SIX NEW TRITERPENOIDS WITH ANTI-INFLAMMATORY ACTIVITY FROM GYPSOPHILA OLDHAMIANA

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Chart S1. Chart of extraction and isolation

Extraction and Isolation of *Gypsophila oldhamiana* Miq.

**Roots of *G. oldhamiana*** 20.0 Kg

- Extracted by 50% acetone aqueous solution under refluxing, concentrated in vacuo

**Concentrated solution**

- Loaded onto a macroporous resin column (30 L) and eluted successively by H2O, 50% EtOH and 95% EtOH

**50% EtOH elution**

- Conducted on column chromatography (CC) over an MCI CHP20P resin and eluted stepwise by H2O, 30% EtOH, 50% EtOH, 70% EtOH and 95% EtOH

**H2O elution**

- Subjected to a silica gel column with a gradient system of CH2Cl2-MeOH (100:0 → 50:50)

Fr. 1 (95:4) Fr. 2 (96:4) Fr. 3 (92:8) Fr. 4 (94:6) Fr. 5 (90:10) Fr. 6 (89:11) Fr. 7 (87:13) Fr. 8 (85:15) Fr. 9 (80:20) Fr. 11-15 (75:25)

- Silica gel CC
- Sephadex LH-20 HPLC
- Compounds 11 (15 mg) and 12 (10 mg)
- Silica gel CC Sephadex LH-20 HPLC prep. TLC HPLC
- Compounds 7 (8 mg), 8 (8 mg) and 6 (5 mg)
- Silica gel CC ODS gel CC Sephadex LH-20 HPLC
- Compounds 3 (6 mg), 4 (25 mg) and 5 (6 mg)
- ODS gel CC Sephadex LH-20 HPLC
- Compounds 9 (12 mg) and 10 (5 mg)
**Table S1.** Crystal data and structure refinement for compound 6.

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identification code</td>
<td>cu_2019112001 (CCDC number: 2169252)</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C(<em>{30})H(</em>{46})O(_{4})</td>
</tr>
<tr>
<td>Formula weight</td>
<td>470.67</td>
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<tr>
<td>Temperature</td>
<td>296(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>1.54178 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Monoclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>P 21</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>a = 7.4419(7) Å</td>
</tr>
<tr>
<td></td>
<td>b = 11.3693(11) Å</td>
</tr>
<tr>
<td></td>
<td>c = 14.9985(14) Å</td>
</tr>
<tr>
<td></td>
<td>(\bar{a} = 90^\circ)</td>
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<td></td>
<td>(\bar{b} = 94^\circ)</td>
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<td></td>
<td>(\bar{c} = 90^\circ)</td>
</tr>
<tr>
<td>Volume</td>
<td>1266.6(2) Å</td>
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<tr>
<td>Z</td>
<td>2</td>
</tr>
<tr>
<td>Density (calculated)</td>
<td>1.234 Mg/m(^3)</td>
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<tr>
<td>Absorption coefficient</td>
<td>0.623 mm(^{-1})</td>
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<tr>
<td>F(000)</td>
<td>516</td>
</tr>
<tr>
<td>Theta range for data collection</td>
<td>4.9 to 66.6°</td>
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<tr>
<td>Index ranges</td>
<td>-8(\leqslant h \leqslant 8), -13(\leqslant k \leqslant 13), -16(\leqslant l \leqslant 17)</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>9188</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>4042 [R(int) = 0.199]</td>
</tr>
<tr>
<td>Completeness to theta = 66.59°</td>
<td>96.1 %</td>
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<tr>
<td>Absorption correction</td>
<td>multi-scan</td>
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<tr>
<td>Max. and min. transmission</td>
<td>0.836 and 0.780</td>
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<tr>
<td>Refinement method</td>
<td>Refinement on F(^2)</td>
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<tr>
<td>Data / restraints / parameters</td>
<td>4042 / 1 / 315</td>
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<tr>
<td>Goodness-of-fit on F(^2)</td>
<td>1.002</td>
</tr>
<tr>
<td>Final R indices [I&gt;2sigma(I)]</td>
<td>R1 = 0.1099, wR2 = 0.2523</td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>R1 = 0.1795, wR2 = 0.3047</td>
</tr>
<tr>
<td>Absolute structure parameter</td>
<td>-0.9(6)</td>
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<tr>
<td>Largest diff. peak and hole</td>
<td>0.45 and -0.65 e.Å(^{-3})</td>
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Table S2 *In vitro* inflammatory activity of compounds 1-12.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}^a$ (µM)</th>
<th>Compound</th>
<th>IC$_{50}^a$ (µM)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>RAW264.7</td>
<td></td>
<td>RAW264.7</td>
</tr>
<tr>
<td>1</td>
<td>29.31±0.86 µM</td>
<td>7</td>
<td>8.61±0.32 µM</td>
</tr>
<tr>
<td>2</td>
<td>2.55±0.49 µM</td>
<td>8</td>
<td>5.51±0.63 µM</td>
</tr>
<tr>
<td>3</td>
<td>0.93±0.21 µM</td>
<td>9</td>
<td>11.26±0.58 µM</td>
</tr>
<tr>
<td>4</td>
<td>37.61±0.74 µM</td>
<td>10</td>
<td>21.47±0.54 µM</td>
</tr>
<tr>
<td>5</td>
<td>29.35±0.67 µM</td>
<td>11</td>
<td>1.71±0.35 µM</td>
</tr>
<tr>
<td>6</td>
<td>18.73±0.68 µM</td>
<td>12</td>
<td>46.3±0.81 µM</td>
</tr>
<tr>
<td>Dexamethasone$^b$</td>
<td>0.86 ±0.08 µM</td>
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<td></td>
</tr>
</tbody>
</table>

