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Bioactive triterpenoids from the traditional Chinese medicine *Swertia mileensis*

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ABSTRACT

Phytochemical investigation on the whole plant of *Swertia mileensis* led to the isolation of two new triterpenoids, including a 28-*nor*-oleanane (**1**) and a 3,4-*seco*-3-*nor*-oleanane (**2**), together with 21 known triterpenoids (**3**–**23**). Their structures were elucidated by extensive spectroscopic analysis. Compounds **2** and **10** exhibited anti-inflammatory activity by inhibiting the secretion of cytokines TNF-α and IL-6 in RAW264.7 cells induced by LPS with IC_{50} values ranging from 32.8 to 69.8 μM. Compounds **1** and **17** showed cytotoxicity against HepG2 and MDA-MB-231 cells with inhibitory rates ranging from 30.34 % to 47.18 % at 10 μM.

1. Introduction

Swertia mileensis, belonging to the family Gentianaceae, is mainly distributed in southwest China (Geng et al., 2010). The whole plant of *S. mileensis* has been documented in the *Chinese Pharmacopoeia* as a traditional Chinese medicine under the name "Qing-Ye-Dan" to treat liver and gallbladder diseases especially viral hepatitis. Previous phytochemical investigation of *S. mileensis* revealed the existence of iridoids, xanthones, flavonoids, and triterpenoids, which showed various biological activities including anti-HBV, anti-cirrhosis and anti-inflammatory effects (Geng et al., 2013).

During our continuous effort to search for novel bioactive terpenoids from medicinal plants, two new oleanane triterpenoids (**1** and **2**) and 21 known analogs (**3**–**23**) were isolated from the whole plant of *S. mileensis* (Fig. 1). Selected isolates were evaluated for their anti-inflammatory and antitumor activities. Herein, the isolation, structural elucidation and biological activities of these triterpenoids are described.

2. Results and discussion

Compound **1** was isolated as a white powder with a molecular formula $C_{30}H_{48}O_3$ as established by HRESIMS (*m/z* 455.3530 $[M - H]^-$, calcd. 455.3530). The ^1H NMR spectrum of **1** (Table 1) displayed seven tertiary methyls [δ_H 0.79 (3 H, s, H_3-24), 0.89 (3 H, s, H_3-26), 0.91 (3H, s, H_3-29), 0.93 (3H, s, H_3-25), 1.00 (3H, s, H_3-23), 1.01 (3H, s, H_3-30), 1.15 (3H, s, H_3-27)], a formyloxy group [δ_H 8.02 (s)], a tri-substituted double bond [δ_H 5.26 (1H, t, J = 3.7 Hz)], and an oxygenated methine [δ_H 3.22 (1H, dd, J = 11.3, 4.3 Hz)]. The ^{13}C NMR and DEPT spectra of **1** (Table 1) revealed 30 carbon resonances, including seven methyls, 10 methylenes, six methines [including one oxygenated (δ_C 79.1) and one olefinic (δ_C 123.5)], and seven quaternary carbons [including one oxygenated (δ_C 87.5) and one olefinic (δ_C 143.1)]. All the aforementioned spectroscopic data suggested that **1** was an oleanane triterpenoid similar to 3β-hydroxy-olean-12-en-28-oic acid (**7**) (Carvaiho and Seita, 1993). **1** differed from **7** by the absence of the carboxylic group and presence of a formyloxy group (δ_C 160.7). The HMBC correlation from the formyloxy proton (δ_H 8.02) to the oxygenated carbon (δ_C 87.5, C-17) (Fig. 2) indicated a formyloxy group, instead of a carboxylic group, at C-17. Accordingly, the planar structure of **1** was assigned, which was further supported by the ^1H–^1H coupling relationships (H_2-1/H_2-2/H-3, H-5/H_2-6/H_2-7, H-9/H_2-11/H-12, H_2-15/H_2-16, H-18/H_2-19, and H_2-21/H_2-22) and HMBC correlations (from H_2-2 to C-3/C-5/C-10, H-3 to C-2/C-4/C-23, H-5 to C-3/C-4/C-6/C-7/C-9/C-10/C-24, H-12 to C-9/C-13/C-14/C-18, and H_2-15 to C-8/C-17) (Fig. 2). The ROESY

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correlations of H-5 with H-3/H_3-23/H-9, H_3-25 with H_3-26/H_3-24, H_3-27 with H-9, and H_3-18 with H_3-26/H-28 further confirmed that the relative configuration of **1** remained unchanged. Therefore, compound **1** was identified as a 28-*nor*-oleanane triterpenoid, and was named 3β-hydroxy-17β-formyloxy-28-*nor*-olean-12-ene.

