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New triterpenes from *Salacia hainanensis* Chun et How with α-glucosidase inhibitory activity

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New triterpenes from *Salacia hainanensis* Chun et How with α-glucosidase inhibitory activity

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Fractionation of the methanol extract from the roots of *Salacia hainanensis* Chun et How showing the potent inhibitory activity on α-glucosidase afforded two new lupane derivatives, 3α,28-dihydroxy-lup-20(29)-en-2-one (**1**) and 3α-hydroxy-lup-20(29)-en-2-one (**2**), a new friedelane derivative, D:A-friedo-oleanane-7α,30-dihydroxy-3-one (**3**), and a novel natural product, 2,3-*seco*-lup-20(29)-en-2,3-dioic acid (**4**), along with four known compounds (**5**–**8**). Their structures were established on the basis of spectral analysis, especially on the data afforded by 2D NMR and high-resolution mass spectra experiments. All of them showed a much stronger inhibiting activity on α-glucosidase than the positive control (acarbose, $IC_{50} = 5.83\ \mu M$). Constituents with α-glucosidase inhibitory activity from this plant are reported for the first time.

Keywords: Hippocrateaceae; *Salacia hainanensis* Chun et How; lupane; friedelane; α-glucosidase; antidiabetes

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1. Introduction

Much attention has been given to the prevention and the treatment of diabetes, which is a complex metabolic disorder caused by insulin insufficiency and/or insulin dysfunction and characterized by aberrant blood glucose and insulin levels, and has an increasingly adverse impact on morbidity, mortality, and overall health care costs worldwide [1]. Complications caused by diabetes are considered as the main reason for health damage, even death. Recently, the inhibitors of glycosidases including intestinal α-glucosidase inhibitors have been postulated to be powerful therapeutic agents in carbohydrate metabolic disorders, especially in diabetes mellitus [2,3].

Salacia hainanensis Chun et How, family Hippocrateaceae, is a special species used as a traditional medicine in Hainan Province of China. Its root and stem have been widely used for the treatment of rheumatoid joint pain, strain of lumbar muscles, weakness, and asthenia [4]. The aqueous extract from the roots of *S. hainanensis* had been reported to show hypoglycemic activity in mice and can significantly reduce the blood glucose levels of alloxan and glucose-loaded mice [5]. However, the pharmacologically active components were unclear. In the course of our studies on antidiabetogenic compounds from natural medicines, the inhibitory activity on α-glucosidase of the methanol (MeOH) extract from its roots

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was evaluated, which showed a very potent inhibitory effect. By means of various chromatographic methods, the extract gave eight triterpene derivatives. This paper describes the isolation and the structural elucidation of the constituents from *S. hainanensis*, together with their α-glucosidase inhibitory activities.

2. Results and discussion

During the process of the chemical study on this plant, two new lupane derivatives, 3α,28-dihydroxy-lup-20(29)-en-2-one (**1**) and 3α-hydroxy-lup-20(29)-en-2-one (**2**), a new friedelane derivative, D:A-friedo-oleanane-7α,30-dihydroxy-3-one (**3**), and a novel natural product, 2,3-*seco*-lup-20(29)-en-2,3-dioic acid (**4**), as well as four known compounds, lup-20(29)-en-3,21-dione (**5**) [6], amyrin (**6**) [7], 30-hydroxy-friedelan-3-one (**7**) [8], and 24*S*,25-dihydroxy-triucall-7-en-3-one (**8**) [9], were isolated (Figure 1).

