Triterpenoids and Steroids with Cytotoxic Activity from *Emmenopterys henryi*

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Key words
- *Emmenopterys henryi*
- Rubiaceae
- ursane-type triterpenoids
- pregnane derivatives
- cytotoxic activity

Abstract

Two new ursane-type triterpenoids, 3*β*,19*α*,23-trihydroxyurs-12-en-24-al-28-oic acid (**1**) and 3*β*,19*α*,24-trihydroxy-23-norurs-12-en-28-oic acid (**2**), two new pregnane derivatives, 3*β*,12*β*-dihydroxy-5*α*-pregnane-14,16-dien-20-one (**9**) and 12*β*-hydroxy-5*α*-pregnane-14,16-dien-3,20-dione (**10**), and eight known compounds were isolated from the twigs and leaves of *Emmenopterys henryi*. The structures of the new compounds were elucidated on the basis of extensive spectroscopic analysis, including 1D and 2D NMR experiments. Compounds **4, 11**, and **12** showed cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW-480 cell lines with IC_{50} values in the range of 3.11–20.12 μM.

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Introduction

Emmenopterys henryi Oliv., a monotypic genus plant of the Rubiaceae family, is indigenous to western and southwestern parts of China [1]. Its roots and barks have been used in traditional Chinese medicine for the treatment of nausea, vomiting, bruises, and injuries from falls [2]. Previous chemical investigations of this plant led to the isolation of a few coumarins, triterpenoids, and steroids [3]. As part of our continuing efforts to discover more naturally occurring bioactive metabolites from the monotypic genus species endemic to China [4–7], a 95% EtOH extract of *E. henryi* was investigated, which led to the isolation of two new ursane-type triterpenoids, 3*β*,19*α*,23-trihydroxyurs-12-en-24-al-28-oic acid (**1**) and 3*β*,19*α*,24-trihydroxy-23-norurs-12-en-28-oic acid (**2**), two new pregnane derivatives, 3*β*,12*β*-dihydroxy-5*α*-pregnane-14,16-dien-20-one (**9**) and 12*β*-hydroxy-5*α*-pregnane-14,16-dien-3,20-dione (**10**), and eight known compounds (**Fig. 1**). Among them, compounds **9** and **10** are two novel C-21 steroids with cyclopentadiene ring (D ring). Herein, we described the isolation, structural elucidation, and the cytotoxicity evaluation of these isolates.

Results and Discussion

The 95% EtOH extract of the twigs and leaves of *E. henryi* was partitioned between EtOAc and water. The EtOAc fraction was subjected repeatedly to column chromatography over silica gel, RP-18, Sephadex LH-20, and semipreparative HPLC to afford two new triterpenoids (**1** and **2**), two new pregnane derivatives (**9** and **10**), and eight known compounds (**Fig. 1**). The known compounds were identified as 3*β*,19*α*,23,24-tetrahydroxyurs-12-en-28-oic acid (**3**) [8], pomolic acid (**4**) [9], 3*β*,6*β*,19*α*,23-tetrahydroxyurs-12-en-28-oic acid (**5**) [10], 3*β*,6*β*,23-trihydroxyolean-12-en-28-oic acid (**6**) [11], 3*β*,6*β*,19*α*,23-tetrahydroxyolean-12-en-28-oic acid (**7**) [11], 3*β*,23,24-trihydroxyolean-12-en-28-oic acid (**8**) [12, 13], 3*β*,12*β*-dihydroxy-5*α*-pregnane-16-en-20-one (**11**) [14], and 12*β*-dihydroxy-5*α*-pregnane-16-en-3,20-dione (**12**) [15], respectively, by comparison of their spectroscopic data with those reported in the literature. All known compounds were isolated from this species for the first time.

Compound **1**, a white powder, had the molecular formula $C_{30}H_{46}O_6$ according to the positive HR-ESI-MS at *m/z* 525.3189 $[M + Na]^+$ (calcd. 525.3192), corresponding to eight degrees of unsaturation. The IR spectrum showed absorption bands for hydroxy (3422 cm^{-1}), carbonyl

Table 1 ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) data of **1** and **2** in CD_3OD (δ in ppm, J in Hz).