Compound **2** was isolated as a colorless solid with a molecular formula $C_{29}H_{44}O_4$ as established by the HRESIMS at *m/z* 457.3308 $[M+H]^+$ (calculated for 457.3318). The ^{1}H NMR spectrum of **2** (Table 1) displayed seven tertiary methyls [δ_H 0.82 (3 H, s, H_3-26), 0.91 (3 H, s, H_3-30), 0.94 (3 H, s, H_3-29), 1.09 (3H, s, H_3-25), 1.17 (3H, s, H_3-27), 1.35 (3H, s, H_3-24), 1.43 (3H, s, H_3-23)] and a tri-substituted double bond [δ_H 5.34 (1H, t, J = 3.7 Hz)]. The ^{13}C NMR and DEPT spectra of **2** (Table 1) revealed 29 carbon resonances arising from seven methyls, nine methylenes, four methines [including an olefinic (δ_C 122.0)], and nine quaternary carbons [including an oxygenated (δ_C 86.0), an olefinic (δ_C 143.8), and two carbonyls (δ_C 170.8, 180.9)]. All the above spectral data suggested that **2** was a *nor*-triterpenoid structurally similar to 3β-hydroxy-olean-12-en-28-oic acid (**7**). However, the highfield methylene (δ_C 27.3, C-2), methine (δ_C 79.2, C-3) and quaternary carbon (δ_C 38.9, C-4) in **7** disappeared in **2**, instead, an ester carbonyl group (δ_C 170.8) and an oxygenated quaternary carbon (δ_C 86.0) appeared. The ester carbonyl carbon (δ_C 170.8) was assignable to C-2 by its HMBC correlation with H_2-1 (δ_H 2.63, 1.86) (Fig. 2). The HMBC correlations from H_3-23/H_3-24/H-5 to C-4, the downfield chemical shift of C-4 (δ_C 86.0), together with the unsaturation degree of its molecular formula, indicated the formation of a lactone between C-2 and C-4 in **2**. The ROESY correlations of H-5 with H_3-23/H-9/H_3-27, H-18 with H_3-30, and H_3-26 with H_3-25/H-18 as well as a consideration of its biogenetic origin assigned the same configuration of **2** as **7**. Therefore, compound **2** was characterized as a 3,4-*seco*-3-*nor*-oleanane triterpenoid, and was named 2-oxo-3-oxaolean-12-en-28-oic acid.

Twenty-one known triterpenoids were also isolated in this study, and were identified as 3β,17β-dihydroxy-olean-12-ene (**3**) (Li et al., 2019), erythrodiol (**4**) (Kim et al., 2019), 3β-hydroxy-28-al-12-en-oleanoic acid (**5**) (Benyahia et al., 2005), 15α,16α-epoxy-olean-12-en-3-ol (**6**) (Chiu et al., 2008), 3β-hydroxy-olean-12-en-28-oic acid (**7**) (Carvaiho and Seita, 1993), momordic acid (**8**) (Xiang et al., 2015), 3-oxo-olean-12-en-28-oic acid (**9**) ((Lu et al., 2015), 28-hydroxy-olean-12-en-3-one (**10**) (Shi et al., 2012), 2α-hydroxy-3-oxo-olean-12-en-28-oic acid (**11**) (Zhang et al., 2019), 2-hydroxy-3-oxo-olean-1,12-dien-28-oic acid (**12**) (Zhang et al., 2020), erythrodiol 3-palmitate (**13**) (He et al., 2015), 3,4-*seco*-olean-12-en-3,28-dioic acid (**14**) (Caldwell et al., 2000), alstoscholarinoid A (**15**) (Hu et al., 2021), 3-epitaraxerol (**16**) (Minh et al., 2017), isomyricadiol (**17**) (Merfort et al., 1992), sweriyunnangenin A (**18**) (Cao et al., 2013), 3β-hydroxy-11α,12α-epoxy-olean-28,13β-olide (**19**) (Xiao et al., 2007), ursolic acid lactone (**20**) (Wang and Fujimoto, 1993), 28-*nor*-urs-12-en-3β,17β-diol (**21**) (Benyahia et al., 2005), ursaldehyde (**22**) (Ngo et al., 2018), and robustanic acid (**23**) (Wang et al., 2016), respectively. Based on their skeleton types, these triterpenoid isolates could be classified as 28-*nor*- (**1** and **3**), 3,4-*seco*- (**14**), (**2**) 3,4-*seco*-3-*nor*- (**2**), 17(18→19)-*abeo*-28-*nor*- (**15**) and 27 (14→13)-*abeo*-oleanane (**16**–**18**), normal oleanane (**4**–**13** and **19**), normal ursane (**20**, **22** and **23**), and 28-*nor*-ursane (**21**). As the major triterpenoid constituent of *S. mileensis*, compound **7** was obtained with a yield of 2.169%. Compounds **13** and **16** were isolated with relatively high contents respectively in 0.228‰ and 0.647‰ yield, while the yield of the other triterpenoids were all less than 0.1‰. Notably, all the triterpenoids except **7**, **16** and **18** are firstly isolated from *S. mileensis*, and all the ones except **4**, **7**, **13**, **16** and **18**–**20** are firstly found in the genus *Swertia*. In addition, 13 triterpenoids (**1**, **2**, **5**, **6**, **8**, **10**–**12**, **14**, **15**, **17**,

