Supporting Information

Diterpenoid Alkaloids from *Delphinium ajacis* and Their Anti-RSV Activities

Li Yang¹, Yu-Bo Zhang¹, Ling Zhuang¹, Tao Li², Neng-Hua Chen¹, Zhong-Nan Wu¹, Pan Li¹, Yao-Lan Li¹, Guo-Cai Wang¹

Affiliations

¹Institute of Traditional Chinese Medicine & Natural Products, College of Pharmacy, Jinan University, Guangzhou, P. R. China
²School of Biomedical Sciences, The University of Hong Kong, Hong Kong

Correspondence

**Prof. Dr. Yao-Lan Li**
Institute of Traditional Chinese Medicine and Natural Products
College of Pharmacy
Jinan University
601 West Huangpu Avenue
510632 Guangzhou
P. R. China
Phone: + 862085221728
Fax: + 862085221559
tliyl@jnu.edu.cn

**Dr. Guo-Cai Wang**
Institute of Traditional Chinese Medicine and Natural Products
College of Pharmacy
Jinan University
601 West Huangpu Avenue
510632 Guangzhou
P. R. China
Phone: + 862085223553
Fax: + 862085221559
twangguocai@jnu.edu.cn
### Contents of the Supporting Information

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction and isolation</td>
<td>1</td>
</tr>
<tr>
<td>Fig. 1S HR-ESI-MS of 1.</td>
<td>3</td>
</tr>
<tr>
<td>Fig. 2S UV spectrum of 1.</td>
<td>3</td>
</tr>
<tr>
<td>Fig. 3S IR spectrum of 1.</td>
<td>4</td>
</tr>
<tr>
<td>Fig. 4S $^1$H NMR spectrum of 1.</td>
<td>4</td>
</tr>
<tr>
<td>Fig. 5S $^{13}$C NMR spectrum of 1.</td>
<td>5</td>
</tr>
<tr>
<td>Fig. 6S DEPT-135 spectrum of 1.</td>
<td>5</td>
</tr>
<tr>
<td>Fig. 7S $^1$H-$^1$H COSY spectrum of 1.</td>
<td>6</td>
</tr>
<tr>
<td>Fig. 8S HSQC spectrum of 1.</td>
<td>6</td>
</tr>
<tr>
<td>Fig. 9S HMBC spectrum of 1.</td>
<td>7</td>
</tr>
<tr>
<td>Fig. 10S NOESY spectrum of 1.</td>
<td>7</td>
</tr>
<tr>
<td>Fig. 11S HR-ESI-MS of 2.</td>
<td>8</td>
</tr>
<tr>
<td>Fig. 12S UV spectrum of 2.</td>
<td>8</td>
</tr>
<tr>
<td>Fig. 13S IR spectrum of 2.</td>
<td>9</td>
</tr>
<tr>
<td>Fig. 14S $^1$H NMR spectrum of 2.</td>
<td>9</td>
</tr>
<tr>
<td>Fig. 15S $^{13}$C NMR spectrum of 2.</td>
<td>10</td>
</tr>
<tr>
<td>Fig. 16S DEPT-135 spectrum of 2.</td>
<td>10</td>
</tr>
<tr>
<td>Fig. 17S $^1$H-$^1$H COSY spectrum of 2.</td>
<td>11</td>
</tr>
<tr>
<td>Fig. 18S HSQC spectrum of 2.</td>
<td>11</td>
</tr>
<tr>
<td>Fig. 19S HMBC spectrum of 2.</td>
<td>12</td>
</tr>
<tr>
<td>Fig. 20S NOESY spectrum of 2.</td>
<td>12</td>
</tr>
<tr>
<td>Fig. 21S HR-ESI-MS of 3.</td>
<td>13</td>
</tr>
<tr>
<td>Fig. 22S UV spectrum of 3.</td>
<td>13</td>
</tr>
<tr>
<td>Fig. 23S IR spectrum of 3.</td>
