Supporting Information

Anti-inflammatory Terpenes from Flowers of *Inula japonica*

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Materials and Methods

Measurement of cell viability

Cell viability was assessed by the MTT assay. RAW264.7 cells and BMMCs plated in 96-well plates were treated with several concentrations of isolates for 20 h and 4 h, respectively. Then, MTT (5mg/mL) was added and incubated for an additional 4 h. The culture medium was discarded, and the formazan blue that formed in the cells was dissolved in DMSO. The optical densities (OD) at 490 nm were measured using a microplate reader. The cytotoxicity of isolates in RAW264.7 cells as shown in Table 1S.

Extraction and isolation

The powdered and dried flowers of *I. japonica* (8.0 kg) were extracted with 75% ethanol (15 L × 3, 2 h each time) under reflux. The extracts were concentrated to give a residue (600 g), which was suspended in water and partitioned with petroleum ether
(PE), EtOAc, and n-BuOH, successively.

The EtOAc-partitioned extract (117 g) was chromatographed on a silica gel column (1.0 kg, 100-200 mesh, 80 × 10 cm), eluted with a gradient solvent system (PE-EtOAc, v/v, 4:1, 3:1, 2:1, 1:1, 1:2, EtOAc, EtOAc-MeOH, v/v, 19:1, 9:1, 4:1, MeOH, 3000 mL each) to produce 23 fractions (fractions 1-23). Fraction 8 (2.26 g) was chromatographed on a Toyopearl HW-40C column (3.5 × 120 cm, CH$_2$Cl$_2$-MeOH, v/v, 2:1, 1200 mL) to produce five fractions (fractions 8.1-8.5). Fraction 8.3 (566.1 mg) was separated by semipreparative HPLC (ODS, 5 µm, 2 × 25 cm, MeOH-H$_2$O, v/v, 9:1, flow rate 3 mL/min) to produce 1 (2.2 mg) and 7 (204.5 mg). Fraction 9 (2.78 g) was chromatographed on a Toyopearl HW-40C column (3.5 × 120 cm, CH$_2$Cl$_2$-MeOH, v/v, 2:1, 1200 mL) to produce six fractions (fractions 10.1-10.6). Fraction 9.2 (1.05 g) was separated by semipreparative HPLC (ODS, 5 µm, 2 × 25 cm, MeOH-H$_2$O, v/v, 8:2, flow rate 3 mL/min) to produce 10 (147.5 mg). Fraction 9.5 (213.0 mg) was separated by semipreparative HPLC (ODS, 5 µm, 2 × 25 cm, MeOH-H$_2$O, v/v, 8:2, flow rate 3 mL/min), and then by gel permeation chromatography (GPC) (2 × 50 cm × 2, MeOH, flow rate 3 mL/min) to yield 6 (33.0 mg). Fraction 12 (2.54 g) was chromatographed on a Toyopearl HW-40C column (3.5 × 120 cm, CH$_2$Cl$_2$-MeOH, v/v, 2:1, 1200 mL) to produce seven fractions (fractions 12.1-12.7). Fractions 12.2 (810.0 mg) and 12.3 (50.0 mg) were recrystallized to give 13 (690.0 mg) and 14 (10.5 mg). Fraction 14 (2.00 g) was chromatographed on a Toyopearl HW-40C column (3.5 × 120 cm, CH$_2$Cl$_2$-MeOH, v/v, 2:1, 1200 mL) to produce seven fractions (fractions 14.1-14.7), in which fraction 14.5 was 15 (200.0 mg). Fraction 14.6 (320.0 mg) was separated by semipreparative HPLC (ODS, 5 µm, 2 × 25 cm, MeOH-H$_2$O, v/v, 7:3, flow rate 3 mL/min), and then by GPC (2 × 50 cm × 2, MeOH, flow rate 3 mL/min) to produce 3 (2.9 mg) and 12 (4.0 mg).
Fraction 15 (1.90 g) was chromatographed on a Toyopearl HW-40C column (3.5 × 120 cm, CH₂Cl₂-MeOH, v/v, 1:1, 1200 mL) to produce six fractions (fractions 15.1-15.6). Fraction 15.2 (400.0 mg) was separated by GPC (2 × 50 cm × 2, MeOH, flow rate 3 mL/min), and then by HPLC (ODS, 5 μm, 2 × 25 cm, MeOH-H₂O, v/v, 6:4, flow rate 3 mL/min) to produce 11 (27.3 mg). Fraction 17 (3.00 g) was chromatographed on a Toyopearl HW-40C column (3.5 × 120 cm, CH₂Cl₂-MeOH, v/v, 1:1, 1200 mL) to produce four fractions (fractions 17.1-17.4). Fraction 17.3 (490.7 mg) was separated by GPC (2 × 50 cm × 2, MeOH, flow rate 3 mL/min), semipreparative HPLC (ODS, 5 μm, 2 × 25 cm, MeOH-H₂O, v/v, 45:55, flow rate 3 mL/min) to produce 8 (6.8 mg) and 9 (17.6 mg). Fraction 19 (1.82 g) was chromatographed on a Toyopearl HW-40C column (3.5 × 120 cm, CH₂Cl₂-MeOH, v/v, 1:1, 1200 mL) to produce six fractions (fractions 19.1-19.6). Fraction 19.4 (219.1 mg) was separated by semipreparative HPLC (ODS, 5 μm, 2 × 25 cm, MeOH-H₂O, v/v, 55:45, flow rate 3 mL/min) to yield 2 (10.0 mg), 4 (26.7 mg), and 5 (24.8 mg).
Table 1S Cytotoxicity of isolates in RAW264.7 cells.

