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Rearranged oleanane type saponins, astraisoolesaponins A_1–A_3 and B, from the stems of *Astragalus membranaceus* (Fisch.) Bge. *var. mongholicus* (Bge.) Hsiao

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ABSTRACT

Four novel saponins, astraisoolesaponins A_1–A_3 and B (**1**–**4**) were isolated from the stems of *Astragalus membranaceus* (Fisch.) Bge. *var. mongholicus* (Bge.) Hsiao. Spectroscopic analysis indicated that **1**–**4** are the first example of saponins possessing a 28-methyl-rearranged carbon skeleton. Their absolute configurations were determined by electronic circular dichroism (ECD) calculations. Both **3** and **4** displayed triglyceride accumulation inhibitory effects in HepG2 cells and glucose uptake increase effects on L6 cells.

1. Introduction

Astragalus genus plants, especially *Astragalus membranaceus*, *Astragalus mongholicus*, and *Astragalus complanatus* have been mainly used in folk medicine for their antidiabetic, antioxidative, *anti*-inflammatory, *anti*-cancer, cardioprotective, hepatoprotective, and antiviral properties, which are owing to the biological active secondary metabolites, such as saponins, flavonoids, and polysaccharides in them.[1]

In our process of continuing to study triglyceride (TG) accumulation inhibitory effective metabolites from the stems of *A. membranaceus* (Fisch.) Bge. *var. mongholicus* (Bge.) Hsiao (AM),[2–4] four novel rearranged oleanane type saponins with 28-methyl-rearranged carbon skeleton, astraisoolesaponins A_1 (**1**), A_2 (**2**), A_3 (**3**), and B (**4**) were obtained, which were firstly found in natural products. The structures of them were established by analysis of their NMR spectroscopic data and experimental and calculated electronic circular dichroism (ECD) spectra. In this paper, we describe their isolation, structure elucidation, and biological activities.

2. Results and discussion

The 70% EtOH extract of AM (8.0 kg) was partitioned with H_2O and $CHCl_3$, the H_2O layer was subjected to D101 macroporous resin column chromatography (CC) and eluted with H_2O and 95% EtOH, successively. Then 95% EtOH eluted fraction was isolated by Silica gel, ODS, Sephadex LH-20 and preparative HPLC CC to afford astraisoolesaponins A_1 (**1**, 31.5 mg),A_2 (**2**, 35.7 mg),A_3 (**3**, 10.7 mg), and B (**4**, 13.7 mg) (Fig. 1).

Astraisoolesaponin A_1 (**1**), was obtained as white powder with negative rotation ($[\alpha]_D^{25}$ −73.5°, in MeOH). The molecular formula, $C_{48}H_{72}O_{21}$, of **1** was established by negative-ion HRESI-TOF-MS (*m/z* 1019.4238 $[M+Cl]^-$, calcd for $C_{48}H_{72}O_{21}Cl$, 1019.4260). The IR spectrum showed absorption bands due to hydroxyl (3400 cm^{-1}), carboxyl group (1719 cm^{-1}), olefinic bond (1642 cm^{-1}), and *O*-glycosidic linkage (1073 cm^{-1}). The UV spectrum displayed maximum absorption at 294 nm, which indicated the presence of α,β,γ,δ-unsaturated carboxyl group. Acid hydrolysis of **1** with 5%

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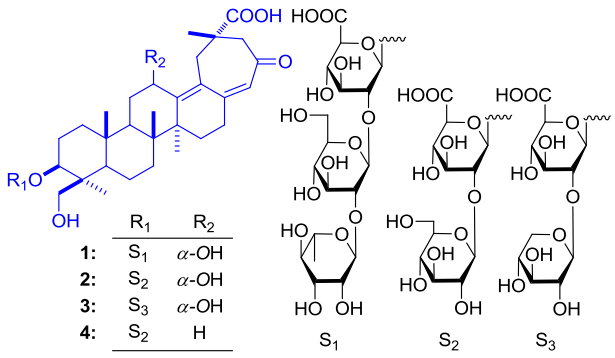

Fig. 1. Structure of compounds **1**–**4**.