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Compound **1** was obtained as a white amorphous powder (CH_2Cl_2). Its molecular formula was assigned to be $C_{30}H_{48}O_3$ by a positive ion peak at *m/z* 479.3495 $[M + Na]^+$ in the high-resolution electrospray ionization mass spectra (HR-ESI-MS). The absorption bands at 3480, 1713, 1640, and 773 cm^{-1} in the IR spectrum indicated the presence of hydroxyl, carbonyl, and double band functions in the structure. Its ^1H NMR spectrum showed signals for six methyl [δ_H 0.70, 0.81, 1.19, 1.70 (each 3H, all s), 1.04 (6H, s)], two olefinic protons [δ_H 4.61 (1H, br s), 4.71 (1H, br s)], an oxygen-substituted methine at δ_H 3.89 (1H, s), one oxomethylene [δ_H 3.36, 3.82 (each 1H, d, $J = 12.0$ Hz)], and other alkyl groups (Table 1). There were 30 carbon signals in the ^{13}C NMR spectrum (Table 1) including a ketone group (δ_C 211.4), one oxygen-substituted methine (δ_C 82.5), two olefinic carbons (δ_C 109.9 and 150.2), one oxomethylene (δ_C 60.5), and other alkyl groups consisting of six methyl, nine methylene, five methine, and five quaternary carbons. These data provided the evidence that **1** was a 3,28-dihydroxy-lup-20(29)-ene derivative, especially from the chemical shifts of its olefinic carbon signals [10]. The ketone group was assigned to the C-2 position by the long-range correlations between H-3 (δ_H 3.89) and carbonyl [δ_C 211.4 (C-2)], which also showed correlations with H-1 [δ_H 2.56 (d, 12.0), 2.04 (d, 12.0)] in the HMBC spectrum. The NOESY cross-peaks of H-3/CH_3-25/CH_3-24, CH_3-25/CH_3-26, and H-19/H-28 were observed, which

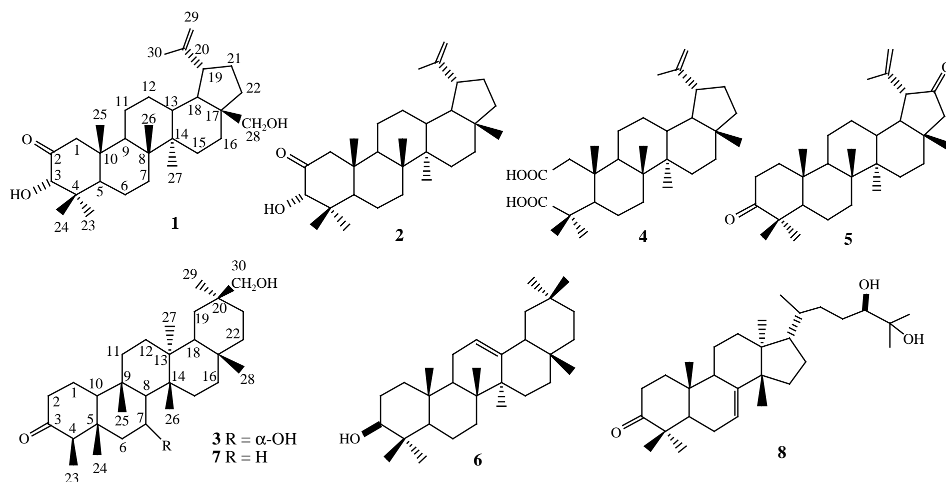

Figure 1. Structures of compounds **1**–**8**.

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Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1**–**4** ($CDCl_3$, J in Hz).