No.	1		2	
	δ_H	δ_C	δ_H	δ_C
1a	1.78 (m)	38.7 t	1.66 (overlap)	39.6 t
1b	1.09 (m)		0.98 (m)	
2a	2.05 (m)	27.8 t	1.76 (m)	27.4 t
2b	1.88 (m)		1.66 (overlap)	
3	3.81 (dd, 12.0, 5.2)	72.1 d	3.84 (m)	75.1 d
4		58.4 s	2.09 (m)	50.0 d
5	1.40 (d, 12.2)	50.9 d	1.26 (m)	49.3 d
6a	1.68 (m)	19.7 t	1.70 (m)	25.2 t
6b	1.29 (m)		1.32 (overlap)	
7a	1.58 (m)	33.6 t	1.68 (m)	33.7 t
7b	1.28 (m)		1.32 (overlap)	
8		40.7 s		41.5 s
9	1.77 (m)	47.4 d	1.71 (m)	47.5 d
10		37.6 s		37.0 s
11a	2.06 (m)	25.1 t	2.01 (m)	24.9 t
11b	1.92 (m)		1.96 (m)	
12	5.29 (t like, 3.5)	129.2 d	5.30 (t like, 3.5)	129.3 d
13		140.1 s		140.0 s
14		42.7 s		42.6 s
15a	1.78 (m)	29.6 t	1.81 (m)	29.6 t
15b	1.00 (m)		1.53 (m)	
16a	2.57 (dt, 13.2, 4.5)	26.6 t	2.59 (dt, 13.2, 4.5)	26.6 t
16b	1.51 (m)		1.50 (m)	
17		48.9 s		48.5 s
18	2.50 (br s)	55.1 d	2.51 (br s)	55.1 d
19		73.5 s		73.5 s
20	1.34 (m)	43.1 d	1.36 (m)	43.0 d
21a	1.72 (m)	27.2 t	1.74 (overlap)	27.2 t
21b	1.29 (m)		1.35 (m)	
22a	1.73 (m)	38.9 t	1.74 (overlap)	39.0 t
22b	1.61 (m)		1.64 (m)	
23a	3.88 (d, 11.0)	62.3 t	3.94 (d, 11.2)	61.0 s
23b	3.74 (d, 11.0)		3.59 (d, 11.2)	
24a	9.99 (s)	208.0 d		
24b				
25	0.83 (s)	16.7 q	0.81 (s)	15.5 q
26	0.76 (s)	17.4 q	0.81 (s)	17.4 q
27	1.34 (s)	24.7 q	1.36 (s)	24.8 q
28		182.7 s		182.2 s
29	1.19 (s)	27.0 q	1.21 (s)	27.0 q
30	0.93 (d, 6.6)	16.6 q	0.94 (d, 6.6)	16.6 q

(1700 cm^{-1}), and olefinic (1647 cm^{-1}) functional groups. The ^{1}H-NMR spectrum (**Table 1**) showed characteristic signals for an olefinic proton (δ_H 5.29 [1H, t like, J = 3.5 Hz, H-12]), an oxymethine proton (δ_H 3.81 [1H, dd, J = 12.0, 5.2 Hz, H-3]), an aldehyde proton (δ_H 9.99 [1H, s, H-24]), two oxymethylene protons (δ_H 3.88, 3.74 [2H, d, J = 11.0 Hz, H-23a and H-23b]), a secondary methyl group (δ_H 0.93 [3H, d, J = 6.6 Hz, H_3-30]), and four tertiary methyl groups. The ^{13}C- and DEPT- NMR data of **1** (**Table 1**) showed signals for 30 carbons, including one trisubstituted double bond (δ_C 129.2, 140.1), five methyls, ten methylenes (one oxygenated), five methines (one oxygenated), and eight quaternary carbons (one oxygenated, one aldehyde, and one carboxylic acid carbons). The above data suggested that **1** was an ursane-type triterpenoid with a hydroxymethyl and an aldehyde groups. The ^{1}H- and ^{13}C-NMR data (**Table 1**) resembled closely those of co-occurring 3β,19α,23,24-tetrahydroxyurs-12-en-28-oic acid (**3**) [8], except for the replacement of one hydroxymethyl group at C-4 in **3** by an aldehyde group in **1**, as deduced from the HMBC correlations (**Fig. 2**) of H-24 with C-3 (δ_C 72.1), C-4 (δ_C 58.4), C-5 (δ_C 50.9), C-23 (δ_C 62.3). Detailed 2D NMR analysis established the plane structure of **1** as shown in **Fig. 1**.