Table 1
^{1}H (700 MHz) and ^{13}C (150 MHz) NMR data of compounds 1 and 2 in CDCl3.

No.	1		2	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1a	1.62 m	38.5 t	2.63 d (16.7)	46.4 t
1b	0.97 m		1.86 m	
2a	1.61 m	27.3 t		170.8 s
2b	1.57 m			
3	3.22 dd (11.3, 4.3)	79.1 d		
4		38.9 s		86.0 s
5	0.74 dd (11.6, 2.0)	55.4 d	1.44 m	52.4 d
6a	1.56 m	18.4 t	1.53 m	21.3 t
6b	1.40 m		1.47 m	
7a	1.47 m	33.0 t	1.54 m	31.8 t
7b	1.36 m		1.39 m	
8		39.7 s		42.0 s
9	1.55 m	47.8 d	1.64 m	45.1 d
10		37.2 s		36.4 s
11a	1.92 m	23.6 t	1.98 m	23.0 t
11b	1.89 m		1.85 m	
12	5.26 t (3.7)	123.5 d	5.34 t (3.7)	122.0 d
13		143.1 s		143.8 s
14		41.5 s		39.2 s
15a	2.08 m	25.9 t	1.71 m	27.7 t
15b	1.07 m		1.14 m	
16a	2.08 m	24.1 t	2.00 m	23.1 t
16b	2.06 m		1.65 m	
17		87.5 s		46.6 s
18	2.72 dd (14.1, 3.9)	45.4 d	2.86 dd (13.9, 4.6)	41.4 d
19a	1.67 t (13.8)	48.0 t	1.62 m	45.9 t
19b	1.24 m		1.17 m	
20		30.9 s		30.8 s
21a	1.88 m	36.2 t	1.34 m	33.9 t
21b	1.32 m		1.24 m	
22a	2.18 dt (13.3, 3.8)	32.3 t	1.79 m	32.5 t
22b	2.01 m		1.60 m	
23	1.00 s, 3 H	28.3 q	1.43 s, 3 H	32.9 q
24	0.79 s, 3 H	15.7 q	1.35 s, 3 H	24.7 q
25	0.93 s, 3 H	15.4 q	1.09 s, 3 H	16.1 q
26	0.89 s, 3 H	17.8 q	0.82 s, 3 H	16.7 q
27	1.15 s, 3 H	25.9 q	1.17 s, 3 H	25.9 q
28	8.02 s	160.7 d		180.9 s
29	1.01 s, 3 H	24.1 q	0.94 s, 3 H	23.7 q
30	0.91 s, 3 H	32.6 q	0.91 s, 3 H	33.2 q