	1		**2**		**3**		**4**	
Position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.56 (d, J = 12.0)	53.5	2.03 (d, J = 12.3)	53.4	2.02 (d, J = 3.9)	21.9	2.63 (d, J = 19.8)	40.9
	2.04 (d, J = 12.0)		2.53 (d, J = 12.3)				2.46 (d, J = 19.8)	
2		211.4		211.5	2.34–2.41[a]	41.2		178.5
3	3.89 (s)	82.5	3.88 (s)	82.9		212.4		187.5
4		45.6		45.6	2.32 (s)	58.2		45.6
5	1.44[a]	54.6	1.43[a]	54.6		42.6	1.36[a]	48.2
6	1.67[a]	18.5	1.63 (d, J = 4.8)	18.5	2.02 (d, J = 3.9)	52.2	1.34[a]	21.3
	1.52[a]		1.52 (d, J = 4.8)		1.41[a]			
7	1.07[a]	34.0	1.06 (m)	33.8	4.12 (t, J = 10.5)	68.6	1.40 (d, J = 4.2)	33.7
	1.88 (m)		1.86 (m)				1.51[a]	
8		41.3		41.2	1.50[a]	58.6		41.8
9	1.61[a]	50.4	1.60[a]	50.4		39.0	2.53[a]	41.7
10		43.9		44.0	1.55[a]	58.9		40.7
11	1.28 (d, J = 12.0)	21.0	1.28 (d, J = 11.7)	21.1	1.41[a]	35.9	1.65[a]	19.2
	1.33 (d, J = 12.0)		1.33 (d, J = 11.7)		1.33[a]		1.50[a]	
12	1.08[a]	25.1	1.09[a]	24.8	1.27[a]	29.6	1.67 (dd, J = 12.6, 4.2)	24.9
	1.68[a]		1.66[a]				1.70 (dd, J = 12.6, 4.0)	
13	1.68[a]	37.2	1.67[a]	37.9		40.3	1.60 (dd, J = 3.6, 11.4)	37.9
14		42.8		42.9		40.0		43.2
15	1.10[a]	27.1	1.50[a]	29.4	1.52[a]	29.5	1.67[a]	27.5
	1.73[a]		1.71[a]				1.04 (d, J = 2.4)	
16	2.04 (2H)[a]	29.3	2.03 (2H, m)	35.4	1.00 (m)	38.3	1.49[a]	35.5
					1.53[a]		1.39 (d, J = 6.0)	
17		47.8		42.9		30.4		43.2
18	1.62[a]	48.7	1.63[a]	48.2	1.53[a]	42.3	2.52 (d, J = 11.4)	48.4
19	2.40 (td, J = 10.9, 6.0)	47.8	2.40 (td, J = 10.7, 6.1)	47.9	1.71[a]	35.3	2.38 (td, J = 11.4, 6.0)	48.0
					2.02 (m)			
20		150.2		150.7		33.5		150.9
21	1.42 (d, J = 12.0)	29.7	1.43 (m)	29.8	1.19 (m)	27.9	1.92 (m)	29.8
	1.97 (d, J = 10.8)		2.03 (m)		1.41 (m)		1.33[a]	

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Table 1 – *continued*

Position	**1**		**2**		**3**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
22	1.50[a]	33.8	1.50 (m)	29.9	1.62[a]	36.1	1.38 (d, $J = 12.0$)	39.9
	1.88 (d, $J = 3.0$)		1.88 (m)				1.39 (d, $J = 12.0$)	
23	1.19 (3H, s)	29.1	1.17 (3H, s)	29.2	0.92 (d, $J = 6.5$)	6.9	1.17 (3H, s)	29.8
24	0.70 (3H, s)	16.4	0.68 (3H, s)	16.4	0.82 (3H, s)	15.9	1.27 (3H, s)	21.3
25	0.81 (3H, s)	17.0	0.79 (3H, s)	17.0	0.94 (3H, s)	18.9	0.93 (3H, s)	20.8
26	1.04 (3H, s)	14.8	1.03 (3H, s)	15.6	1.10 (3H, s)	19.1	1.03 (3H, s)	15.9
27	1.04 (3H, s)	15.6	0.99 (3H, s)	14.5	1.24 (3H, s)	20.8	0.94 (3H, s)	14.6
28	3.36 (d, $J = 12.0$)	60.5	0.79 (3H, s)	18.0	1.20 (3H, s)	31.8	0.78 (3H, s)	18.0
	3.82 (d, $J = 12.0$)							
29	4.61 (br s)	109.9	4.57 (1H, s)	109.5	1.01 (3H, s)	29.1	4.57 (br s)	109.4
	4.71 (br s)		4.69 (1H, s)				4.09 (br s)	
30	1.70 (3H, s)	19.1	1.68 (3H, s)	19.3	3.40 (d, $J = 10.2$)	71.3	1.69 (3H, s)	19.2
					3.46 (d, $J = 10.2$)			

Note: [a] Overlapped signals were reported without designating multiplicity.