aqueous H_2SO_4–1,4-dioxane (1:1, v/v) afforded D-glucuronic acid, D-glucose, and L-rhamnose, whose absolute configurations were determined by GC–MS analysis of their trimethylsilyl thiazolidine derivatives.[2] The ^{13}C NMR spectrum (C_5D_5N, Table 1) displayed 48 carbon signals. In addition to the carbon signals represented by the above mentioned sugars, the other 30 ones indicated **1** was a triterpenoid saponin. The analysis of the ^1H, ^{13}C and HSQC NMR data suggested the presences of five methyl groups [δ 0.70, 0.91, 1.48, 1.48, 1.74 (3H each, all s, H_3-25, 26, 23, 30, 27)], one oxygenated methylene proton [δ 3.32, 4.32 (1H each, both d, J=12.0 Hz), H_2-24], and two oxygenated methine protons [δ 3.40 (1H, dd, J=4.5, 11.5 Hz, H-3), 5.33 (1H, br s, H-12)], together with nine methylenes, three methines, and ten quaternary carbons for the aglycon of **1**. And the presences of α,β,γ,δ-unsaturated carboxyl group and olefinic bond were further certified by NMR data [δ_H 6.01 (1H, s, H-28); δ_C 150.7 (C-13), 159.5 (C-17), 130.7 (C-18), 126.7 (C-28)]. The ^1H–^1H COSY spectrum of **1** indicated the presence of seven partial structures written in bold lines (Fig. 2). The planar structure of the aglycon was determined based on the key HMBC correlations from H_2-16 to C-17, C-18, C-28; H_2-19 to C-13, C-17, C-18, C-21; H_2-21 to C-22, C-28, C-29; H_3-23 to C-3–C-5, C-24; H_3-25 to C-1, C-5, C-9, C-10; H_3-26 to C-7–9, C-14; H_3-27 to C-8, C-13–15; H-28 to C-16, C-18, C-21; H_3-30 to C-19–C-21, C-29 (Fig. 2). Moreover, the ^1H and ^{13}C NMR data on A, B rings and glycosyl groups in **1** were superimposable on those of azukisaponin V,[5] which indicated **1** was an oleanane type saponin with 3β-hydroxyl-24-hydroxymethyl. Meanwhile, NOE correlations between H-3 and H-5, H_3-23; H-5 and H-9; H-9 and H_3-27; H_2-24 and H_3-25; H_3-25 and H_3-26, H-11β; H_3-26 and H-11β observed in the 2D NOESY experiment, suggested A, B, C rings were in chair conformations and an α-orientation for H-3, H-5, H-9, 23-CH_3, 27-CH_3, as well as a β-orientation for 24-CH_2OH, 25-CH_3, and 26-CH_3. On the other hand, β-orientation of H-12 was proved by the NOE correlation between H-11β and H-12 and the coupling constant between H-11 and H-12 (near to zero). The NOE correlation between H_3-27 and H_2-16 was clearly observed, which suggested the conformation of D ring was semichair. The geometric configurations for olefinic bonds of $^{\Delta}$13(18)*E* and $^{\Delta}$17(28)*Z* were certified by NOE correlations found between H-12 and H_2-19; H_2-16 and H-28, respectively. Meanwhile, a β-orientation for 30-CH_3 was indicated by the NOE correlations between H-12 and H_3-30, H_2-19; H_3-30 and H-19β. Then, the relative configuration of **1** was assigned unambiguously (Fig. 3). The absolute configuration, 3*S*,4*S*,5*R*,8*R*,9*R*,10*R*,12*S*,$^{\Delta}$13(18)*E*,14*S*,$^{\Delta}$17(28)*Z*,20*S* of it was determined by comparing the experimental with the computational

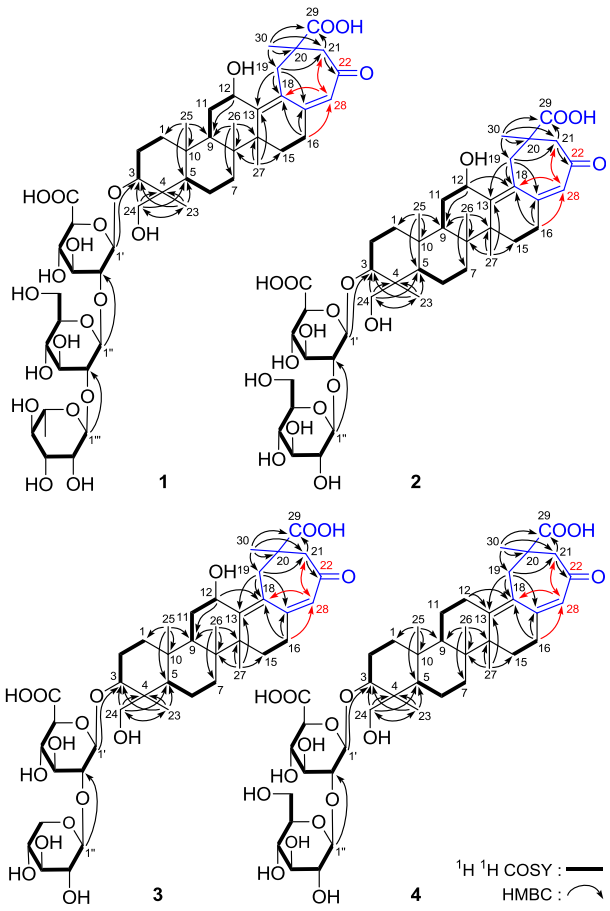

Fig. 2. Key ^1H–^1H COSY and HMBC correlations of **1**–**4**.

Table 1
^1H NMR data (500 MHz) for **1** in C_5D_5N (J in Hz)

No.	δ_H	No.	δ_H
1	1.00, m; 1.55, m	24	3.32, d (12.0)
2	1.95, m; 2.38, m		4.32, d (12.0)
3	3.40, dd (4.5, 11.5)	25	0.70, s
4	—	26	0.91, s
5	0.97, dd (3.0, 11.5)	27	1.74, s
6	1.21, m; 1.57, m	28	6.01, s
7	1.36, m; 1.54, m	29	—
8	—	30	1.48, s
9	2.08, br d (ca. 13)	1′	5.02, d (7.0)
10	—	2′	4.41, dd (7.0, 9.0)
11	1.66, m; 1.95, m	3′	4.71, t (9.0)
12	5.33, br s	4′	4.49, dd (9.0, 9.5)
13	—	5′	4.67, d (9.5)
14	—	6′	—
15	1.40, m	1″	5.86, d (7.5)
	1.86, dd (12.0, 14.0)	2″	4.34, dd (7.5, 9.0)
16	2.16, br d (ca. 12)	3″	4.25, t (9.0)
	2.54, t (12.0)	4″	4.51, t (9.0)
17	—	5″	3.62, m
18	—	6″	4.28, dd (5.0, 11.5)
19	3.02, d (14.0)		4.41, br d (ca. 12)
	3.44, d (14.0)	1‴	6.40, br s
20	—	2‴	4.77, br d (ca. 3)
21	2.70, d (12.5)	3‴	4.72, dd (3.0, 9.0)
	3.33, d (12.5)	4‴	4.37, t (9.0)
22	—	5‴	5.08, m
23	1.48, s	6‴	1.83, d (6.0)