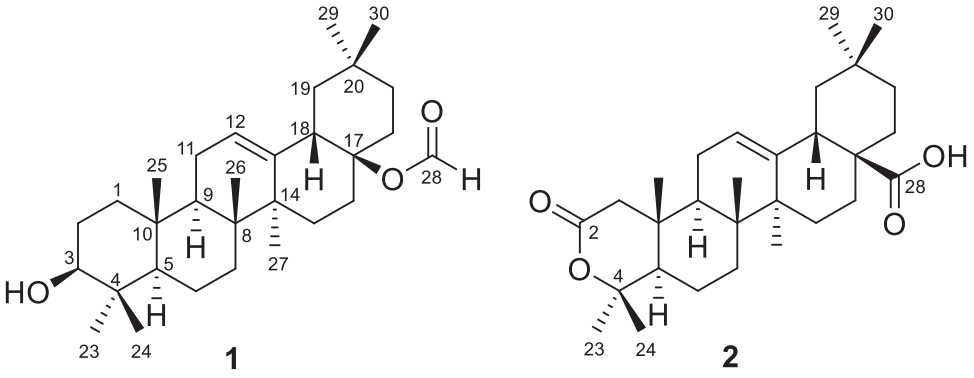

Fig. 1. Chemical structures of compounds 1 and 2.

21 and **23**) were discovered in the Gentianaceae plants for the first time. The principal existence of structurally diversified triterpenoids in *S. mileensis* might provide meaningful chemotaxonomic characteristics for this medicinal herb and reference significance for other plants from the genus *Swertia*.

All isolates except compounds **13** and **16** (due to their scarce amount) were evaluated for their anti-inflammatory activity by testing their inhibition against the secretion of cytokines TNF-α and IL-6 in RAW264.7 cells induced with LPS, and their antitumor activity by testing their inhibition against the proliferation of HepG2 and MDA-MB-231 cells. The results showed that compounds **2** and **10** could suppress the secretion of cytokines TNF-α (IC_{50}, 40.9 and 69.8 μM, respectively) and IL-6 (IC_{50}, 34.8 and 32.8 μM, respectively), while the IC_{50} values of the positive control dexamethasone were 1.17×10^{-3} and 9.42×10^{-3} μM, respectively. Preliminary structure and activity relationship suggested that the lactone ring in compound **2** could be positive for the anti-inflammatory activity (compared with **7** and **9**), while the coexistence of C-3 carbonyl and C-28 hydroxyl in compound **10** (compared with **4** and **9**) likewise contributed to its activity. In addition, compounds **1** and **17** exhibited weak cytotoxicity against HepG2 cells (inhibitory rates, 31.97 % and 31.39%, respectively) and MDA-MB-231 cells (inhibitory rates, 30.34 % and 47.18 %, respectively) at 10 μM.

3. Experimental

3.1. General experimental procedures

Column chromatography (CC) was carried out on silica gel (200 −300 mesh, Qingdao Dingkang Silicone Co., Ltd., Qingdao, P. R. China), neutral Al_2O_3 (200 −300 mesh, Shanghai Titan Scientific Co., Ltd., Shanghai, P. R. China), Sephadex LH-20 (20 −100 μm, Amersham Pharmacia Biotech, Sweden), and MCI gel CHP-20P (70 −150 μm, Mitsubishi Chemical Corp., Tokyo, Japan). Thin-layer chromatography (TLC) was performed on silica gel (GF_{254}, 10 −40 μm, Qingdao Dingkang Silicone Co., Ltd.). TLC spots were detected under UV light or by heating after spraying with 5 % H_2SO_4 in EtOH (v/v). NMR experiments were carried out using either a Bruker AvanceNeo-600 or AvanceNeo-700 spectrometer. Semipreparative HPLC was performed on a HITACHI Primaide series instrument with a Shimadzu Shim-pack GIST-C_{18} column (5 μm, 10 × 250 mm, 3 mL/min). Mass spectra were obtained on either an Agilent G6230 spectrometer or a Waters Synapt XS spectrometer. IR spectra were recorded on a Bruker-Tensor-27 spectrometer with KBr pellets. UV spectra were measured on a Chirascan qCD spectrograph. Optical rotations were obtained on an Autopol-I spectropolarimeter.

3.2. Plant material

The whole plant of *Swertia mileensis* (Gentianaceae) was purchased from Kunming Bencaotang Medicinal Materials Co., Ltd. (Yunnan, P. R. China), and was identified by Xiao Luo at the Chinese Medicine Office of Chengdu Food and Drug Inspection Research Institute, Sichuan, P. R. China. An authentic sample (Sm-201904) was kept at the Institute of Chinese Medicine and Pharmacy, Chengdu University of Traditional Chinese Medicine.