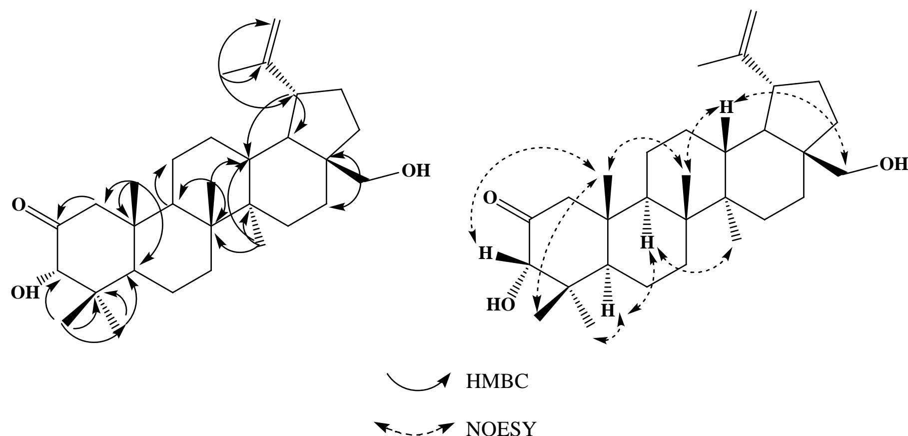

Figure 2. Key HMBC and NOESY correlations of compound **1**.

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suggested that the hydroxyl group linked with C-3 was in the α-oriented form (Figure 2). Based on the above analysis, compound **1** was elucidated as 3α,28-dihydroxy-lup-20(29)-en-2-one (Figure 1).

Compound **2** was isolated as a white solid powder (CH_2Cl_2) with a molecular formula of $C_{30}H_{48}O_2$ determined by a positive $[M + Na]^+$ ion peak at *m/z* 463.3545 in HR-ESI-MS. Its IR spectrum displayed absorption bands arising from the hydroxyl (3500 cm^{-1}), carbonyl (1707 cm^{-1}), and double bond (1642 cm^{-1}) groups. The data afforded by the ^1H and ^{13}C NMR (Table 1) spectra suggested that compound **2** was a derivative of **1** with the difference being that C_{28}—OH was reduced to be a methyl group. In the HMBC spectrum, the long-range correlations between CH_3-28 [δ_H 0.79 (3H, s)] and C-17, C-18, and C-22 (δ_C 42.9, 48.2, and 29.9, respectively) were also found; therein, compound **2** was determined to be 3α-hydroxy-lup-20(29)-en-2-one (Figure 1).

Compound **3** was obtained as white needles (CH_2Cl_2). Its molecular formula was assigned to be $C_{30}H_{50}O_3$ by a positive ion peak at *m/z* 481.3649 in the HR-ESI-MS. The IR spectrum showed absorption bands at 3501 and 1707 cm^{-1} ascribable to hydroxyl and carbonyl functions. Its ^1H (300 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) spectral data (Table 1) suggested that compound **3** was a hydroxylated derivative of D:A-friedo-oleanane-30-hydroxy-3-one (**3**) [8], which was also obtained in this work as compound **7**. In the HMBC experiment, the presence of the long-range correlations between H-2 [δ_H 2.34–2.41 (2H, m)] and C-3 (δ_C 212.4); H-6 [δ_H 2.02 (1H, d, $J = 3.9$ Hz) and 1.41 (1H, overlap)] and C-7 (δ_C 68.6) suggested that the hydroxyl group should be assigned to the C-7 position, and the key correlations are exhibited in Figure 3. Moreover, the NOESY correlations of H-7/CH_3-24, 25, 26, CH_3-28/H-30, CH_3-29/CH_3-27 suggested that the orientation of C_7—OH was in the α form. Thus, compound **3** was elucidated to be D:A-friedo-oleanane-7α,30-dihydroxy-3-one.