<td>14</td>
</tr>
<tr>
<td>Fig. 24S $^1$H NMR spectrum of 3.</td>
<td>14</td>
</tr>
<tr>
<td>Fig. 25S $^{13}$C NMR spectrum of 3.</td>
<td>15</td>
</tr>
<tr>
<td>Fig. 26S DEPT-135 spectrum of 3.</td>
<td>15</td>
</tr>
<tr>
<td>Fig. 27S $^1$H-$^1$H COSY spectrum of 3.</td>
<td>16</td>
</tr>
<tr>
<td>Fig. 28S HSQC spectrum of 3.</td>
<td>16</td>
</tr>
<tr>
<td>Fig. 29S HMBC spectrum of 3.</td>
<td>17</td>
</tr>
<tr>
<td>Fig. 30S NOESY spectrum of 3.</td>
<td>17</td>
</tr>
<tr>
<td>Fig. 31S HR-ESI-MS of 4.</td>
<td>18</td>
</tr>
<tr>
<td>Fig. 32S UV spectrum of 4.</td>
<td>18</td>
</tr>
<tr>
<td>Fig. 33S IR spectrum of 4.</td>
<td>19</td>
</tr>
<tr>
<td>Fig. 34S $^1$H NMR spectrum of 4.</td>
<td>19</td>
</tr>
<tr>
<td>Fig. 35S $^{13}$C NMR spectrum of 4.</td>
<td>20</td>
</tr>
<tr>
<td>Fig. 36S DEPT-135 spectrum of 4.</td>
<td>20</td>
</tr>
</tbody>
</table>
Fig. 37S $^1$H-$^1$H COSY spectrum of 4. ................................................................. 21
Fig. 38S HSQC spectrum of 4. .............................................................................. 21
Fig. 39S HMBC spectrum of 4. .............................................................................. 22
Fig. 40S NOESY spectrum of 4. .............................................................................. 22
Fig. 41S HR-ESI-MS of 5. ..................................................................................... 23
Fig. 42S UV spectrum of 5. ..................................................................................... 23
Fig. 43S IR spectrum of 5. ..................................................................................... 24
Fig. 44S $^1$H NMR spectrum of 5. ........................................................................ 24
Fig. 45S $^{13}$C NMR spectrum of 5. ..................................................................... 25
Fig. 46S DEPT-135 spectrum of 5. ....................................................................... 25
Fig. 47S $^1$H-$^1$H COSY spectrum of 5. ............................................................. 26
Fig. 48S HSQC spectrum of 5. .............................................................................. 26
Fig. 49S HMBC spectrum of 5. .............................................................................. 27
Fig. 50S NOESY spectrum of 5. .............................................................................. 27
Extraction and isolation