The relative configuration of **1** was deduced from the ROESY spectrum (**Fig. 2**). From the biosynthetic point of view, H-5 was assumed to be α-oriented [3]. The ROESY correlations of H-3/H-5, H-5/H_2-23, H-5/H-9, H-9/H_3-27 revealed the α-orientation of H-3, H-9, H_3-27, and the hydroxymethyl group. Meanwhile, the cross peaks of H-24/H_3-25, H_3-25/H_3-26, H-18/H_3-29, and H-20/H_3-29 in ROESY spectrum indicated that these protons were β-oriented. Thus, compound **1** was assigned to be 3β,19α,23-trihydroxyurs-12-en-24-al-28-oic acid.

Compound **2** had the molecular formula $C_{29}H_{46}O_5$ based on the positive HR-ESI-MS at m/z 497.3233 $[M + Na]^+$ (calcd. 497.3242), indicating seven degrees of unsaturation. Comparison of the NMR spectroscopic data of **2** (**Table 1**) with those of 2β,3β,19α,24-tetrahydroxy-23-norurs-12-en-28-oic acid showed many similarities [16], except that the oxymethine signals at C-2

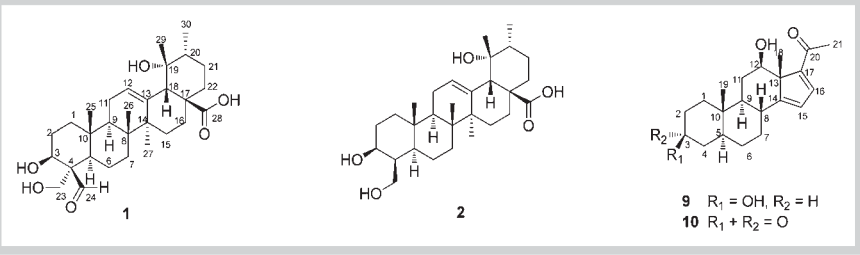

Fig. 1 Chemical structures of new compounds **1, 2, 9**, and **10** isolated from *E. henryi*.

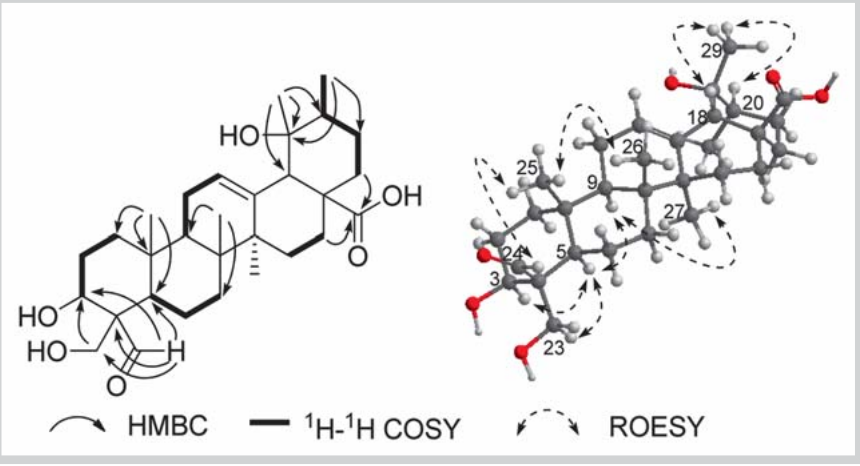

Fig. 2 Key 2D NMR correlations of compound **1**. (Color figure available online only.)

was missing and a methylene unit was present in **2**. This was supported by the key correlations from H_2-2 (δ_H 1.66, 1.76, m, each 1H) to C-1 (δ_C 39.6), C-3 (δ_C 75.1), C-4 (δ_C 50.0), and C-10 (δ_C 37.0) in the HMBC experiment, as well as the ^{1}H-^{1}H COSY correlations of H_2-1/H_2-2/H-3/H-4/H_3-24. The *β*-orientation of 3-OH and the hydroxymethyl was deduced from the ROESY correlations of H-3/H-5, H-5/H-9, and H_2-24/H_3-25. Hence, compound **2** was established to be 3*β*,19*α*,24-trihydroxy-23-norurs-12-en-28-oic acid.

