Supporting Information

Down-regulation of matrix metalloproteinase-13 by the root extract of Cyathula officinalis Kuan and its constituents in IL-1β-treated chondrocytes

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Abbreviations

V/V volume/volume
General Procedures

MS spectra were measured on an Autospec M363 (Micromass). NMR spectra were recorded on DPX 400 and AVANCE 600 spectrometers (Bruker). The chemical shifts were represented as parts per million (ppm) referenced to the residual solvent signal. Column chromatography was carried out using Kieselgel 60, 63–200 μm and 40–63 μm (Merck), and YMC gel ODS-A, 150 μm (YMC instruments). TLC was performed on glass packed Kieselgel 60 F\textsubscript{254} and RP F\textsubscript{254s} plates. GC and HPLC analysis were performed using Acme 6000 (Young-Lin) and Spectra system (Thermo Separation Products), respectively.

Isolation procedures of compounds 1–6

The CHCl\textsubscript{3} fraction (10 g) was subjected to column chromatography on a silica gel (63–200 μm, 5 × 60 cm) using isocratic elution with CHCl\textsubscript{3} : MeOH (15 : 1; 100 mL each) to divide it into five fractions (Fr. 1–Fr. 5). Fr. 1 (0–1000 mL, 2 g) was re-chromatographed on silica gel (63–200 μm, 4 × 50 cm) by elution with benzene : ethyl acetate 9 : 1 (100 mL each), yielding six sub-fractions (Fr. 1-1–Fr. 1-6). Fr. 1-2 (500–800 mL, 0.8 g) was re-chromatographed on silica gel (40–63 μm, 4 × 50 cm) by
elution with *n*-hexane : ethyl acetate (9 : 1) to give compound 1 (110 mg). Fr. 1-3 (1100–1700 mL, 0.4 g) was re-chromatographed on silica gel (40–63 μm, 3 × 20 cm) by elution with *n*-hexane : ethyl acetate (4 : 1) to give compounds 2 (450–550 mL, 17 mg) and 3 (650–750 mL, 19 mg). Fr. 2 (1100–1400 mL, 2 g) was re-chromatographed on silica gel (63–200 μm, 4 × 50 cm) by elution with benzene : ethyl acetate (9 : 1; 100 mL each) and then divided into six sub-fractions (Fr. 2-1–Fr. 2-6). Fr. 2-2 (500–700 mL, 0.3 g) was re-chromatographed on silica gel (40–63 μm, 4 × 50 cm) by elution with benzene : ethyl acetate (9 : 1; 50 mL each) and divided into four sub-fractions (Fr. 2-2-1–Fr. 2-2-4). Fr. 2-2-3 (250–350 mL, 0.1 g) was re-chromatographed on silica gel (40–63 μm, 3 × 20 cm) by elution with CHCl₃ (30 mL each) to give compound 4 (330–450 mL, 15 mg). Fr. 2-4 (800–1000 mL, 0.15 g) was re-chromatographed on silica gel (40–63 μm, 3 × 20 cm) by elution with *n*-hexane : ethyl acetate (5 : 1; 30 mL each) to give compound 5 (120–210 mL, 13 mg). Fr. 4 (2000–2800 mL, 1 g) was re-chromatographed on silica gel (40–63 μm, 4 × 50 cm) by elution with CHCl₃ : MeOH (18 : 1; 30 mL each) to give compound 6 (120–180 mL, 29.7 mg).

The purity of the isolated compounds was determined by GC (1: 16.2 min, 96.7%; 2: 17.5 min, 89.7%; 3: 16.9 min, 95.2%) and HPLC (4: 8.7 min, 95.8%, 5: 14.7 min, 95.9%, 6: 7.3 min, 95.1%) under the following conditions: GC – GC chromatographic
separations were performed on a HP-5 column (30 m × 0.32 mm, 0.25 μm). Nitrogen was the carrier gas flowing at 1.0 mL/min. The temperature programme started at 80 °C for 3 min, heated to 250°C at a rate of 8°C/min and held for 4 min, for a total time of 25 min; detection with FID detector, respectively; HPLC – Intersil OS-2 column (150 mm × 4.5 mm, 5 μm); elution with acetonitrile : water (40 : 60 V/V); rate 0.7 mL/min; detection with UV detector at 254 nm, respectively. The purity of tested compounds is expressed as area %.