3.3. Extraction and isolation

The whole plant of *S. mileensis* was air-dried (100.0 kg, dry weight), milled, and soaked in MeOH (500 L × 3, 4 h) at 65 − 67 °C. The extract was concentrated and suspended in distilled water, then extracted with EtOAc (30 L × 3) and dried under vacuum to give 710.0 g of residue. The EtOAc extract was subjected to silica gel column chromatography (CC) and eluted with a CH_2Cl_2-acetone gradient (10:1, 4:1, 7:3, 1:1,

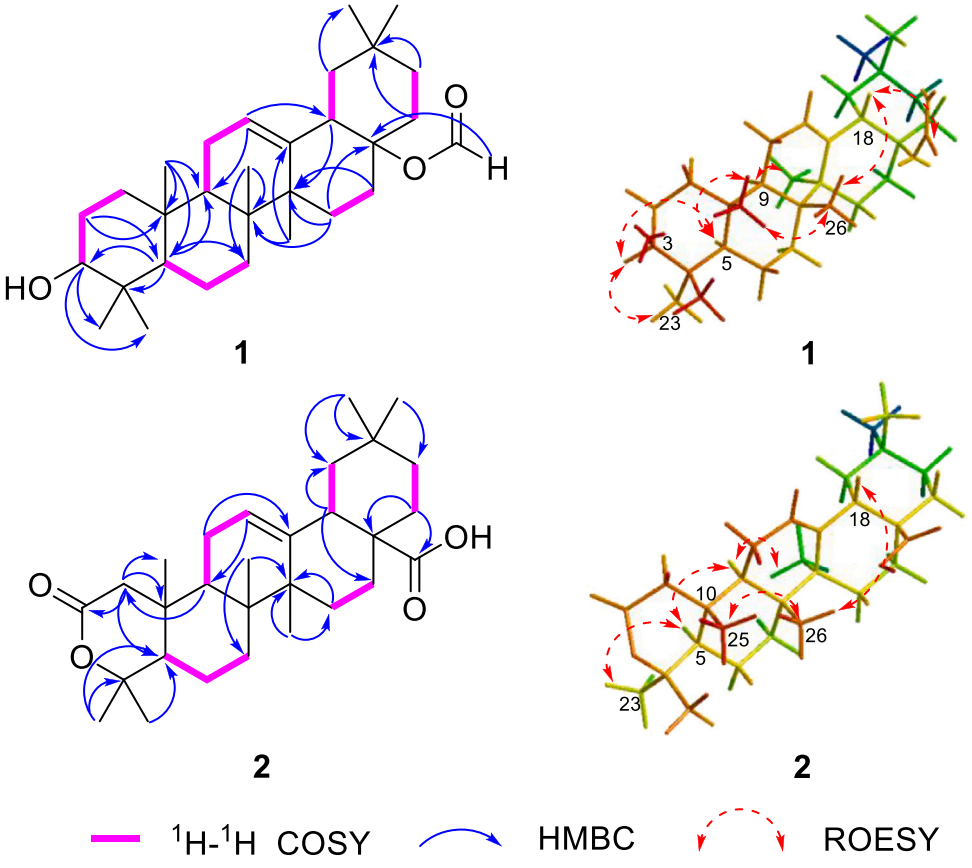

Fig. 2. Key ^{1}H–^{1}H COSY, HMBC and ROESY correlations of compounds 1 and 2.

0:10, v/v) to give five fractions (Fr. 1 −Fr. 9).

Fr. 1 (35.2 g) was separated using MCI gel CC eluted with a MeOH/H_2O stepwise-gradient system (from 5:5–10:0, v/v) to obtain eight subfractions (Fr. 1–1 −Fr. 1–8). Separation of Fr.1–4 (361.0 mg) was performed on silica gel CC eluted with PE/EtOAc (6:1, v/v) to afford four subfractions (Fr. 1–4–1 −Fr. 1–4–4). Fr. 1–4–3 (51.0 mg) was applied to Sephadex LH-20 CC eluted with $CHCl_3$/MeOH (1:1, v/v) and then purified by semipreparative RP-HPLC using MeCN-H_2O (85:15, v/v) as the mobile phase to yield compound **15** (t_R 15.0 min, 2.0 mg, in 0.003‰ yield). Fr 1–5 (9.5 g) was separated by C_{18} silica gel CC eluted with a MeOH/H_2O stepwise-gradient system (from 5:5–10:0, v/v) to obtain six subfractions (Fr. 1–5–1 −Fr. 1–5–6). Fr. 1–5–4 (285 mg) was further separated by Sephadex LH-20 CC eluted with $CHCl_3$/MeOH (1:1, v/v) to give four additional subfractions (Fr. 1–5–4a−Fr. 1–5–4d). Fr. 1–5–4a (24.0 mg) was purified by semipreparative RP-HPLC using MeCN-H_2O (95:5, v/v) as the mobile phase to yield compounds **3** (t_R 19.2 min, 2.0 mg, in 0.003‰ yield) and **21** (t_R 21.6 min, 3.1 mg, in 0.004‰ yield).