The molecular formula of compound **9** was determined as $C_{21}H_{30}O_3$ on the basis of the HR-EI-MS at *m/z* 330.2199 $[M]^+$ (calcd. 330.2195), implying seven degrees of unsaturation. The IR spectrum showed absorption at 1636, 1647, and 3422 cm^{-1} indicating the presences of hydroxy, carbonyl, and double bond groups. The ^{13}C- and DEPT NMR data of **9** (**Table 2**) exhibited 21 carbon signals, including three methyls, six methylenes, seven methines (two oxygenated and two olefinic ones), and five quaternary carbons (one carbonyl and two olefinic ones). The 1D NMR data of **9** were similar to those of co-occurring 3*β*,12*β*-dihydroxy-5*α*-pregnane-16-en-20-one (**11**) [14], and the obvious difference was the presence of an additional double bond (δ_C 122.1, 173.6) in **9**. The double bond was located at C-14 and C-15, as inferred from the ^{1}H-^{1}H COSY correlations (**Fig. 3**) between H-15 and H-16 and the HMBC correlations (**Fig. 3**) from H-15 (δ_H 6.20, d, J = 2.4 Hz) to C-8 (δ_C 36.6), C-14 (δ_C 173.6), C-16 (δ_C 148.6), and C-17 (δ_C 154.9). The ROESY correlations (**Fig. 3**) of H-3/H-5, H-5/H-9, and H-9/H-12 suggested that such groups were on the same side of the molecule, and they were arbitrarily assigned as *α*-oriented. In addition, H-8, H_3-18, and H_3-19 were on the other side of the structure and established as *β*-oriented based on the ROESY correlations of H-8/H_3-18 and H-8/H_3-19. Accordingly, compound **9** was deduced to be 3*β*,12*β*-dihydroxy-5*α*-pregnane-14,16-dien-20-one.

The positive HR-EI-MS of **10** exhibited a molecular ion peak at *m/z* 328.2041 $[M]^+$ (calcd. 328.2038), suggesting a molecular formula of $C_{21}H_{28}O_3$, with eight degrees of unsaturation. Comparison of the 1D NMR data of **10** (**Table 2**) with those of **9**, demonstrated that the oxymethine at C-3 in **9** was replaced by a carbonyl group (δ_C 213.9) in **10**. This assumption was subsequently confirmed by the HMBC correlations from H_2-1 (δ_H 1.42, 2.12, m, each 1H), H_2-2 (δ_H 2.28, 2.57, m, each 1H), and H-5 (δ_H 1.66) to the carbonyl carbon. Therefore, compound **10** was elucidated to be 12*β*-hydroxy-5*α*-pregnane-14,16-dien-3,20-dione. Structurally, compounds **9** and **10** are two novel pregnane derivatives with a cyclopentadiene D ring, which were isolated from natural resources for the first time. To the best of our knowledge, only several pregnane derivatives with cyclopentadiene D ring were obtained by chemical transformation from other steroids [17, 18].

All compounds were evaluated for their cytotoxicity against HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines using the methyl thiazolyl tetrazolium (MTT) method as previously reported [19], with cisplatin as the positive control. Compounds **11** and **12** showed significant cytotoxicity against above five cancer cell lines, and compound **4** exhibited moderate cytotoxicity (**Table 3**). The other compounds were inactive with IC_{50} values of more than 40 μM. Previously, compound **12** and some related pregnane derivatives with *α,β*-unsaturated carbonyl moiety at C-16, C-17, and C-20 have been reported as cytotoxic [15, 20]. Our current study and the previous reported results suggested that the *α,β*-unsaturated carbonyl unit at C-16, C-17, and C-20 should be an important feature responsible for the cytotoxicity of this kind of pregnane derivatives.

