 dd (11.2 5.2) 3.77 m	67.5 t	4.35 m 3.74 m	67.5 t
4′-OAc	2.01 s s	171.2 s 21.5 q				
23-OAc					2.06 s	171.0 s 21.3 q
2′-OAc					2.19 s	170.4 s 21.6 q

[a] Signals overlapped. [b] Recorded at 600 MHz in Pyridine-d_5. [c] Recorded at 150 MHz in Pyridine-d_5.

▶ **Table 3** Cytotoxic activities (IC_{50}, μM) of compounds **1–4** against five human cancer cell lines.[a]

Compounds	HL-60	SMMC-7721	A549	MCF-7	SW480
1	> 40	> 40	> 40	> 40	> 40
2	> 40	> 40	> 40	> 40	> 40
3	> 40	> 40	27.75 (26.44–29.06)	22.45 (21.25–23.65)	> 40
4	26.54 (25.51–27.57)	> 40	36.98 (35.88–38.08)	21.34 (20.37–22.31)	> 40
DDP	1.24 (1.19–1.29)	7.14 (7.08–7.20)	6.30 (6.23–6.37)	17.65 (16.25–19.05)	13.50 (12.40–14.60)

[a] DDP (cisplatin) was used as a positive control. All data are present as the mean of IC_{50} values with lower and upper 95 % CI from triplicate measurement (n = 3).

Materials and Methods

General experimental procedures

Optical rotations were recorded on a Horiba SEPA-300 polarimeter. UV spectra were acquired on a Shimadzu UV-2401A instrument. IR spectra were collected on Bruker Tensor 27 FTIR spectrometers with KBr pellets. NMR spectra were recorded on Bruker Avance III-600 spectrometers with tetramethylsilane as an internal standard at room temperature. HRTOF-ESIMS were recorded on an Agilent G6230 TOF spectrometer. TLC was performed on precoated TLC plates (200–250 µm thickness, silica gel 60 F_{254}, Qingdao Marine Chemical Inc.), and the spots were visualized by heating after spraying with 10% aqueous H_2SO_4. Semi-preparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Zorbax SB-C18 (5 µm, 9.4 mm × 250 mm, 3 mL/min) column.

Plant material

The aerial parts of *A. heracleifolia* were collected from Yichun County, Heilongjiang Province, China, in September 2012 and were identified by Prof. Zongyu Wang, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (ZDSQ20120901) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, China.

Extraction and isolation

The air-dried and powdered aerial parts of *A. heracleifolia* (10 kg) were extracted with MeOH (3 × 30 L × 24 h) at room temperature to give a residue (265 g) after concentration under vacuum at 50 °C. The extract was subjected to silica gel CC (200–300 mesh, 3 kg, 20 × 150 cm) and eluted with $CHCl_3$-MeOH [100 : 0 (2 L), 80 : 1 (5 L), 50 : 1 (5 L), 20 : 1 (8 L), and 10 : 1 (8 L)] to afford fractions A (3 g), B (5 g), C (2.5 g), D (120 g), and E (10 g). Fraction B was divided into four sub-fractions (B.1–B.4) by RP-18 CC (20–45 µm, 250 g, 5 × 50 cm) eluted with MeOH-H_2O (gradient from 50 : 50 to 100 : 0, 20 L). Fraction B.3 (2.5 g) was subjected to repeated silica gel columns (200–300 mesh, 50 g, 5 × 25 cm) eluting with $CHCl_3$-Me_2CO (gradient from 20 : 1 to 5 : 1, 5 L) and then purified by semi-preparative HPLC (eluting with CH_3CN-H_2O, gradient from 50 : 50 to 75 : 25, 3.0 mL/min, 40 min, 210 nm) to give compounds **1** (2 mg), **5** (4 mg), **8** (4 mg), and **9** (2 mg). Compounds **2** (9 mg), **3** (4 mg), **4** (8 mg), **6** (10 mg), and **7** (7 mg) were isolated from fraction C by silica gel CC (200–300 mesh, 50 g, 5 × 25 cm) eluted with $CHCl_3$-Me_2CO (15 : 1 to 5 : 1, 5 L) followed by repeated semi-preparative HPLC (eluted with CH_3CN-H_2O, gradient from 50 : 50 to 75 : 25, 3.0 mL/min, 40 min, 210 nm).

