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→2)-**α**-L-arabinopyranosyl-(1→6)-**β**-D-glucopyranosyl oleanolic acid 28-**O**-**β**-D-xylopyranosyl-(1→4)-**α**-L-rhamnopyranosyl-(1→4)-**β**-D-xylopyranosyl-(1→4)-**α**-L-rhamnopyranosyl-(1→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. IC<sub>50</sub> values against the two cell lines were 9.5 and 11.6 μM, 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 (V1/V2 = 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 (V1/V2 = 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 IC$_{50}$ 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. $^1$H, $^{13}$C-NMR data of 1 at 400 and 100 MHz in DMSO-d$_6$ ($\delta$ in ppm; $J$ in Hz)

**Legends for Figures**

Figure S1. $^1$H-NMR spectrum of compound 1

Figure S2. $^{13}$C-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
| Position | $\delta_C$ | $\delta_H$ |
|----------|-----------|-----------|
| **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 |
| **C3-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 |
| **C28-Glc** | | |
| 1 | 93.41 | 5.31 m |
| 2 | 74.20 | 3.19 m |
| 3 | 76.11 | 3.18 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. $^1$H-NMR Spectrum of compound 1

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