Table 2 ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) data of **9** and **10** in CD_3OD (δ in ppm, J in Hz).

No.	9		10	
	δ_H	δ_C	δ_H	δ_C
1a	1.77 (m)	39.5 t	2.12 (m)	39.8 t
1b	1.02 (m)		1.42 (dt, 13.9, 4.9)	
2a	1.78 (m)	32.0 t	2.57 (dt, 13.9, 6.6)	38.8 t
2b	1.44 (m)		2.28 (m)	
3	3.51 (m)	71.6 d		213.9 s
4a	1.59 (m)	38.6 t	2.46 (m)	45.2 t
4b	1.32 (m)		2.11 (m)	
5	1.17 (m)	45.7 d	1.60 (m)	47.4 d
6a	1.52 (m)	29.2 t	1.57 (m, 2H)	29.9 t
6b	1.44 (m)			
7a	2.03 (m)	30.3 t	2.10 (m)	29.3 t
7b	1.41 (m)			
8	2.31 (m)	36.6 d	2.41 (m)	36.5 d
9	0.65 (dt, 12.6, 3.4)	53.2 d	0.79 (dt, 12.5, 3.4)	52.7 d
10		37.0 s		37.1 s
11a	1.82 (m)	30.1 t	1.89 (m)	30.3 t
11b	1.53 (m)		1.57 (m)	
12	2.96 (dd, 11.2, 4.4)	75.9 d	3.01 (dd, 11.2, 4.4)	75.8 d
13		60.3 s		60.3 s
14		173.6 s		172.9 s
15	6.20 (d, 2.4)	122.1 d	6.25 (d, 2.5)	122.3 d
16	7.62 (d, 2.4)	148.6 d	7.65 (d, 2.5)	148.4 d
17		154.9 s		155.1 s
18	1.03 (s)	14.3 q	1.11 (s)	14.3 q
19	0.96 (s)	12.6 q	1.20 (s)	11.6 q
20		198.3 s		198.4 s
21	2.42 (s)	26.3 q	2.46 (s)	26.3 q

Materials and Methods

General experimental procedures

Optical rotations were measured with a Horiba SEPA–300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. IR spectra were obtained by a Tensor 27 spectrophotometer with KBr pellets. 1D and 2D spectra were run on a Bruker AM-400 or an Avance III 600 spectrometer with TMS as the internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. ESI-MS and HR-ESI-MS were performed on an API QSTAR time-of-flight spectrometer. EI-MS and HR-EI-MS were recorded on a Waters AutoSpec Premier P776 spectrometer. Semi-preparative HPLC was performed on an Agilent 1200 apparatus equipped with a UV detector and a Zorbax SB-C-18 (Agilent, 9.4 mm × 25 cm) column. MPLC was performed on a Lisui EZ Purify III system including pump manager P03, detector modules P02, and fraction collector P01 (Shanghai Li Sui Chemical Engineering Co., Ltd.). Column chromatography (CC) was performed using silica gel (Qingdao Marine Chemical Co. Ltd.), RP-18 gel (40–63 µm; Merck), and Sephadex LH-20 (Amersham Pharmacia Biotech). All isolated compounds had a degree of purity more than 95% based on TLC, HPLC, and NMR methods.