Compound **4** had the molecular formula of $C_{30}H_{48}O_4$ determined by its pseudomolecular ion $[M + Na]^+$ at *m/z* 495.3445 in its HR-ESI-MS spectrum. Its ^1H NMR spectrum (Table 1) revealed the presence of seven methyls [δ_H 0.78, 0.93, 0.94, 1.03, 1.17, 1.27, and 1.69 (each 3H, s)], two olefinic protons [δ_H 4.57 (1H, br s) and 4.09 (1H, br s)], and other alkyl groups. Thirty carbon signals were shown in its ^{13}C NMR spectrum (Table 1), and two olefinic carbons at δ_C 109.4 and 150.9 were characteristic of the lup-20(29)-en

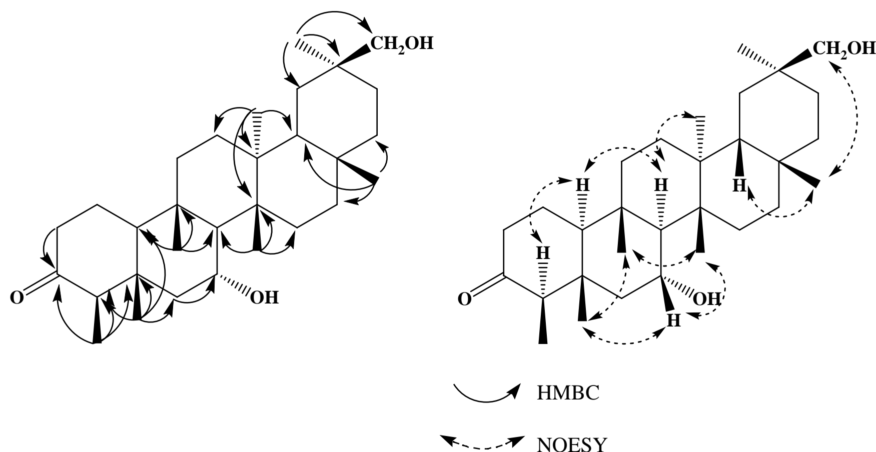

Figure 3. Key HMBC and NOESY correlations of compound **3**.

skeleton. In addition, signals at δ_C 178.5 and 187.5 indicated the existence of two carboxylic groups in compound **4**. Its HMBC spectrum gave the key correlations of H-1 (δ_H 2.46, 2.63) and C-2 (δ_C 178.5); CH_3-23 [δ_H 1.17 (3H, s)] and C-3, 5 (δ_C 187.5, 48.2); CH_3-24 (δ_H 1.27) and C-4 (δ_C 45.6), C-5; and CH_3-25 [δ_H 0.93 (3H, s)] and C-1 (δ_C 40.9), C-10 (δ_C 40.7), which suggested that compound **4** had a 2,3-*seco*-A ring in the structure and two carboxyl signals were assigned to the C-2 and C-3 positions, respectively. Thus, compound **4** was determined to be 2,3-*seco*-lup-20(29)-en-2,3-dioic acid (Figure 4), as a novel natural product, which was found as a medium product during a chemical reaction

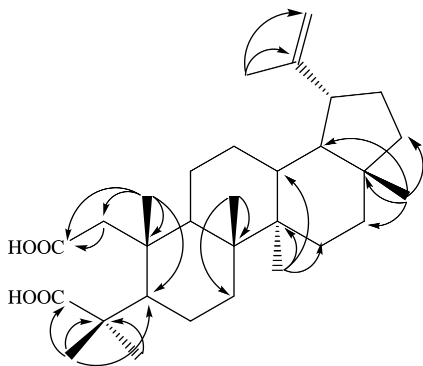

Figure 4. Key HMBC correlations of compound **4**.

[11] firstly and its proton and carbon signals were assigned completely for the first time here.