Compound 1

$^1$H-NMR (400 MHz, CDCl$_3$) δ: 0.83 (3H, t, $J = 7.0$ Hz, terminal CH$_3$), 1.26 (22H, CH$_3$-CH$_2$-(CH$_2$)$_{10}$, m), 1.32 (2H, CH$_3$-CH$_2$-(CH$_2$)$_{10}$, m), 1.63 (2H, m, -CH$_2$-CH$_2$-COOH), 2.35 (2H, t, $J = 7.5$ Hz, CH$_2$-COOH); $^{13}$C-NMR (100 MHz, CDCl$_3$) δ: 14.52 (C-16), 23.01 (C-15), 25.01 (C-3), 29.47–30.09 (C-4–C-14), 32.33 (C-14), 34.46 (C-2), 180.54 (C-1); EIMS $m/z$ : 256 [M]$^+$.  

Compound 2

$^1$H-NMR (400 MHz, CDCl$_3$) δ: 5.35 (1H, m, H-6), 3.52 (1H, m, H-3), 1.01 (3H, s, H-19), 0.91 (3H, d, $J = 6.4$ Hz, H-21), 0.84 (3H, t, $J = 7.2$ Hz, H-29), 0.80 (3H, d, $J = 6.8$...
Hz, H-27), 0.79 (3H, d, J = 6.8 Hz, H-26), 0.69 (3H, s, H-18); ¹³C-NMR (100 MHz, CDCl₃) δ : 33.1 (C-1), 33.5 (C-2), 71.4 (C-3), 39.4 (C-4), 140.3 (C-5), 121.3 (C-6), 31.5 (C-7), 31.5 (C-8), 49.7 (C-9), 36.1 (C-10), 20.7 (C-11), 41.9 (C-12), 41.9 (C-13), 56.4 (C-14), 23.9 (C-15), 27.9 (C-16), 55.6 (C-17), 11.6 (C-18), 19.4 (C-19), 35.7 (C-20), 18.6 (C-21), 39.4 (C-22), 25.6 (C-23), 45.4 (C-24), 29.0 (C-25), 19.0 (C-26), 18.4 (C-27), 22.9 (C-28), 11.5 (C-29); EIMS m/z : 412 [M]⁺.

Compound 3

¹H-NMR (400 MHz, CDCl₃) δ : 5.16 (1H, m, H-7), 5.02 (1H, dd, J = 15.2, 8.6 Hz, H-23), 3.59 (1H, m, H-3), 1.03 (3H, d, J = 6.6 Hz,H-21), 0.85 (3H, d, J = 6.2 Hz, H-26), 0.80 (3H, s, H-19), 0.55 (3H, s, H-18); ¹³C-NMR (100 MHz, CDCl₃) δ : 139.96 (C-8), 138.57 (C-22), 129.86 (C-23), 117.87 (C-7), 71.46 (C-3), 56.32 (C-17), 55.54 (C-14), 51.66 (C-24), 49.87 (C-9), 43.63 (C-13), 41.22 (C-20), 40.68 (C-5), 39.88 (C-4), 38.41 (C-12), 37.56 (C-1), 34.63 (C-10), 32.28 (C-25), 31.89 (C-2), 30.05 (C-6), 28.94 (C-16), 25.79 (C-28), 23.43 (C-15), 21.96 (C-11), 21.78 (C-26), 21.45 (C-21), 19.40 (C-27), 13.44 (C-19), 12.65 (C-29), 12.38 (C-18); EIMS m/z : 412 [M]⁺.