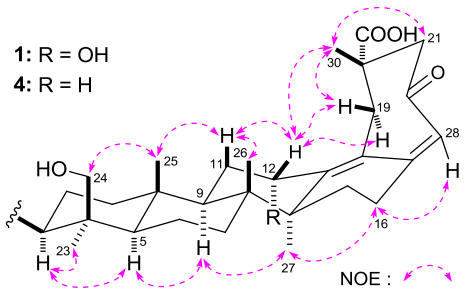

Fig. 3. Key NOESY correlations for aglycons of **1** and **4**.

electronic circular dichroism (ECD) spectra (Fig. 4).[6,7] Furthermore, the connectivity of oligoglycoside moiety to the aglycon part was characterized by HMBC experiment on **1**, in which long-range correlations were found from H-1′ to C-3, H-1″ to C-2′, H-1‴ to C-2″.

The molecular formula for astraisoolesaponins A_2 (**2**) and A_3 (**3**) were $C_{42}H_{62}O_{17}$ {*m/z* 873.3651 $[M+Cl]^-$ (calcd for $C_{42}H_{62}O_{17}Cl$, 873.3681)} and $C_{41}H_{60}O_{16}$ {*m/z* 843.3562 $[M+Cl]^-$ (calcd for $C_{41}H_{60}O_{16}Cl$, 843.3575)} obtained from HRESI-TOF-MS, respectively. The UV, CD, ECD, IR, ^1H, ^{13}C NMR (DMSO-d_6, Table 2) and 2D-NMR spectra (Fig. 2) suggested the aglycon of them was the same as that of **1**. Sugar analysis result indicated D-glucuronic acid and D-glucose presented in **2**, meanwhile D-glucuronic acid and D-xylose presented in **3**.[2] The linkage positions of sugar parts with aglycon were elucidated by the HMBC data, which showed long-range correlations from δ_H 4.45 (d, *J*=7.5 Hz, H-1′) to δ_C 89.5 (C-3); δ_H 4.62 (d, *J*=7.5 Hz, H-1″) to δ_C 79.9 (C-2′) for **2**, and δ_H 4.40 (d, *J*=8.0 Hz, H-1′) to δ_C 89.4 (C-3); δ_H 4.49 (d, *J*=7.5 Hz, H-1″) to δ_C 79.4 (C-2′) for **3**.

The molecular formula of astraisoolesaponin B (**4**) was found to be $C_{42}H_{62}O_{16}$ determined by negative-ion HRESI-TOF-MS (*m/z* 821.3987 $[M-H]^-$, calcd for $C_{42}H_{61}O_{16}$, 821.3965). The UV, IR, CD, ^1H, ^{13}C NMR (DMSO-d_6, Table 2) and $^1H-^1H$ COSY, HSQC, HMBC, and NOESY spectra indicated the aglycon of **4** was similar to that of **1**–**3**. According to the main ^{13}C NMR data differences between **4** and **1**–**3** aglycons (Table 2, in DMSO-d_6) displayed at C-8/C-9/C-11/C-12/C-18/C-27 taking C ring as the centre, we could deduce that the methine at C-12 was replaced by methylene. The correlations observed in $^1H-^1H$ COSY between δ_H 1.26, 1.68 (H_2-11) and δ_H 1.56 (H-9), 2.15, 2.64 (H_2-12), and in HMBC from δ_H 2.15, 2.64 (H_2-12) to δ_C 126.7 (C-18), 150.3 (C-13) confirmed the correctness of the above conjecture. The NOE correlations in NOESY (Fig. 3) spectra and the Cotton effect showed in CD spectra proved the absolute configuration in **4** was 3*S*,4*S*,5*R*,8*R*,9*R*,10*R*,^{4}13(18)*Z*,14*S*,^{4}17(28)*Z*,20*S*.

Liver and skeleton muscles are major organs in energy metabolism. In insulin resistance condition, insulin action is delayed and the ability of insulin to stimulate glucose uptake is blunted in skeleton muscle, insulin fails to suppress blood sugar production by the liver while allowing the production of hepatic triglycerides. This combination results in high blood sugar and fatty liver disease.[8] Compounds **1**–**4** showed significant inhibitory effects on oleic acid induced triglyceride accumulation in HepG2 cells, especially **3** and **4**, suppressing 20% intracellular triglyceride levels at 10 μM (Fig. 5). Moreover, compared with control group, compounds **3** and **4** significantly increased glucose uptake on L6 cell, but **1** and **2** had no effect on it (Fig. 6).

3. Conclusion

In summary, our current study describes the discovery of four novel saponins, astraisoolesaponins A_1–A_3 (**1**–**3**) and B (**4**) from AM, which possess a 28-methyl-rearranged carbon skeleton. Their absolute configurations were determined by ECD calculations. Moreover, compounds **3** and **4** displayed TG inhibitory effects in HepG2 cells and glucose uptake increase effects on L6 cells. The results of the present study indicated that **3** and **4** could be possible lead candidates for improvement of insulin resistance.