Fr. 2 (9.9 g) was applied to MCI gel CC eluted with a MeOH/H_2O stepwise-gradient system (from 5:5–10:0, v/v) to obtain nine subfractions (Fr. 2–1 −Fr. 2–9). Compound **18** (9.0 mg, in 0.013‰ yield) was crystallized from Fr. 2–9 as a colorless crystal. Fr. 2–9 (1.3 g) was separated by Sephadex LH-20 CC eluted with $CHCl_3$/MeOH (1:1, v/v) to provide eight additional subfractions (Fr. 2–9–1 −Fr. 2–9–8). Fr. 2–9–2 (258.2 mg) was fractionated by silica gel CC eluted with PE/EtOAc (6:1, 3:1, v/v) to afford five subfractions (Fr. 2–9–2a−Fr. 2–9–2e). Fr. 2–9–2c (21.0 mg) was purified by semipreparative RP-HPLC using MeCN-H_2O (100:0, v/v) as the mobile phase to get compounds **1** (t_R 11.2 min, 6.0 mg, in 0.008‰ yield), **5** (t_R 13.1 min, 8.0 mg, in 0.011‰ yield) and **22** (t_R 15.0 min, 2.0 mg, in 0.003‰ yield). Compounds **13** (162.1 mg, in 0.228‰ yield) and **16** (459.2 mg, in 0.647‰ yield) were directly crystallized from Fr. 3 (54.7 g).

Fr. 4 (60.5 g) was subjected to silica gel CC eluted with PE/EtOAc (15:1, 8:1, v/v) to afford five subfractions (Fr. 4–1 −Fr. 4–5). Fr. 4–2 (12.2 g) was separated by silica gel CC eluted with petroleum ether/CH_2Cl_2 (1:1, v/v) to afford compound **6** (1.0 mg, in 0.001‰ yield) and five additional subfractions (Fr. 4–2–1 −Fr. 4–2–5). Fr. 4–2–4 (29.0 mg) was further separated by Sephadex LH-20 CC eluted with $CHCl_3$/MeOH (1:1, v/v) and then purified by semipreparative RP-HPLC using MeCN-H_2O (70:30, v/v) as the mobile phase to yield compound **14** (t_R 10.6 min, 8.0 mg, in 0.011‰ yield).

Fr. 4–3 (15.1 g) was separated by C_{18} silica gel CC eluted with a MeOH/H_2O gradient system (from 5:5–10:0, v/v) to give seven subfractions (Fr. 4–3–1 −Fr. 4–3–7). Compound **9** (2.0 mg, in 0.003‰ yield) was crystallized from Fr. 4–3–7 as a colorless crystal. Fr. 4–3–7 (1.1 g) was isolated by Al_2O_3 CC eluted with PE/EtOAc (6:1, 3:1, v/v) to provide four additional subfractions (Fr. 4–3–7a−Fr. 4–3–7d), and compound **4** (3 mg, in 0.004‰ yield) was crystallized from Fr. 4–3–7–4. Fr. 4–3–7–3 (255.9 mg) was applied to Sephadex LH-20 CC eluted with $CHCl_3$/MeOH (1:1, v/v) and then purified by semipreparative RP-HPLC using MeCN-H_2O (100:0, v/v) as the mobile phase to acquire compounds **10** (t_R 19.0 min, 6.0 mg, in 0.008‰ yield), **17** (t_R 20.0 min, 5.0 mg, in 0.007‰ yield) and **19** (t_R 22.6 min, 3.0 mg, in 0.004‰ yield).