An assay was carried out for the inhibition of α-glucosidase using the main extract (MeOH) and subsequent partitions with ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH), and the aqueous extract (Table 2). It was found that the activity occurred in all fractions. The IC_{50} values of each fraction indicated their potent inhibitory effects. In addition, compounds **1**–**8** were tested for their inhibitory activities (Table 3). All of them showed a much stronger inhibitory activity than the positive control. Among these, the most potent inhibitor was **4**, with an IC_{50} value of 0.01 μM. Its structural features including the 2,3-*seco* of ring A and the existence of two carboxylic groups may be responsible for its significant inhibitory property. This result would be helpful to clarify the importance of exploiting the useful resource for *S. hainanensis*.

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained on a Perkin-Elmer 341 polarimeter at room temperature. IR spectra were measured on a Bruker IFS-55 Fourier transform infrared spectrometer.

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Table 2. Inhibition activities of the extracts of *S. hainanensis* on α-glucosidase (IC_{50}).

	IC_{50}[a] (mg/ml)				
	Main extract	Fractions			Positive control
Assay	MeOH	EtOAc	*n*-BuOH	H_2O	Acarbose
α-Glucosidase	0.26	0.27	0.03	0.05	3.76

Note: [a] IC_{50} values were calculated from dose-dependent inhibition curves.

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NMR spectral data were recorded on Bruker AV-600 and ARX-300 spectrometers and chemical shifts were shown as δ-values (ppm) with tetramethyl silane as an internal standard, including NOE, HMQC, and HMBC. HR-ESI-MS were recorded on a Bruker micro-TOF-Q mass spectrometer. HPLC separation was carried out on a reversed-phase Mightysil packed column using the gradient CH_3CN-H_2O and $MeOH-H_2O$ solvent systems with detection at 210 nm. Silica gel for column chromatography (CC, 200–300 mesh) and TLC plates (GF_{254}) were purchased from Qingdao Marine Chemical Ltd (Qingdao, China), and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating. All chemical agents used were of biochemical reagent grade. A molecular device spectrophotometer was used for the measurement of enzyme inhibition.

3.2 Plant material

The roots of *S. hainanensis* Chun et How were collected from Baoting County in Hainan Province of China in September 2007 and taxonomically identified by Prof. Qi-shi Sun (School of Traditional Chinese Medicine, Shenyang Pharmaceutical University). The voucher specimen (No. ZB-2007-26) has been deposited at the same department.

Table 3. Inhibitory activities of compounds **1**–**8** on α-glucosidase (IC_{50}).

Compound	IC_{50} (μM)
1	0.26
2	0.09
3	0.87
4	0.01
5	0.07
6	0.19
7	0.08
8	0.30
Acarbose	5.83

3.3 Extraction and isolation

Air-dried roots of *S. hainanensis* (5.5 kg) were extracted with MeOH under reflux for two times. The solution was evaporated under vacuum to obtain a brown viscous residue (491 g), which was suspended in water (1200 ml) and extracted with EtOAc and BuOH successively, to yield the EtOAc (SMA, 78 g), *n*-BuOH (SMB, 100 g), and aqueous (SMW, 232.8 g) soluble fractions.

SMA (75 g) was fractionated using silica gel CC with a gradient of $CHCl_3$–MeOH (100:0, 100:2, 100:3, 100:5, 100:10, 100:20, and 0:100) to give seven fractions (Fr. 1–7). Fr. 4 (2.6 g) was subjected to silica gel CC (petroleum ether (PE)–acetone, 30:1) again to afford four subfractions (Fr. 4.1–4.4). Then, subfraction Fr. 4.1 (0.4 g) was purified with reversed-phase HPLC [$MeOH-H_2O$ (86:14)] to furnish compound **7** (15 mg, t_R 50.3 min). Fr. 4.2 (0.7 g) was also applied to HPLC [$MeOH-H_2O$ (85:15) to afford compounds **1** (4 mg, t_R 27.0 min) and **3** (5 mg, t_R 38.5 min). Compound **8** (5 mg) was crystallized from Fr. 7 (1.4 g) subjected to silica gel CC and eluted with CH_2Cl_2–MeOH (30:1). From Fr. 5 (2 g), compounds **2** (6 mg, t_R 60.5) and **5** (3 mg,

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t_R 28.0 min) were obtained after purification with HPLC [MeOH–H_2O (95:5)].