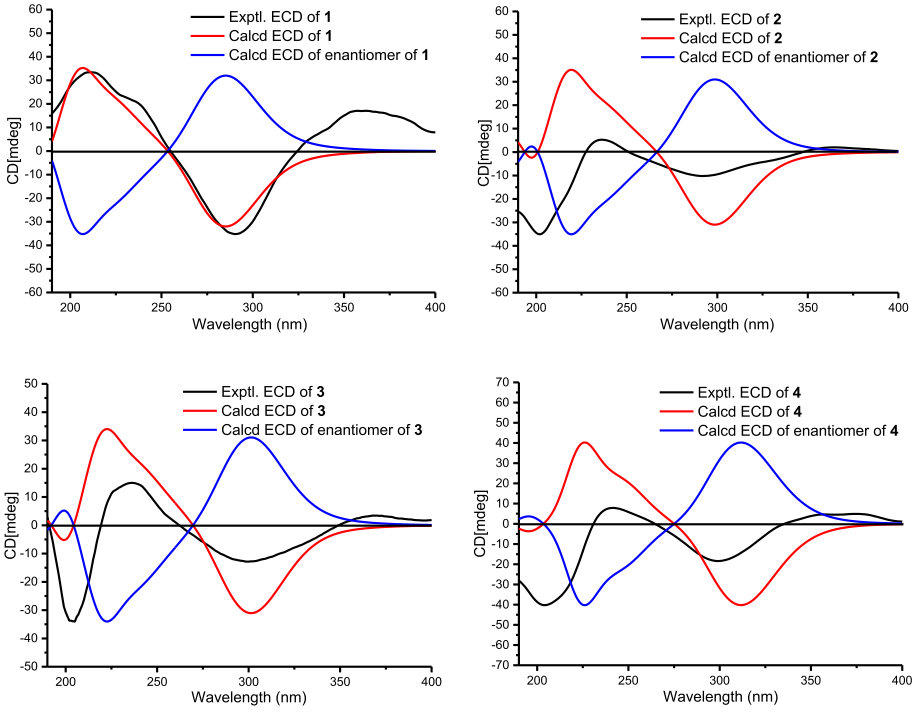

Fig. 4. Calculated (both the isolates and their enantiomers) and experimental ECD spectra of **1**–**4**.

Table 2
^{13}C NMR data of **1**–**4**

No.	1[a]	1[b]	2[b]	3[b]	4[b]	No.	1[a]	1[b]	2[b]	3[b]	4[b]
1	38.6	37.9	38.0	38.3	38.0	25	16.3	15.6	15.6	15.5	15.7
2	26.6	25.6	25.6	25.6	25.6	26	17.6	16.9	16.9	17.0	16.7
3	91.5	90.4	89.5	89.4	89.5	27	22.9	21.7	21.7	21.7	20.1
4	43.8	42.8	42.9	43.2	42.9	28	126.7	125.3	125.3	125.3	124.3
5	56.4	55.3	55.4	55.7	55.1	29	179.0	177.1	177.0	177.0	177.0
6	18.5	17.7	17.9	18.1	17.8	30	25.5	24.5	24.5	24.5	24.4
7	34.4	33.5	33.5	33.6	33.3	1′	105.3	103.7	103.0	103.3	103.0
8	41.4	44.1	44.1	44.1	40.7	2′	78.5	76.4	79.9	79.4	80.0
9	45.3	44.1	44.2	44.2	49.1	3	78.6	76.2	76.4	76.2	76.4
10	36.6	35.7	35.8	35.9	36.2	4′	73.8	71.8	71.2	71.4	71.3
11	30.5	29.6	29.5	29.6	21.6	5′	77.3	74.9	75.1	75.1	75.0
12	65.6	64.1	64.1	64.1	25.6	6′	173.1	170.5	170.4	170.3	170.6
13	150.7	149.8	149.7	149.8	150.3	1″	102.0	99.9	103.0	103.7	103.0
14	45.8	44.9	44.9	44.9	45.5	2″	77.8	76.7	74.0	74.0	74.1
15	32.7	31.8	31.8	31.8	30.8	3″	79.2	77.4	76.4	76.4	76.4
16	31.5	30.3	30.3	30.3	30.6	4″	69.8	68.7	68.7	69.1	68.7
17	159.5	159.4	159.3	159.3	159.8	5″	78.0	76.4	76.7	65.6	76.7
18	130.7	129.2	129.2	129.1	126.7	6″	61.4	60.0	60.2		60.2
19	40.9	40.4	39.4	39.4	39.7	1‴	102.0	100.0			
20	45.6	44.1	44.1	44.1	44.4	2‴	72.4	70.5			
21	53.3	52.2	52.1	52.2	52.0	3‴	72.7	70.5			
22	200.0	198.7	198.7	198.7	198.7	4‴	74.4	72.2			
23	22.8	22.0	21.9	21.9	21.9	5‴	69.5	67.9			
24	63.4	62.0	62.0	61.5	62.0	6‴	19.0	17.8			

[a] Determined in C_5D_5N.
[b] Determined in DMSO-d_6; **1**^{a}, 2^b–**4**^{b} were measured at 125 MHz and **1**^{b} at 125 MHz.