$^a$ Means ± S.D. From three independent experiments (n=3)

$^b$ Positive control

Figure S1. Structures of compounds 7-12
Figure S2. HPLC spectrum of compound 1

The sample was separated on InertSustain® C18 column with water (A)- acetonitrile (B) as the mobile phase for gradient elution. The analysis was performed at a flowrate of 0.8 mL/min, column temperature of 45°C and the detection wavelength was set at 210 nm and 254nm.

Figure S3. IR spectrum of compound 1
Figure S4. HR-ESI-MS spectrum of compound 1

Figure S5. $^1$H-NMR spectrum of compound 1 in C$_5$D$_5$N (600 MHz)
Figure S6. $^{13}$C-NMR spectrum of compound 1 in C$_5$D$_5$N (150 MHz)

Figure S7. DEPT spectrum of compound 1 in C$_5$D$_5$N
Figure S8. HSQC spectrum of compound 1 in C$_5$D$_5$N

Figure S9. HMBC spectrum of compound 1 in C$_5$D$_5$N
Figure S10. HPLC spectrum of compound 2
The sample was separated on InertSustain® C18 column with water (A)- acetonitrile (B) as the mobile phase for gradient elution. The analysis was performed at a flowrate of 0.8 mL/min, column temperature of 45 ℃ and the detection wavelength was set at 210 nm.

Figure S11. IR spectrum of compound 2
Figure S12. HR-ESI-MS spectrum of compound 2

Figure S13. $^1$H-NMR spectrum of compound 2 in C$_2$D$_2$N (600 MHz)
Figure S14. $^{13}$C-NMR spectrum of compound 2 in C$_5$D$_5$N (150 MHz)

Figure S15. HSQC spectrum of compound 2 in C$_5$D$_5$N
Figure S16. DEPT spectrum of compound 2 in C$_5$D$_5$N
Figure S17. HMBC spectrum of compound 2 in C$_5$D$_5$N

Figure S18. HPLC spectrum of compound 3

The sample was separated on InertSustain® C18 column with water (A)- acetonitrile (B) as the mobile phase for gradient elution. The analysis was performed at a flowrate of 0.8 mL/min, column temperature of 45°C and the detection wavelength was set at 210 nm.
**Figure S19.** IR spectrum of compound 3

**Figure S20.** HR-ESI-MS spectrum of compound 3
Figure S21. $^1$H-NMR spectrum of compound 3 in C$_5$D$_5$N (600 MHz)

Figure S22. $^{13}$C-NMR spectrum of compound 3 in C$_5$D$_5$N (150 MHz)
Figure S23. DEPT spectrum of compound 3 in C₅D₅N

Figure S24. HSQC spectrum of compound 3 in C₅D₅N
Figure S25. HMBC spectrum of compound 3 in C$_5$D$_5$N

Figure S26. HPLC spectrum of compound 4
The sample was separated on InertSustain® C18 column with water (A)-acetonitrile (B) as the mobile phase for gradient elution. The analysis was performed at a flowrate of 0.8 mL/min, column temperature of 45°C and the detection wavelength was set at 210 nm.
Figure S27. IR spectrum of compound 4

Figure S28. HR-ESI-MS spectrum of compound 4
Figure S29. $^1$H-NMR spectrum of compound 4 in C$_5$D$_5$N (600 MHz)

Figure S30. $^{13}$C-NMR spectrum of compound 4 in C$_5$D$_5$N (150 MHz)
Figure S31. DEPT spectrum of compound 4 in C₅D₅N
Figure S32. HSQC spectrum of compound 4 in C$_5$D$_5$N

Figure S33. HMBC spectrum of compound 4 in C$_5$D$_5$N
**Figure S34.** HPLC spectrum of compound 5

The sample was separated on InertSustain® C18 column with water (A)-acetonitrile (B) as the mobile phase for gradient elution. The analysis was performed at a flowrate of 0.8 mL/min, column temperature of 45°C and the detection wavelength was set at 210 nm.