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Spectra of compounds 1-5.

**Fig. 1.1S** HRESIMS spectrum of compound 1.

**Fig. 1.2S** IR spectrum of compound 1.
Fig. 1.3S $^1$H NMR spectrum of compound 1.

Fig. 1.4S $^{13}$C NMR spectrum of compound 1.
**Fig. 1.5S** DEPT135 spectrum of compound 1.

**Fig. 1.6S** $^1$H–$^1$H COSY spectrum of compound 1.
Fig. 1.7S HSQC spectrum of compound 1.

Fig. 1.8S HMBC spectrum of compound 1.
**Fig. 1.9S** ROESY spectrum of compound 1.

**Fig. 2.1S** HRESIMS spectrum of compound 2.
Fig. 2.2S IR spectrum of compound 2.

Fig. 2.3S $^1$H NMR spectrum of 2.

Fig. 2.4S $^{13}$C NMR of compound 2.

Fig. 2.5S DEPT135 spectrum of compound 2.
Fig. 2.6S $^1H$ $^1H$ COSY spectrum of compound 2.

Fig. 2.7S HSQC spectrum of compound 2.
Fig. 2.8S HMBC spectrum of compound 2.

Fig. 2.9S ROESY spectrum of compound 2.
Fig. 3.1S HRESIMS spectrum of compound 3.

Fig. 3.2S IR spectrum of compound 3.
Fig. 3.3S ¹H NMR spectrum of compound 3.

Fig. 3.4S ¹³C NMR spectrum of compound 3.
Fig. 3.5S DEPT135 spectrum of compound 3.

Fig. 3.6S $^1$H $^1$H COSY spectrum of compound 3.
Fig. 3.7S HSQC spectrum of compound 3.

Fig. 3.8S HMBC spectrum of compound 3.
Fig. 4.1S HRESIMS spectrum of compound 4.

Fig. 4.2S IR spectrum of compound 4.
Fig. 4.3S ¹H NMR spectrum of compound 4.

Fig. 4.4S ¹³C NMR spectrum of compound 4.
**Fig. 4.5S** DEPT135 spectrum of compound 4.

**Fig. 4.6S** $^1$H $^1$H COSY spectrum of compound 4.
Fig. 4.7S HSQC spectrum of compound 4.

Fig. 4.8S HMBC spectrum of compound 4.
Fig. 4.9S ROESY spectrum of compound 4.

Fig. 5.1S HRESIMS spectrum of compound 5.
Fig. 5.2S IR spectrum of compound 5.

Fig. 5.3S $^1$H NMR spectrum of compound 5.
Fig. 5.4S $^{13}$C NMR spectrum of compound 5.

Fig. 5.5S $^{13}$C NMR spectrum of compound 5.
**Fig. 5.6S** $^1$H $^1$H COSY spectrum of compound 5.

**Fig. 5.7S** HSQC spectrum of compound 5.
**Fig. 5.8S** HMBC spectrum of compound 5.

**Fig. 5.9S** ROESY spectrum of compound 5.
RP-HPLC analysis for compound 3

Condition

HPLC: Agilent 1200 series
Analytical column: Agilent XDB-C18 150 × 4.6 mm 5 μm
Detection wavelength: 220 nm
Column temperature: 30°C
Flow: 1 mL/min

Timetable:

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Chromatogram

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### Compound 3

![Graph](image)

### Result

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RP-HPLC analysis for compound 14

Condition

HPLC: Agilent 1200 series

Analytical column: Agilent XDB-C18 150 × 4.6 mm 5 μm

Detection wavelength: 227 nm

Column temperature: 30°C

Flow: 1 mL/min

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Chromatogram

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Compound 14

Result

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