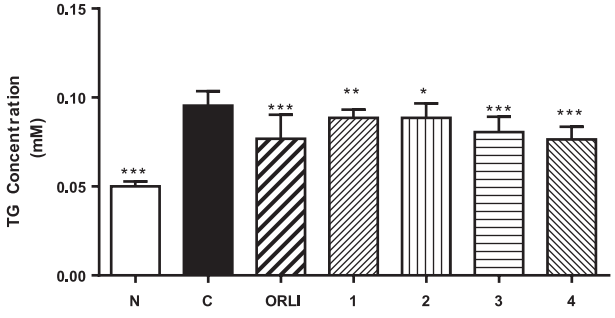

Fig. 5. Inhibitory effects of compounds **1**–**4** on oleic acid induced triglyceride accumulation in HepG2 cells. N: Normal group; C: Control group; ORLI: Orlistat 0.5 μM treated group; **1**–**4**: **1**–**4** 10 μM treated group. Values represent the mean±SD of six determinations. *P<0.05; **P<0.01, ***P<0.001 versus control group. N=6.

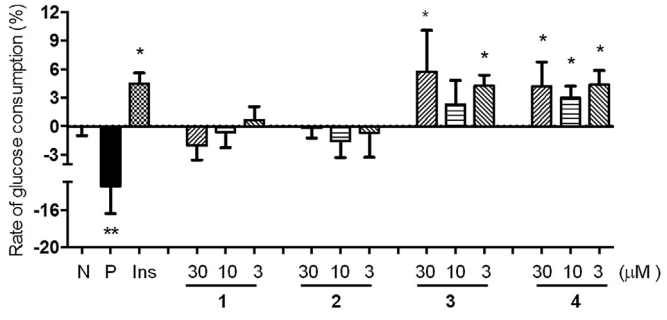

Fig. 6. Effects of compounds **1**–**4** on glucose uptake in L6 cells. N: Normal group; P: Cytochalasin B 10 μM treated group; Ins: insulin 10 μg/mL treated group. **1**–**4**: **1**–**4** treated group in different dose. Values represent the mean±SD of six determinations. *P<0.05 versus control group. N=6.

4. Experimental

4.1. General

The following instruments were used to obtain physical data: UV spectra were recorded on a Varian Cary 50 UV–vis spectrophotometer. IR spectra were obtained on a Varian 640-IR FTIR spectrophotometer. Optical rotations were determined on a Rudolph Autopol® IV automatic polarimeter. NMR spectra were measured on a Bruker AVANCE III 500 MHz and/or 400 MHz NMR spectrometer with TMS as an internal standard. Negative-ion HRESI-TOF-MS were determined on an Agilent 6520 Accurate-Mass Q-Tof MS spectrometer. Column chromatographies (CC) were performed on macroporous resin D101 (Haiguang Chemical Co., Ltd., Tianjin, China), Silica gel (48–75 μm, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), ODS (40–63 μm, YMC Co., Ltd., Tokyo, Japan), and Sephadex LH-20 (Ge Healthcare Bio-Sciences, Uppsala, Sweden), and Preparative HPLC (PHPLC) column, Cosmosil 5C18-MS-II (20 mm i.d.×250 mm, 5 μM, Nakalai Tesque, Inc., Tokyo, Japan) were used to purify the constituents.

4.2. Plant material

The stems of *A. membranaceus* (Fisch.) Bge. *var. mongholicus* (Bge.) Hsiao. were collected from Gansu province, China, and identified by Dr. Li Tianxiang. The voucher specimen was deposited at the Academy of Traditional Chinese Medicine of Tianjin University of TCM.

4.3. Extraction and isolation

The stems of AM (8.0 kg) were cut and refluxed with 70% ethanol–water. Then, the 70% ethanol–water extract (580 g) was partitioned in a $CHCl_3$–H_2O mixture (1:1, v/v), and $CHCl_3$ (190 g) and H_2O (390 g) layers were given. Then the H_2O layer (300 g) was subjected to D101 macroporous resin CC (H_2O→95% EtOH). As a result, H_2O (179 g) and 95% EtOH (116 g) eluted fractions were obtained. The 95% EtOH eluate (90.6 g) was subjected to [$CHCl_3$–MeOH (100:5, v/v)→$CHCl_3$–MeOH–H_2O (10:3:1→7:3:1→6:4:1, v/v/v, lower layer)→MeOH] to yield fourteen fractions (Fr. 1–14). Fraction 9 (7.0 g) was isolated by ODS CC [MeOH–H_2O (10:90→20:80→30:70→40:60→50:50→70:30→80:20→100:0, v/v)] to afford twenty fractions (Fr. 9-1–9–20). Fraction 9–12 was separated by PHPLC [MeOH–H_2O (40:60, v/v)] to obtain six fractions (Fr. 9–12-1–9–12-6). Fractions 9–12-4 (56.5 mg) and 9–12-5 (81.0 mg) were further purified by PHPLC [CH_3CN–H_2O (24:76, v/v)+1% HAc] to give astraisoolesaponins A_2 (**2**, 35.7 mg) and A_3 (**3**, 10.7 mg), respectively. Fraction 9–14 (734.0 mg) was isolated by PHPLC [MeOH–H_2O (55:45, v/v)+1% HAc] to yield seven fractions (Fr. 9–14-1–9–14-7). Fraction 9–14-3 (47.2 mg) was subjected to [$CHCl_3$–MeOH–H_2O (10:3:1→7:3:1, v/v/v, lower layer)], and three fractions (Fr. 9–14-3-1–9–14-3-3) were obtained. Fraction 9–14-3-2 (54.2 mg) was purified by PHPLC [CH_3CN–H_2O (26:74, v/v)+1% HAc] to afford astraisoolesaponin B (**4**, 13.7 mg). Fraction 12 (8.2 g) was separated by PHPLC [CH_3CN–H_2O (30:70→40:60→50:50→60:40→80:20, v/v)+1% HAc] to give nineteen fractions (Fr. 12-1–12–19). Fraction 12–8 (687.8 mg) was isolated by PHPLC [MeOH–H_2O (32:68, v/v)+1% HAc], to gain six fractons (Fr. 12–8-1–12–8-6). Fraction 12–8-6 (237.8 mg) was subjected to SiO_2 gel CC [$CHCl_3$–MeOH–H_2O (7:3:1→6:4:1, v/v/v, lower layer)], and three fractions (Fr. 12–8-6-1–12–8-6-3) were obtained. Fraction 12–8-6-3 (123.6 mg) was further purified by PHPLC [MeOH–H_2O (23:77, v/v)+1% HAc] to afford astraisoolesaponin A_1 (**1**, 31.5 mg).