Physicochemical properties of 1–4

Cimiheraclein E (**1**): white powder; $[\alpha]_D^{25}$ = − 69.25 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.89) nm; IR (KBr) ν_{max} 3440, 2942, 2871, 1700, 1632, 1457, 1380, 1032, 977 cm^{-1}; ^1H and ^{13}C NMR data (C_5D_5N) see ▶ **Table 1**; HRTOF-ESIMS *m/z* 525.3180 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_6Na$, 525.3192).

Cimiheraclein F (**2**): white powder; $[\alpha]_D^{25}$ = − 10.49 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.78), 268 (3.07) nm; IR (KBr) ν_{max} 3440, 2964, 2872, 1734, 1632, 1456, 1383, 1055, 1001 cm^{-1}; ^1H and ^{13}C NMR data (C_5D_5N) see ▶ **Table 2**; HRTOF-ESIMS *m/z* 699.3715 $[M + Na]^+$ (calcd for $C_{37}H_{56}O_{11}Na$, 699.3720).

Cimiheraclein G (**3**): white powder; $[\alpha]_D^{25}$ = − 80.57 (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ε) 203 (4.00) nm; IR (KBr) ν_{max} 3440, 2964, 2869, 1721, 1631, 1455, 1382, 1045, 969 cm^{-1}; ^1H and ^{13}C NMR data (C_5D_5N) see ▶ **Table 2**; HRTOF-ESIMS *m/z* 553.3138 $[M + Na]^+$ (calcd for $C_{31}H_{46}O_7Na$, 553.3141).

23-O-Acetyl-7(8)-en-shengmanol-3-O-[2′-O-acetyl]-α-L-arabinopyranoside (**4**): white powder; $[\alpha]_D^{25}$ = − 49.33 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.96) nm; IR (KBr) ν_{max} 3434, 2926, 2853, 1738, 1631, 1459, 1379, 1044, 989 cm^{-1}; ^1H and ^{13}C NMR data (C_5D_5N) see ▶ **Table 2**; HRTOF-ESIMS *m/z* 725.3877 $[M + Na]^+$ (calcd for $C_{39}H_{58}O_{11}Na$, 725.3877)

Hydrolysis and identification of the sugar moieties in compounds 2 and 4

Compounds **2** and **4** (2.5 mg of each) were dissolved in methanol (3 mL) and refluxed with 1.0 N HCl (2 mL) at 90 °C for 2 h. After neutralizing with 1.0 N NaOH, the reaction mixtures were extracted with $CHCl_3$, and the aqueous layers were concentrated under reduced pressure to give the monosaccharides, which had *Rf* values (EtOAc/$CHCl_3$/MeOH/H_2O, 3 : 2 : 2 : 1) and specific rotations ($[\alpha]_D^{20}$ + 82.78, *c* 0.05, MeOH) that were consistent with those of L-arabinopyranose (Sigma-Aldrich).

Biological assays

Cytotoxic activity was investigated using five human cancer cell lines, human leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A549, breast cancer MCF-7, and colon cancer SW480 (Cell Bank of Chinese Academy of Sciences). Cells were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in RPMI-1640 medium (HyClone) supplemented with 10% (v/v) FBS (HyClone) and dispersed in identical 96-well plates. Compounds were dissolved in DMSO and serially diluted in saline to give final DMSO concentrations below 1%. Each tumor cell line was exposed to the test compounds at concentrations of 0.064, 0.32, 1.6, 8, and 40 μM for 48 h with cisplatin (DPP; Sigma, > 98%) as the positive control; cell viability was determined by MTT cytotoxicity assay by measuring the absorbance at 570 nm with a microplate reader (Bio-Rad 680) [23]. Three independent trials were conducted for each compound (n = 3). The IC_{50} values and 95% confidence interval (CI) were estimated using GraphPad Prism 6.

Supporting Information

The NMR, IR, UV, and HRTOF-ESIMS spectra of compounds **1**–**5**, as well as the dose-response curves of cytotoxic activity, are available as Supporting Information.

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Conflict of Interest

The authors declare no conflicts of interest.

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