Plant material

The twigs and leaves of *E. henryi* were collected from Kunming Botany Garden, Yunnan province, People's Republic of China, in December 2010, and identified by one of the authors (Prof. X. Gong), Kunming Institute of Botany. A voucher specimen (KIB20090911e) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The air-dried and powdered twigs and leaves of *E. henryi* (15.0 kg) were extracted three times with 95% EtOH (50 L × 3) at room temperature and concentrated in vacuo to yield a residue, which was suspended in water (3 L) and then extracted with EtOAc (6 L × 3). The EtOAc fraction (750 g) was subjected to silica gel CC (100–200 mesh, 10.0 × 150.0 cm, 4 kg) with a gradient elution of petroleum ether–Me_2CO (1 : 0, 9 : 1, 8 : 2, 7 : 3, 6 : 4, 0 : 1, each 40 L) to afford five fractions (A–E). Fraction D (80 g) was separated by MPLC (RP-18, 15.0 × 110.0 cm, 1 kg) eluting with MeOH–H_2O (65 : 35, 70 : 30, 75 : 25, 80 : 20, 85 : 15, 90 : 10, 95 : 5, 100 : 0, each 6 L, 50 mL/min) to provide seven fractions (D1–D7). Fraction D2 (3.1 g) was subjected to silica gel CC (200–300 mesh, 3.0 × 40.0 cm, 50 g), eluted with $CHCl_3$–Me_2CO (20 : 1, 10 : 1, 9 : 1, 8 : 2, 7 : 3, each 600 mL), then followed by Sephadex LH-20 (2.0 × 135.0 cm, 80 g, MeOH) to obtain **3** (20 mg), **5** (19 mg), and **7** (50 mg). Fraction D3 (2.1 g) was chromatographed over silica gel (200–300 mesh, 3.0 × 40.0 cm, 35 g) to provide four fractions (D3.1–D3.4) eluting with $CHCl_3$–Me_2CO (10 : 1, 9 : 1, 8 : 2, each 350 mL). Compounds **10** (3 mg, t_R 18.6 min) and **12** (7 mg, t_R 22.4 min) were afforded from fraction D3.1 (180 mg) by semi-preparative HPLC (MeOH–H_2O, 63 : 37, 3 mL/min). Compound **1** (20 mg) was obtained from fraction D3.2 (90 mg) by Sephadex LH-20 (1.4 × 100 cm, 50 g, $CHCl_3$: MeOH = 1 : 1). Fraction D3.3 (200 mg) was further chromatographed over silica gel (200–300 mesh, 1.5 × 25.0 cm, 17 g), using $CHCl_3$–MeOH (20 : 1, 400 mL) as eluent and then purified by semipreparative HPLC (MeOH–H_2O, 60 : 40, 3 mL/min) to yield **9** (5 mg, t_R 18.6 min) and **11** (8 mg, t_R

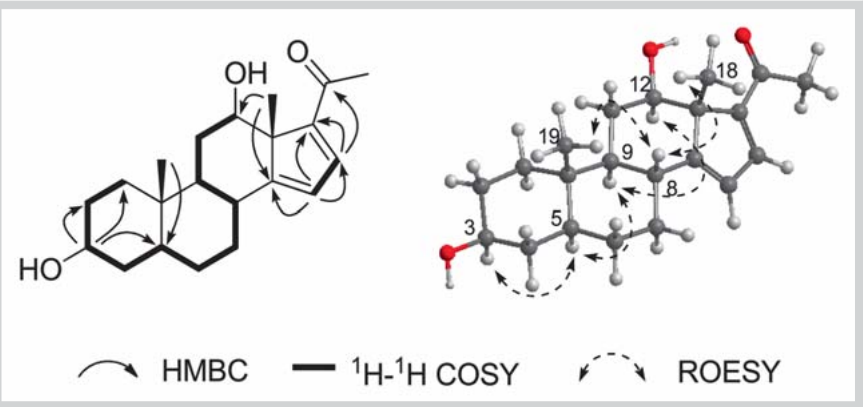

Fig. 3 Key 2D NMR correlations of compound **9**. (Color figure available online only.)

Compound	HL-60	SMMC-7721	A-549	MCF-7	SW-480
4	20.12	16.13	15.58	17.56	15.32
11	4.74	4.21	3.11	3.28	3.75
12	10.53	5.59	10.39	5.39	9.56
Cisplatin[a]	1.25	16.18	14.05	16.95	18.05

[a] Positive control

Table 3 Cytotoxic activities of compounds **4, 11**, and **12** against tumor cell lines with IC_{50} values.