*4.3.1. Astraisoolesaponin A_1 (**1**).* White powder. $[\alpha]_D^{25}$ −73.5° (c=0.65, MeOH); IR ν_{max} (KBr) cm^{-1}: 3400, 2928, 2858, 1719, 1642, 1455, 1405, 1269, 1170, 1128, 1073, 1041; UV λ_{max} (MeOH) nm (log ε): 294 (4.08). CD (CH_3CN, c=0.0011) mdeg (λ_{nm}): +0.49 (211), −0.51 (294), +0.25 (356). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.92, 1.64 (1H each, both m, H_2-1), 1.70, 1.91 (1H each, both m, H_2-2), 3.26 (1H, dd, J=4.0, 12.4 Hz, H-3), 0.93 (1H, dd, J=2.8, 11.6 Hz, H-5), 1.24, 1.58 (1H each, both m, H_2-6), 1.31, 1.43 (1H each, both m, H_2-7), 1.76 (1H, dd,

J=6.0, 10.4 Hz, H-9), 1.46, 1.64 (1H each, both m, H_2-11), 4.74 (1H, br s, H-12), 1.34, 1.75 (1H each, both m, H_2-15), [2.17 (1H, br d, ca. *J*=12 Hz), 2.45 (1H, m), H_2-16], 2.35, 3.01 (1H each, both d, *J*=8.0 Hz, H_2-19), 2.33, 2.62 (1H each, both d, *J*=12.0 Hz, H_2-21), 1.11 (3H, s, H_3-23), 3.10, 3.89 (1H each, both d, *J*=10.8 Hz, H_2-24), 0.74 (3H, s, H_3-25), 0.77 (3H, s, H_3-26), 1.38 (3H, s, H_3-27), 5.65 (1H, s, H-28), 1.14 (3H, s, H_3-30), 4.27 (1H, d, *J*=6.4 Hz, H-1′), 3.44 (2H, m, H-2′ and 3′), 3.22 (1H, dd, *J*=8.0, 9.2 Hz, H-4′), 3.26 (1H, d, *J*=9.2 Hz, H-5′), 4.81 (1H, d, *J*=7.6 Hz, H-1″), 3.17 (1H, dd, *J*=7.6, 8.0 Hz, H-2″), 3.29 (1H, t, *J*=8.0 Hz, H-3″), 3.19 (1H, dd, *J*=8.0, 10.4 Hz, H-4″), 3.01 (1H, m, H-5″), [3.45 (1H, dd, *J*=5.6, 11.6 Hz), 3.61 (1H, br d, ca. *J*=12 Hz), H_2-6″], 5.03 (1H, br s, H-1‴), 3.68 (1H, br d, ca. *J*=4 Hz, H-2‴), 3.54 (1H, dd, *J*=4.0, 8.0 Hz, H-3‴), 3.42 (1H, t, *J*=8.0 Hz, H-4‴), 3.98 (1H, m, H-5‴), 1.12 (3H, d, *J*=6.0 Hz, H_3-6‴); ^{13}C NMR (100 MHz, DMSO-d_6) spectroscopy data, see Table 2. HRESI-TOF-MS: Negative-ion mode *m/z* 1019.4238 $[M+Cl]^-$ (calcd for $C_{48}H_{72}O_{21}Cl$ 1019.4260).

