SUPPLEMENTARY MATERIAL

A new triterpenoid saponin from *Gleditsia sinensis* and its antiproliferative activity

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Abstract

Chemical investigation of the anomalous fruits of *Gleditsia sinensis* led to the isolation and identification of a new triterpenoid saponin, 3-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl oleanolic acid 28-*O*- β -D-xylopyranosyl-(1 $\rightarrow$ 4)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 4)- β -D-xylopyranosyl-(1 $\rightarrow$ 4)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 3)- β -D-glucopyranosyl ester (**1**), along with other nine known compounds (**2–10**). All the isolates from this species were reported for the first time. The structure of Compound **1** was determined by detailed analysis using various analytical techniques including 1D and 2D NMR. *In vitro* anti-proliferative activities of Compounds **1** on MCF-7 and Hep-G2 tumor cell lines were evaluated. IC₅₀ values against the two cell lines were 9.5 and 11.6 μ M, respectively.

Keywords: *Gleditsia sinensis*; triterpenoid saponin; antiproliferative

1. Extraction and isolation

The anomalous fruits (10 kg) of *G. sinensis* were ground into powder and then extracted with 90% ethanol (50.0 L) by heating reflux for 3 hrs using a reaction still. Following filtration and vacuum-concentration of the combined solution yielded a crude extract (919.0 g), which was then suspended in water (3000 mL) and extracted successively with petroleum ether (3×1000 mL), EtOAc (3×1000 mL) and *n*-BuOH (3×1000 mL) to afford three organic fractions.

The *n*-BuOH fraction (638.0 g) was submitted to column chromatography (CC) over HP-20, eluted with methanol – water ($V_1/V_2 = 0:1, 3:7, 5:5, 7:3, 9:1$) gradient to yield five subfractions (Fr. 1 42.3 g, Fr. 2 65.8 g, Fr. 3 32.5 g, Fr. 4 22.1 g, Fr. 5 117.2 g). The Fr. 3 was separated on silica gel CC eluted with dichloromethane-methanol to yield compounds **2** (132.1 mg), **3** (14.9 mg) and fraction 3.1 – 3.4. Fr. 3.1 (4.2 g) was submitted to ODS CC eluted with methanol – water ($V_1/V_2 = 3:7$) to yield **4** (15.3 mg) and **5** (8.4 mg). Purification of Fr. 3.2 (3.6 g) by ODS CC eluted with methanol – water to afford compound **6** (9.2 mg). Part of fraction 3.3 (5.9 g) was also chromatographed over ODS columns and then repeatedly subjected to semi-preparative HPLC (MeOH: H₂O) purification to afford **1** (142 mg) and **7** (9.1 mg). By the same method, fraction 3.4 (2.7 g) furnished **10** (10.6 mg), **8** + **9** (49 mg). Following repeated HPLC [MeOH- H₂O (38:62)] purification, **8** + **9** gave **8** (15.2 mg) and **9** (3.8 mg).

2. Cytotoxicity activity experiments

The cytotoxic effects of compounds **1–4** were estimated *in vitro* against the MCF-7 and Hep-G2 cancer cell lines by MTT assay. Briefly, the cell suspensions (200 mL) at a density of 5×10^4 cells·mL⁻¹ were distributed into 96-well cell culture plates and cultured at 37°C in incubator with 5% CO₂ for 24 h. The solution of five different concentrations of the test compounds (2 mL in DMSO) was added to each well and further incubated for 48 h under the same conditions. The MTT solution (20 mL) was then added to each well and incubated for 4 hrs. Finally, the supernatant was discarded and limited DMSO was added to each well to dissolve the blue-violet

crystals completely, the optical density (OD) values were then read on the microplate reader at 490 nm. All tests and analyses were carried out in triplicate. Dose-response curves were generated and the IC₅₀ values were defined as the concentration of compound required to inhibit cell proliferation by 50%. Martrine, an approved agent for the treatment of many tumors, was applied as a positive control.