18.6 min). Fraction D4 (4.4 g) was applied to Sephadex LH-20 (3.0 × 150 cm, 180 g) eluted with $CHCl_3$: MeOH (1 : 1) to give five fractions (D4.1–D4.5). Fraction D4.2 (180 mg) was further subjected to silica gel CC (200–300 mesh, 1.5 × 30.0 cm, 20 g) with petroleum ether–Me_2CO (9 : 1, 8 : 2, 7 : 3, each 200 mL) and $CHCl_3$–Me_2CO (9 : 1, 500 mL) as eluent, respectively, to furnish **2** (3 mg) and **6** (28 mg). Fraction D5 (1.4 g) was fractionated by silica gel (200–300 mesh, 2.0 × 35.0 cm, 25 g) with elution of petroleum ether–EtOAc (7 : 3, 700 mL) and $CHCl_3$–Me_2CO (15 : 1, 300 mL), respectively, to afford **4** (18 mg) and **8** (12 mg).

Isolates

3β,19α,23-Trihydroxyurs-12-en-24-al-28-oic acid (**1**): white powder; mp 178–182 °C; $[\alpha]_D^{23.0}$ + 59.50 (*c* 0.24, MeOH); IR (KBr) ν_{max} 3422, 2933, 1700, 1647, 1541, 1457, 1386, 1286, 1117, 1074, 1046, 956, 767 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see **Table 1**; positive ESI-MS: *m/z* 525 $[M + Na]^+$; positive HR-ESI-MS $[M + Na]^+$ *m/z* 525.3189 (calcd. for $C_{30}H_{46}O_6Na$, 525.3192).

3β,19α,24-Trihydroxy-23-norurs-12-en-28-oic acid (**2**): white powder; mp 222–226 °C; $[\alpha]_D^{23.4}$ + 29.09 (*c* 0.11, MeOH); IR (KBr) ν_{max} 3422, 2929, 1717, 1697, 1541, 1457, 1375, 1288, 1123, 1076, 1039, 956, 767 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see **Table 1**; positive ESI-MS: *m/z* 497 $[M + Na]^+$; positive HR-ESI-MS $[M + Na]^+$ *m/z* 497.3233 (calcd. for $C_{29}H_{46}O_5Na$, 497.3242).

3β,12β-Dihydroxy-5α-pregnane-14,16-dien-20-one (**9**): colorless oil; $[\alpha]_D^{23.9}$ + 331.74 (*c* 0.49, MeOH); UV (MeOH) λ_{max} (log ε): 314 (4.34) nm; IR (KBr) ν_{max} 3422, 2925, 2853, 1733, 1647, 1636, 1541, 1473, 1316, 1045, 780 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see **Table 2**; EI-MS: *m/z* 330 $[M]^+$ (100), 315 (28), 287 (18), 161 (18), 136 (31), 57 (70); HR-EI-MS $[M]^+$ *m/z* 330.2199 (calcd. for $C_{21}H_{30}O_3$, 330.2195).

12β-Hydroxy-5α-pregnane-14,16-dien-3,20-dione (**10**): colorless oil; $[\alpha]_D^{22.8}$ + 387.18 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε): 313 (4.13) nm; IR (KBr) ν_{max} 3421, 2938, 2865, 1713, 1624, 1519, 1381, 1253, 065, 858, 647 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see **Table 2**; ESI-MS: *m/z* 351 $[M + Na]^+$; HR-EI-MS $[M]^+$ *m/z* 328.2041 (calcd. for $C_{21}H_{28}O_3$, 328.2038).

Cytotoxicity assay

The cytotoxicity of compounds **1**–**12** against human myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW-480) cell lines was assessed using the MTT method [19]. Cells were plated in 96-well plates 12 h before treatment and continuously exposed to different concentrations of compounds. After 48 h, 20 µL of MTT solution were added to each well, which were incubated for another 4 h. Then 20% SDS (100 µL) were added to each well. After 12 h at room temperature, the OD value of each well was recorded at 595 nm. The IC_{50} value of each compound was calculated by the Reed and Muench method [21]. Cisplatin (Sigma, 99% purity) was used as a positive control.

Supporting information

1D and 2D NMR spectra, UV, IR, HR-ESI-MS, and HR-EI-MS spectra of the four new compounds, and structures of known compounds are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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