*4.3.2. Astraisoolesaponin A$_2$ (**2**).* White powder. $[\alpha]_D^{25}$ −31.1° (*c*=0.84, MeOH); IR ν_{max} (KBr) cm^{-1}: 3339, 2924, 2855, 1715, 1637, 1458, 1402, 1276, 1199, 1168, 1074, 1039; UV λ_{max} (MeOH) nm (log ε): 293 (3.89). CD (CH_3CN, *c*=0.0019) mdeg (λ_{nm}): +2.42 (236), −4.69 (292), +0.92 (365). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.92, 1.64 (1H each, both m, H_2-1), 1.70, 1.87 (1H each, both m, H_2-2), 3.31 (1H, dd, *J*=4.5, 11.5 Hz, H-3), 0.91 (1H, dd, *J*=3.0, 11.0 Hz, H-5), 1.32, 1.57 (1H each, both m, H_2-6), 1.33, 1.42 (1H each, both m, H_2-7), 1.76 (1H, dd, *J*=6.0, 9.0 Hz, H-9), 1.48, 1.64 (1H each, both m, H_2-11), 4.74 (1H, br s, H-12), 1.34, 1.75 (1H each, both m, H_2-15), [2.17 (1H, br d, ca. *J*=13 Hz), 2.46 (1H, m), H_2-16], 2.38, 2.98 (1H each, both d, *J*=10.0 Hz, H_2-19), 2.33, 2.63 (1H each, both d, *J*=12.0 Hz, H_2-21), 1.11 (3H, s, H_3-23), 3.15, 3.89 (1H each, both d, *J*=11.0 Hz, H_2-24), 0.76 (3H, s, H_3-25), 0.77 (3H, s, H_3-26), 1.38 (3H, s, H_3-27), 5.65 (1H, s, H-28), 1.15 (3H, s, H_3-30), 4.45 (1H, d, *J*=7.5 Hz, H-1′), 3.37 (1H, dd, *J*=7.5, 9.0 Hz, H-2′), 3.47 (1H, t, *J*=9.0 Hz, H-3′), 3.35 (1H, dd, *J*=9.0, 9.5 Hz, H-4′), 3.65 (1H, d, *J*=9.5 Hz, H-5′), 4.62 (1H, d, *J*=7.5 Hz, H-1″), 2.98 (1H, dd, *J*=7.5, 9.0 Hz, H-2″), 3.14 (1H, t, *J*=9.0 Hz, H-3″), 3.15 (1H, t, *J*=9.0 Hz, H-4″), 3.07 (1H, m, H-5″), [3.52 (1H, dd, *J*=4.0, 11.5 Hz), 3.62 (1H, br d, ca. *J*=12 Hz), H_2-6″]; ^{13}C NMR (125 MHz, DMSO-d_6) spectroscopy data, see Table 2. HRESI-TOF-MS: Negative-ion mode *m/z* 873.3651 $[M+Cl]^-$ (calcd for $C_{42}H_{62}O_{17}Cl$ 873.3681).

*4.3.3. Astraisoolesaponin A$_3$ (**3**).* White powder. $[\alpha]_D^{25}$ −51.2° (*c*=0.13, MeOH); IR ν_{max} (KBr) cm^{-1}: 3430, 2924, 2854, 1719, 1631, 1460, 1401, 1170, 1046; UV λ_{max} (MeOH) nm (log ε): 293 (3.70). CD (CH_3CN, *c*=0.0014) mdeg (λ_{nm}): +2.25 (236), −1.84 (294), +0.51 (370). ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.93, 1.64 (1H each, both m, H_2-1), 1.74, 1.83 (1H each, both m, H_2-2), 3.21 (1H, dd, *J*=5.0, 11.0 Hz, H-3), 0.88 (1H, dd, *J*=3.0, 11.5 Hz, H-5), 1.35, 1.58 (1H each, both m, H_2-6), 1.33, 1.43 (1H each, both m, H_2-7), 1.75 (1H, dd, *J*=6.0, 11.0 Hz, H-9), 1.46, 1.65 (1H each, both m, H_2-11), 4.74 (1H, br s, H-12), 1.37 1.74 (1H each, both m, H_2-15), [2.16 (1H, br d, ca. *J*=13 Hz), 2.46 (1H, m), H_2-16], 2.39 2.98 (1H each, both d, *J*=9.5 Hz, H_2-19), 2.33, 2.63 (1H each, both d, *J*=12.0 Hz, H_2-21), 1.08 (3H, s, H_3-23), 3.17, 3.76 (1H each, both d, *J*=9.0 Hz, H_2-24), 0.77 (3H, s, H_3-25), 0.78 (3H, s, H_3-26), 1.37 (3H, s, H_3-27), 5.65 (1H, s, H-28), 1.15 (3H, s, H_3-30), 4.40 (1H, d, *J*=8.0 Hz, H-1′), 3.33 (1H, dd, *J*=8.0, 9.0 Hz, H-2′), 3.41 (1H, t, *J*=9.0 Hz, H-3′), 3.33 (1H, m, overlapped, H-4′), 3.62 (1H, m, overlapped, H-5′), 4.49 (1H, d, *J*=7.5 Hz, H-1″), 2.97 (1H, dd, *J*=7.5, 9.0 Hz, H-2″), 3.08 (1H, t, *J*=9.0 Hz, H-3″), 3.25 (1H, m, H-4″), [2.98 (1H, t, *J*=11.0 Hz), 3.62 (1H, m, overlapped), H_2-5″]; ^{13}C NMR (125 MHz, DMSO-d_6) spectroscopy data, see Table 2. HRESI-TOF-MS: Negative-ion mode *m/z* 843.3562 $[M+Cl]^-$ (calcd for $C_{41}H_{60}O_{16}Cl$ 843.3575).