Legends for Table

Table S1. ¹H, ¹³C-NMR data of **1** at 400 and 100 MHz in DMSO-d₆ (δ in ppm; J in Hz)

Legends for Figures

Figure S1. ¹H-NMR spectrum of compound **1**

Figure S2. ¹³C-NMR spectrum of compound **1**

Figure S3. HSQC spectrum of compound **1**

Figure S4-1. HMBC spectrum of compound **1**

Figure S4-2. Key HMBC (H→C) correlations of **1**

Figure S5-1. ROESY spectrum of compound **1**

Figure S5-2. Key ROESY correlations of **1**

Figure S6. HR-ESI-MS of compound **1**

Table S1. ^1H , ^{13}C -NMR data of **1** at 400 and 100 MHz in DMSO- d_6 (δ in ppm; J in Hz)

Position	δ_{C}	δ_{H}
Aglycon		
1	38.55	1.35 m, 0.84 m
2	26.20	2.24 m, 1.83 m
3	88.10	3.10 m
4	39.20	
5	55.50	0.81 m
6	18.41	1.67 m, 1.32 m
7	33.26	1.63 m, 1.58 m
8	39.61	
9	47.49	1.62 m
10	36.76	
11	23.43	2.03 m, 2.08 m
12	122.20	5.18 br s
13	143.67	
14	41.86	
15	27.99	1.23 m, 1.57 m
16	22.73	1.85 m, 2.05 m
17	46.72	
18	41.39	3.17 dd ($J = 9.4, 2$ Hz), 3.43 brd
19	46.13	1.78 m, 1.26 m
20	30.78	
21	33.65	1.35 m, 1.16 m
22	32.04	2.09 m, 1.70 m
23	27.98	0.98 s
24	16.97	0.75 s
25	15.76	0.85 s
26	17.14	0.68 s
27	25.78	1.08 s
28	175.92	
29	32.71	0.84 s
30	23.92	0.82 s
C ₃ -Glc		
1	105.66	4.15 d ($J = 7.6$ Hz)
2	74.39	3.02 m
3	77.22	3.38 m
4	71.14	3.36 m
5	75.52	3.25 m
6	68.68	3.46 m, 3.84 m
C ₂₈ -Glc		
1	93.41	5.31 m
2	74.20	3.19 m
3	76.11	3.18 m

4	69.97	3.25 m
5	76.50	3.13 m
6	65.96	3.06 m, 3.68 m
Ara		
1	101.41	4.45 d ($J = 4.8$ Hz)
2	79.35	3.54 m
3	72.36	3.15 m
4	66.26	3.60 m
5	63.17	3.30 m
Rha		
1	100.10	5.13 br s
2	70.14	3.75 m
3	71.02	3.38 m
4	83.67	3.39 m
5	67.31	3.60 m
6	18.40	1.09 d ($J = 6$ Hz)
Rha'		
1	100.57	4.48 br s
2	69.88	3.28 m
3	70.93	3.08 m
4	86.26	3.32 m
5	68.28	3.34 m
6	18.20	1.16 d ($J = 5.6$ Hz)
Xyl		
1	105.08	4.25 d ($J = 7.2$ Hz),
2	74.35	3.09 m
3	76.53	3.38 m
4	69.82	3.22 m
5	66.18	3.67 t, 3.09 m
Xyl'		
1	105.65	4.42 d ($J = 7.6$ Hz),
2	74.10	3.02 m
3	75.91	3.29 m
4	68.50	3.35 m
5	66.21	3.48 t, 3.37 m
Xyl''		
1	104.80	4.38 d ($J = 7.6$ Hz)
2	74.25	3.18 m
3	77.71	3.38 m
4	70.97	3.58 m
5	66.02	3.68 t, 3.07 m

Figure S1. ^1H -NMR Spectrum of compound **1**

Figure S2. ^{13}C -NMR Spectrum of compound **1**

Figure S4-1. HMBC Spectrum of compound **1**

Figure S4-2. Key HMBC (H→C) correlations of **1**

Figure S5-1. ROESY Spectrum of compound **1**

Figure S5-2. Key ROESY correlations of **1**

Figure S6. HR-ESI-MS of compound **1**