**Figure S35.** IR spectrum of compound 5
Figure S36. HR-ESI-MS spectrum of compound 5

Figure S37. $^1$H-NMR spectrum of compound 5 in C$_6$D$_5$N (600 MHz)
Figure S38. $^{13}$C-NMR spectrum of compound 5 in $\text{C}_6\text{D}_5\text{N}$ (150 MHz)

Figure S39. HSQC spectrum of compound 5 in $\text{C}_6\text{D}_5\text{N}$
**Figure S40.** HMBC spectrum of compound 5 in C$_5$D$_5$N

**Figure S41.** HPLC spectrum of compound 6

The sample was separated on InertSustain® C18 column with water (A)- acetonitrile (B) as the mobile phase for gradient elution. The analysis was performed at a flowrate of 0.8 mL/min, column temperature of 45°C and the detection wavelength was set at 210 nm.
Figure S42. IR spectrum of compound 6

Figure S43. HR-ESI-MS spectrum of compound 6
Figure S44. $^1$H-NMR spectrum of compound 6 in C$_5$D$_5$N (600 MHz)

Figure S45. $^{13}$C-NMR spectrum of compound 6 in C$_5$D$_5$N (150 MHz)
Figure S46. DEPT spectrum of compound 6 in C₅D₅N

Figure S47. HSQC spectrum of compound 6 in C₅D₅N
Figure S48. HMBC spectrum of compound 6 in C$_5$D$_5$N

Figure S49. NOESY spectrum of compound 6 in C$_5$D$_5$N
Figure S53. HPLC spectrum of compound 7
The sample was separated on InertSustain® C18 column with water (A)-acetonitrile (B) as the mobile phase for gradient elution. The analysis was performed at a flowrate of 0.8 mL/min, column temperature of 45°C and the detection wavelength was set at 210 nm.

Figure S54. 1H-NMR spectrum of compound 7 in C₅D₅N (600 MHz)
**Figure S55.** 13C-NMR spectrum of compound 7 in C$_5$D$_5$N (150 MHz)

**Figure S50.** HPLC spectrum of compound 8

The sample was separated on InertSustain® C18 column with water (A)- acetonitrile (B) as the mobile phase for gradient elution. The analysis was performed at a flowrate of 0.8 mL/min, column temperature of 45°C and the detection wavelength was set at 210 nm.
Figure S51. 1H-NMR spectrum of compound 8 in C$_5$D$_5$N (600 MHz)

Figure S52. 13C-NMR spectrum of compound 8 in C$_5$D$_5$N (150 MHz)
Figure S56. HPLC spectrum of compound 9
The sample was separated on InertSustain® C18 column with water (A)-acetonitrile (B) as the mobile phase for gradient elution. The analysis was performed at a flowrate of 0.8 mL/min, column temperature of 45 °C and the detection wavelength was set at 210 nm.

Figure S57. 1H-NMR spectrum of compound 9 in C$_5$D$_5$N (600 MHz)
Figure S58. 13C-NMR spectrum of compound 9 in C\textsubscript{5}D\textsubscript{5}N (150 MHz)

Figure S59. HPLC spectrum of compound 10
The sample was separated on InertSustain\textsuperscript{®} C18 column with water (A)-acetonitrile (B) as the mobile phase for gradient elution. The analysis was performed at a flowrate of 0.8 mL/min, column temperature of 45°C and the detection wavelength was set at 210 nm.
Figure S60. 1H-NMR spectrum of compound 10 in C$_5$D$_5$N (600 MHz)

Figure S61. 13C-NMR spectrum of compound 10 in C$_5$D$_5$N (150 MHz)
Figure S62. HPLC spectrum of compound 11
The sample was separated on InertSustain® C18 column with water (A)-acetonitrile (B) as the mobile phase for gradient elution. The analysis was performed at a flowrate of 0.8 mL/min, column temperature of 45°C and the detection wavelength was set at 210 nm.

Figure S63. 1H-NMR spectrum of compound 11 in C$_5$D$_5$N (600 MHz)
Figure S64. 13C-NMR spectrum of compound 11 in C$_5$D$_5$N (150 MHz)

Figure S65. HPLC spectrum of compound 12
The sample was separated on InertSustain® C18 column with water (A)- acetonitrile (B) as the mobile phase for gradient elution. The analysis was performed at a flowrate of 0.8 mL/min, column temperature of 45℃ and the detection wavelength was set at 210 nm and 254nm.
Figure S66. 1H-NMR spectrum of compound 12 in C$_6$D$_5$N (600 MHz)

Figure S67. 13C-NMR spectrum of compound 12 in C$_6$D$_5$N (150 MHz)