*4.3.4. Astraisoolesaponin B (**4**).* White powder. $[\alpha]_D^{25}$ −92.1° (*c*=0.84, MeOH); IR ν_{max} (KBr) cm^{-1}: 3339, 2933, 1715, 1635, 1604, 1404, 1381, 1270, 1192, 1069, 1040; UV λ_{max} (MeOH) nm (log ε): 301 (3.98). CD (CH_3CN, *c*=0.0013) mdeg (λ_{nm}): +0.27 (240), −0.63 (298), +0.17 (375). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.00, 1.64 (1H each, both m, H_2-1), 1.70, 1.90 (1H each, both m, H_2-2), 3.27 (1H, dd, *J*=5.0, 11.0 Hz, H-3), 0.90 (1H, dd, *J*=3.0, 11.0 Hz, H-5), 1.35, 1.56 (1H each, both m, H_2-6), 1.33, 1.38 (1H each, both m, H_2-7), 1.56 (1H, dd, *J*=6.0, 11.0 Hz, H-9), 1.26, 1.68 (1H each, both m, H_2-11), 2.15, 2.64 (1H each, both m, H_2-12), 1.40, 1.71 (1H each, both m, H_2-15), [2.16 (1H, br d, ca. *J*=13 Hz), 2.33 (1H, m), H_2-16], 2.38 2.75 (1H each, both d, *J*=14.0 Hz, H_2-19), 2.34, 2.60 (1H each, both d, *J*=12.0 Hz, H_2-21), 1.10 (3H, s, H_3-23), 3.15, 3.87 (1H each, both d, *J*=10.5 Hz, H_2-24), 0.74 (3H, s, H_3-25), 0.80 (3H, s, H_3-26), 1.19 (3H, s, H_3-27), 5.59 (1H, s, H-28), 1.20 (3H, s, H_3-30), 4.42 (1H, d, *J*=8.0 Hz, H-1′), 3.35 (1H, dd, *J*=8.0, 9.0 Hz, H-2′), 3.45 (1H, t, *J*=9.0 Hz, H-3′), 3.32 (1H, t, *J*=9.0 Hz, H-4′), 3.60 (1H, d, *J*=9.0 Hz, H-5′), 4.60 (1H, d, *J*=7.5 Hz, H-1″), 2.97 (1H, dd, *J*=7.5, 9.0 Hz, H-2″), 3.14 (2H, m, H-3″ and 4″), 3.06 (1H, m, H-5″), [3.51 (1H, dd, *J*=4.0, 12.0 Hz), 3.61 (1H, br d, ca. *J*=12 Hz), H_2-6″]; ^{13}C NMR (125 MHz, DMSO-d_6) spectroscopy data, see Table 2. HRESI-TOF-MS: Negative-ion mode *m/z* 821.3987 $[M-|-H]^-$ (calcd for $C_{42}H_{61}O_{16}$ 821.3965).

4.4. Acid hydrolysis of 1–4

Solution of new compounds **1**–**4** (each 2.0 mg) in 5% aqueous H_2SO_4-1,4-dioxane were treated by using the known method:[2] heated under reflux for 1 h, respectively, neutralized with Amberlite IRA-400 (OH^- form), removed by filtration, dealed with ODS column ($H_2O \rightarrow$ MeOH), reacted with L-cysteine methyl ester hydrochloridein pyridine and *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), successively. Finally, the reaction product was determined by GC–MS analysis (GC conditions, column: RESTEK Rxi-5ms, 30 m×0.25 mm (i.d.) capillary column; column temperature: 230 °C; carrier gas: He.). As result, the identification for derivatives of D-glucuronic acid, D-glucose, and L-rhamnose from **1**; D-glucuronic acid and D-glucose from **2** and **4**; D-glucuronic acid and D-xylose from **3** present in the supernatant were performed by comparison of their retention times with the hydrolysate with their authentic samples treated in the same way, t_R: (i) 13.3 min (D-glucuronic acid), (ii) 11.4 min (D-glucose), (iii) 7.7 min (L-rhamnose), and (IV) 6.4 min (D-xylose).

4.5. Computations

The ECD spectra for the optimized conformers were calculated at the CAM-B3LYP/SVP level with a CPCM solvent model in acetonitrile, and the calculated ECD spectra of different conformers were simulated with a half bandwidth of 0.3–0.4 eV. The ECD curves were extracted by SpecDis 1.62 software. The overall ECD curves of all the compounds were weighed by Boltzmann distribution after UV correction.

4.6. Bioassay

4.6.1. Inhibitory effects on oleic acid induced triglyceride accumulation in HepG2 cells. The hepatic cell line HepG2 (IBMS, CAMS/PUMC, Beijing China) were maintained in high glucose Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin under a humidified atmosphere of 5% CO_2 in air. After growth to 80% confluence, cells were seeded at 1×10^4 cells/mL on 48-well dish. After 24 h incubation, the medium was switched to high glucose MEM and supplemented with 10% FBS and 0.2 mM oleic acid sodium salt, together with sample DMSO solution (final concentration of DMSO was less than 0.1%). After 48 h incubation, the amount of intracellular triglycerides was determined with the Triglycerides kit (BioSino Bio-technology and Science Inc., China) after cell lysis.

4.6.2. Increase effects on glucose uptake in L6 cells. Differentiated L6 mature myotubes were cultured on 48-well plates (with 2×10^4 cells/

mL), incubated in glucose and serum-free HEPES-buffered saline (HBS) for 2 h. The medium was switched to HBS containing 1 mg/mL glucose, together with sample DMSO solution (final concentration of DMSO was less than 0.1%). After 4 h incubation, the amount of glucose in culture supernatant was determined with the glucose kit (BioSino Bio-technology and Science Inc., China). Insulin (10 μg/mL) was used as positive control and Cytochalasin B (10 μM), a glucose transporter inhibitor, was used as negative control.

4.6.3. Statistical analysis. Values are expressed as mean±S.D. All the grouped data were statistically performed with SPSS 11.0. Significant differences between means were evaluated by one-way analysis of variance (ANOVA) and Tukey's Studentized range test was used for post hoc evaluations. $P<0.05$ was considered to indicate statistical significance.

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Supplementary data

Supplementary data (NMR spectroscopic data for all new compounds) associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2016.09.034.

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