BuOH extract (100 g) was fractionated using silica gel CC with a gradient of CH_2Cl_2–MeOH (100:2, 100:4, 100:10, 100:20, 100:30, and 0:100) to give six fractions (Fr. 1–6). Fr. 1 (3 g) was further separated on silica gel CC with solvents PE–acetone (40:1) to give compound **6** (6 mg). In addition, the rechromatography of Fr. 2 (1.5 g) on a silica gel column with PE–acetone (40:1) generated three subfractions (Fr. 2.1–2.3), and then, subfraction 2.3 (0.2 mg) was purified by HPLC [MeOH–H_2O (65:35)] to afford compound **4** (16 mg, t_R 71.0 min).

*3.3.1 3α,28-Dihydroxy-lup-20(29)-en-2-one (**1**)*

A white powder (CH_2Cl_2), $[\alpha]_D^{21}$ + 42.9 ($c = 0.05$, CH_2Cl_2); IR (KBr) ν_{max}: 3480 (—OH), 1713 (C=O), 1640 (C=C), 1454, 1219, 1025, 773 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see Table 1. HR-ESI-MS: m/z 479.3495 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_3Na$, 479.3496.

*3.3.2 3α-Hydroxy-lup-20(29)-en-2-one (**2**)*

A white powder (CH_2Cl_2); $[\alpha]_D^{23}$ + 49.7 ($c = 0.08$, CH_2Cl_2); IR (KBr) ν_{max}: 3500 (—OH), 3078 (=CH), 2936, 2872, 1708 (C=O), 1642 (C=C), 1454, 1383, 1056, 881 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) spectral data, see Table 1; HR-ESI-MS: m/z 463.3545 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_2Na$, 463.3547)].

*3.3.3 D:A-friedo-oleanane-7α,30-dihydroxy-3-one (**3**)*

White needles (CH_2Cl_2); $[\alpha]_D^{23}$ − 17.5 (CH_2Cl_2, $c = 0.85$); IR (KBr) ν_{max}: 3501, 1708 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) spectral data, see Table 1; HR-ESI-MS: m/z 481.3649 $[M + Na]^+$ (calcd for $C_{30}H_{50}O_3Na$, 481.3652).

*3.3.4 2,3-seco-Lup-20(29)-en-2,3-dioic acid (**4**)*

A white powder (CH_2Cl_2); ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) spectral data, see Table 1; HR-ESI-MS: m/z 495.3445 $[M + Na]^+$ (calcd for $C_{30}H_{48}O_4Na$, 495.3445).

3.4 Glycosidase inhibition assay

The α-glucosidase (Sigma Chemical Company, Shanghai, China) inhibition assay was performed according to the reported methods with slight modification [12]. Thirty microliters of a 0.02 U/μl enzyme in 0.2 M phosphate buffer (pH 6.8) and in the presence or absence of various concentrations of the test compounds in 0.2 M phosphate buffer (pH 6.8) were incubated in 96-well plates at 37°C for 10 min. Then, 140 μl of 0.02 M *p*-nitrophenyl-α-D-glucosidase (PNPG; Sigma Chemical Company) in 0.2 M phosphate buffer (pH 6.8) was added, and the plate was incubated at 37°C for another 30 min. The reaction was quenched by the addition of 0.2 M Na_2CO_3 solution (2 ml). Acarbose (Germany Bayer Chemical Ltd, Shanghai, China) was tested as a positive control. The increment in absorption at 450 nm due to the hydrolysis of PNPG by glycosidase was monitored on a microplate spectrophotometer (Bio-Rad, Hercules, CA, USA). The concentration of samples required to inhibit 50% of their activities under the assay conditions was defined as the IC_{50} value.

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