Compound 4
$^1$H-NMR (600 MHz, CDCl$_3$) $\delta$: 0.93 (3H, s, CH$_3$), 1.89 (3H, d, $J$ = 1.2 Hz, CH$_3$), 4.62 (1H, s, C = CH$_2$), 4.90 (1H, s, C = CH$_2$), 5.59 (1H, s, C = CH); $^{13}$C-NMR (150 MHz, CDCl$_3$) $\delta$: 8.46 (C-13), 18.57 (C-14), 22.66 (C-6), 23.00 (C-2), 36.18 (C-3), 38.13 (C-10), 39.10 (C-1), 48.41 (C-5), 107.48 (C-15), 119.16 (C-9), 120.45 (C-11), 147.99 (C-4), 148.09 (C-8), 148.34 (C-7), 171.38 (C-12); EIMS $m/z$: 230 [M]$^+$.  

Compound 5

$^1$H-NMR (600 MHz, CDCl$_3$) $\delta$: 1.70 (2H, m, H-4), 1.83 (3H, dd, $J$ = 1.2, 6.8 Hz, H-14), 1.89 (2H, m, H-2), 2.05 (3H, s, OCOCH$_3$), 2.06 (3H, s, OCOCH$_3$), 2.16 (2H, dt, $J$ = 7, 7 Hz, H-5), 4.09 (2H, t, $J$ = 6.5 Hz, H-1), 4.99 (1H, tt, $J$ = 6.8, 6.5 Hz, H-3), 5.57 (2H, d, $J$ = 15.8 Hz, H-7, H-12), 6.26 (1H, dt, $J$ = 7.2, 15.8 Hz, H-6), 6.31 (1H, dq, $J$ = 7.2, 15.8 Hz, H-13); $^{13}$C-NMR (150 MHz, CDCl$_3$) $\delta$: 18.88 (C-14), 20.90 (COCH$_3$), 21.08 (COCH$_3$), 29.13 (C-5), 33.04 (C-4), 33.07 (C-2), 60.62 (C-1), 70.35 (C-3), 72.31 (C-10), 73.15 (C-9), 79.29 (C-11), 80.02 (C-8), 109.54 (C-5), 109.91 (C-12), 143.46 (C-13), 146.51 (C-6), 170.57 (COCH$_3$), 170.95 (COCH$_3$); EIMS $m/z$: 302[M]$^+$.  

Compound 6

$^1$H-NMR (400 MHz, MeOH-d$_4$) $\delta$: 2.76 (2H, t, $J$ = 7.1 Hz, H-7'), 3.49 (2H, t, $J$ = 7.1 Hz, H-8'), 3.81, 3.85 (each 3H, s, OCH$_3$), 6.42 (1H, d, $J$ = 15.7 Hz, H-8), 6.66 (1H, dd, $J$ = 1.7, 8.0 Hz, H-6'), 6.73 (1H, d, $J$ = 8.0 Hz, H-5'), 6.79 (1H, d, $J$ = 8.2 Hz, H-5), 6.81 (1H, s, H-2'), 7.01 (1H, dd, $J$ = 1.7, 8.2 Hz, H-6), 7.09 (1H, d, $J$ = 1.7 Hz, H-2), 7.45 (1H, d, $J$ = 15.7 Hz, H-7); $^{13}$C-NMR (100 MHz, MeOH-d$_4$) $\delta$: 36.22 (C-7'), 42.49 (C-8'), 56.35 (OCH$_3$), 56.36 (OCH$_3$), 111.53 (C-2), 113.42 (C-2'), 116.22 (C-5'), 116.52 (C-
5), 118.75 (C-8), 122.26 (C-6'), 123.23 (C-6), 128.19 (C-1), 132.02 (C-1'), 142.08 (C-7),
146.01 (C-3'), 148.93 (C-4'), 149.29 (C-3), 149.91 (C-4), 169.18 (C-9); EIMS m/z : 343
[M]+.