Fr 4–4 (13.1 g) was applied to MCI gel CC eluted with a MeOH/H_2O stepwise-gradient system (from 5:5–10:0, v/v) to obtain 10 subfractions (Fr. 4–4–1 −Fr. 4–4–10). Fr. 4–4–9 (626.7 mg) was separated by Sephadex LH-20 CC eluted with $CHCl_3$/MeOH (1:1, v/v) and then purified by semipreparative RP-HPLC using MeCN-H_2O (65:35, v/v) as the mobile phase to yield compounds **2** (t_R 16.0 min, 1.0 mg, in 0.001‰ yield), **20** (t_R 10.0 min, 3.0 mg, in 0.004‰ yield), **23** (t_R 16.1 min, 2.0 mg, in 0.003‰ yield) and three additional subfractions (Fr. 4–4–9a−Fr. 4–4–9c). Fr. 4–4–9–3 (42.3 mg) was purified by semipreparative RP-HPLC using MeCN-H_2O (80:20, v/v) as the mobile phase to give compounds **11** (t_R 9.8 min, 19.0 mg, in 0.027‰ yield), **12** (t_R 13.0 min, 3.0 mg, in 0.004‰ yield) and **8** (t_R 16.7 min, 4.0 mg, in 0.006‰ yield). Fr. 5 (65.1 g) was isolated by MCI gel CC eluted with a MeOH/H_2O stepwise-gradient system (from 5:5–10:0, v/v) to obtain five subfractions (Fr. 5–1 −Fr. 5–5). Fr. 5–5 (16.9 g) was purified by silica gel CC eluted with PE/EtOAc (4:1, 2:1, v/v) to afford compound **7** (15.4 g, in 2.169% yield).

3.3.1. 3β-Hydroxy-17β-formyloxy-28-nor-olean-12-ene

White powder, $[\alpha]_D^{20}$ + 25.0 (*c* 0.02, MeOH); IR (KBr) ν_{max} 3432, 2944, 1719, 1462, 1385, 1199, 1177 cm^{-1}; UV (MeOH) λ_{max} (log ε) 220 (1.44) nm; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 455.3530 $[M - H]^-$ (calcd for $C_{30}H_{47}O_3$, *m/z* 455.3530).

3.3.2. 2-Oxo-3-oxaolean-12-en-28-oic acid

Colorless solid, $[\alpha]_D^{20}$ − 50.0 (*c* 0.01, MeOH); IR (KBr) ν_{max} 3435, 2919, 1636, 1431, 1384, 1164, 1056 cm^{-1}; UV (MeOH) λ_{max} (log ε) 220 (0.33) nm; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 457.3308 $[M + H]^+$ (calcd for $C_{29}H_{45}O_4$, *m/z* 457.3318).

3.4. Bioassays

3.4.1. Cell culture

RAW264.7, HepG2, and MDA-MB-231 cells were obtained from Kunming Institute of Zoology (Chinese Academy of Sciences, CAS), American Type Culture Collection, and Kunming Institute of Botany (CAS), respectively. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) (VivaCell) containing 10% fetal bovine serum (FBS) (Cell-Box), penicillin (50 kU/L) and streptomycin (50 mg/L) (VivaCell) at 37 °C in the presence of 5 % CO_2 in a humidified atmosphere under control. Exponentially growing cells were used for subsequent assay experiments.

3.4.2. Anti-inflammatory assay

RAW264.7 cells were placed in 96-well plates at initial densities of 2×10^4 cells per well. After 12 h of culture, RAW264.7 cells were treated with different concentrations (80, 40, 20 and 10 μM, dissolved in DMSO) of compounds **1**−**12**, **14**, **15** and **17**−**23**. Dexamethasone (Dex, Solarbio) was assayed as a positive control. After 1 h, the cells were treated with LPS (1 μg/mL) (Beyotime Biotechnology). After another 24 h of culture, the supernatant was used for cytokines TNF-α and IL-6 determination by using the murine TNF-α and IL-6 ELISA kit according to the manufacturer's protocol (BD biosciences) (Guo et al., 2021). The optical density was subsequently measured using a microplate reader (Molecular Devices). The half-maximal inhibitory concentrations (IC_{50}) of TNF-α and IL-6 were determined by GraphPad Prism (GraphPad). Triplicate experiments were conducted.

3.4.3. Antitumor assay

HepG2 and MDA-MB-231 cells were separately seeded in 96-well plates at initial densities of 1.4×10^4 cells per well. After 24 h of culture, the cells were separately treated with compounds **1**−**12**, **14**, **15** and **17**−**23** at a concentration of 10 μM. After 24 h, a total of 10 μL of CCK-8 (Bioground) was added to each well. After 30 min of incubation, the optical density at 450 nm was subsequently measured using a microplate reader (Molecular Devices) (Valashedi et al., 2022). Triplicate experiments were carried out.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phytol.2023.03.001.

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