The dried and powdered roots and stems of *D. ajacis* (10 kg) were powdered and percolated with 95% EtOH at room temperature. The ethanolic solution was concentrated under reduced pressure to yield a residue (505 g). The crude extract was suspended in water, and the pH was adjusted to 4~5 with 5% HCl. Lipophilic impurities were removed from acidified extracts by extracting with CHCl₃. The pH of the aqueous layer was adjusted to 9~10 with 10% NH₃ · H₂O and re-extracted with CHCl₃ to obtain a total alkaloid fraction (80 g).

The total alkaloid was subjected to silica gel (1.0 kg; 200-300 mesh; 10 × 100 cm) CC eluting with gradient mixtures of CHCl₃-MeOH (100:0; 100:1; 50:1; 40:1; 30:1; 20:1; 10:1; 0:1; v/v; each 4.0 L; fraction size: 1000 mL/flask; flow rate: 5 mL/min). The fractions were examined by TLC and combined to give seven fractions (Frs.A-G). Fr.B (10.5 g, eluted by CHCl₃-MeOH 100:1) was loaded on an ODS CC (100.0 g; 5 × 40 cm) which was eluted with a gradient of MeOH-H₂O (50:50; 60:40; 70:30; 80:20; 90:10; 100:0; v/v; each 1.5 L; fraction size: 500 mL/flask; flow rate: 3 mL/min) to afford four subfractions (Frs.B.1-4). Fr.B.2 (75 mg) was separated by preparative HPLC (CH₃CN/H₂O 45:55; v/v; 3 mL/min) to yield compounds 1 (5.0 mg; purity > 95 %, HPLC, Rᵣ = 20.5 min) and 5 (8.5 mg; purity > 95 %, HPLC, Rᵣ = 29.5 min). Fr.B.3 (240 mg) was purified by preparative HPLC with an eluent of CH₃CN/H₂O (55:45; v/v; 3 mL/min) to yield compounds 3 (6.0 mg; purity > 94%, HPLC, Rᵣ = 18.0 min) and 8 (23.5 mg; purity > 95 %, HPLC, Rᵣ = 25.5 min). Fr.C (15.4 g, eluted by CHCl₃-MeOH 50:1) was separated by silica gel (200.0 g; 300-400 mesh; 5 × 80 cm) CC and eluted with gradient petroleum ether/acetone (100:0; 50:1; 40:1; 30:1; 10:1; 0:1; v/v; each 2.0 L; fraction size: 500 mL/flask; flow rate: 4 mL/min) to afford five fractions (Frs.C.1-5). Fr.C.3 (4.5 g) was loaded on an ODS CC (50.0 g; 3 × 40 cm) which was eluted with a gradient of MeOH-H₂O (65:35; 75:25; 85:15; 100:0; v/v; each 500 mL; fraction size: 200 mL/flask; flow rate: 3 mL/min) to afford four subfractions (Frs.C.3.1-3.4). Fr.C.3.2 (87 mg)
was purified by HPLC with an eluent of CH$_3$CN/H$_2$O (40:60; v/v; 3 mL/min) to yield compounds 2 (4.5 mg; purity > 95%, HPLC, R$_t$ = 14.0 min) and 4 (7.4 mg; purity > 94 %, HPLC, R$_t$ = 26.5 min). Fr.C.3.3 (310 mg) was separated by HPLC with an eluent of CH$_3$CN/H$_2$O (60:40; v/v; 3 mL/min) to yield compounds 6 (150.0 mg; purity > 95%, HPLC, R$_t$ = 18.0 min) and 9 (10.5 mg; purity > 95 %, HPLC, R$_t$ = 28.5 min). Fr.C.4 (1.8 g, eluted by petroleum ether/acetone 40:1) was purified by preparative HPLC (MeOH/ H$_2$O 70:30; v/v; 3 mL/min) to give compounds 7 (29.0 mg; purity > 94%, HPLC, R$_t$ = 14.5 min) and 10 (25.4 mg; purity > 95 %, HPLC, R$_t$ 30.5 min). Fr.D (8.7 g, eluted by CHCl$_3$-MeOH 40:1) was partitioned on a Sephadex LH-20 (80.0 g, 2 × 160 cm; MeOH/CHCl$_3$ 1:1) to yield two fractions (Frs.D.1-2). Fr.D.2 (2.8 g) was further purified by ODS CC (50.0 g, 2 × 35 cm; MeOH/H$_2$O 75: 25; 100 mL per fraction) to give three fractions (Frs.D.2.1-2.3). Fr.D.2.2 (66.5 mg) was purified by preparative HPLC (MeOH/ H$_2$O 70:30; v/v; 3 mL/min) to give compounds 11 (2.5 mg; purity > 95%, HPLC, R$_t$ = 9.5 min) and 12 (2.5 mg; purity > 95 %, HPLC, R$_t$ 18.0 min).
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Fig. 5S $^{13}$C NMR spectrum of 1.

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Fig. 11S HR-ESI-MS of 2.

Fig. 12S UV spectrum of 2.
Fig. 13S IR spectrum of 2.

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Fig. 15S $^{13}$C NMR spectrum of 2.

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Fig. 17S $^1$H-$^1$H COSY spectrum of 2.

Fig. 18S HSQC spectrum of 2.
Fig. 19S HMBC spectrum of 2.

Fig. 20S NOESY spectrum of 2.
Fig. 21S HR-ESI-MS of 3.

Fig. 22S UV spectrum of 3.
Fig. 23S IR spectrum of 3.

Fig. 24S $^1$H NMR spectrum of 3.
Fig. 25S $^{13}$C NMR spectrum of 3.

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Fig. 27S $^1$H-$^1$H COSY spectrum of 3.

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Fig. 29S HMBC spectrum of 3.

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Fig. 31S HR-ESI-MS of 4.

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**Fig. 35S** $^{13}$C NMR spectrum of 4.

**Fig. 36S** DEPT-135 spectrum of 4.
Fig. 37S $^1$H-$^1$H COSY spectrum of 4.

Fig. 38S HSQC spectrum of 4.
Fig. 39S HMBC spectrum of 4.

Fig. 40S NOESY spectrum of 4.
Fig. 41S HR-ESI-MS of 5.

Fig. 42S UV spectrum of 5.
Fig. 43S IR spectrum of 5.

Fig. 44S $^1$H NMR spectrum of 5.
**Fig. 45S** $^{13}$C NMR spectrum of 5.

**Fig. 46S** DEPT-135 spectrum of 5.
Fig. 47S $^1$H-$^1$H COSY spectrum of 5.

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