Supporting Information to:

Antiproliferative and Apoptotic Activities of Linear Furocoumarins from Notopterygium incisum on Cancer Cell Lines

Shi-Biao Wu¹, Fei Pang², Ying Wen³, Hong-Feng Zhang², Zheng Zhao³, Jin-Feng Hu¹

Affiliation
¹ Key Laboratory of Brain Functional Genomics, East China Normal University, Shanghai, P.R. China
² Department of Biomedicine, School of Life Science, East China Normal University, Shanghai, P.R. China
³ Department of Neuropharmacology and Toxicology, Key Laboratory of Brain Functional Genomics, Ministry of Education and Shanghai Key Laboratory of Brain Functional Genomics, East China Normal University, Shanghai, P.R. China

Correspondence
Prof. Dr. Jin-Feng Hu
Department of Natural Products for Chemical Genetic Research
Key Laboratory of Brain Functional Genomics
Ministry of Education

and

Shanghai Key Laboratory of Brain Functional Genomics
East China Normal University
3663 Zhongshan Road N
Shanghai 200062
People’s Republic of China
Tel.: +86-21-6260-7510
Fax: +86-21-6260-6791
jfhu@brain.ecnu.edu.cn
Apoptosis detection by staining with annexin V-FITC and propidium iodide

Flow cytometry is the technique of choice for the quantitative measurement of apoptosis. By using propidium iodide (PI) (Sigma-Aldrich) in combination with fluorescein isothiocyanate–labeled annexin V (annexin V-FITC) (Sigma-Aldrich), apoptotic, viable, and dead cells can be readily distinguished. In this study, MCF-7 cells were used to test the apoptotic activities of the isolated compounds. Adriamycin (a widely used anticancer drug; Sigma-Aldrich, catalog No. D1317) was used as the positive control. The cells were seeded at $1 \times 10^6$ cells per dish. After 24 h preincubation, the cells were exposed to compounds for either 6 h or 12 h. The supernatants were collected and centrifuged at 1500 rpm for 5 min to obtain the detached cells, which may contain a considerable number of apoptotic cells. The adherent cells were detached using a DMEM/trypsin solution and collected by centrifugation at 1500 rpm by following the methods of Sasaki et al. [1] and Ormerod et al. [2]. Annexin V-FITC (ann) and PI co-labeling allows the differentiation of early apoptotic cells (ann+/PI−) from viable cells (ann−/PI−) as well as those in the later stages of apoptosis (ann+/PI+) from those that are already dead (ann−/PI+). Measurements were carried out on a flow cytometer (Becton-Dickinson FACScan; BD Biosciences), and data acquisition and analysis were performed with the BD CellQuest software package.

Cell cycle analysis

After a period of exposure (24 h or 48 h) to the isolated compounds 1 or 2 or to the positive control compound adriamycin, the MCF-7 cells were harvested, centrifuged at 1500 rpm, and fixed in cold 75% ethanol at 4 °C overnight. After washing once with D-Hanks solution, 400 µL RNase (100 µg/mL) was added and incubated for 10 min at 37 °C. This was followed by addition of 400 µL of PI solution (20 µg/mL), and the incubation was continued for another 10 min at 37 °C. An additional 500 µL of D-Hanks solution was used to dilute the cell suspension in each tube for testing.
Fig. 1S Apoptotic effects of compounds 1 and 2 (150 µM) on MCF-7 cells. (A1) Control (6 h); (A2) Control (12 h); (B1) Notopol (1) (6 h); (B2) Notopol (1) (12 h); (C1) Notopterol (2) (6 h); (C2) Notopterol (2) (12 h). All experiments were performed in triplicate and gave similar results.
Fig. 2S HPLC spectra of (A) crude extract, (B) notopterol (2), (C) 5-[(2E, 5Z)-7-hydroxy-3,7-dimethyl-2,5-octadienoxy]psoralene (3), (D) notopel (1), and (E) 5-[(2,5)-epoxy-3-hydroxy-3,7-dimethyl-6-octenoxy]psoralene (4).
**Table 1S** Apoptosis effects of compounds 1 and 2 on MCF-7 cells {Authors: Please verify title}

<table>
<thead>
<tr>
<th>Assays</th>
<th>Time</th>
<th>Viable cells</th>
<th>Dead cells</th>
<th>Early apoptotic cells</th>
<th>Late apoptotic cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank control</td>
<td>6 h</td>
<td>94.5 ± 2.8</td>
<td>0.6 ± 0.1</td>
<td>2.7 ± 0.7</td>
<td>2.2 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>90.5 ± 3.4</td>
<td>1.9 ± 0.7</td>
<td>1.2 ± 0.4</td>
<td>6.4 ± 2.3</td>
</tr>
<tr>
<td>1</td>
<td>6 h</td>
<td>86.4 ± 2.4</td>
<td>2.8 ± 1.0</td>
<td>4.6 ± 1.0</td>
<td>6.3 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>82.5 ± 2.0</td>
<td>5.0 ± 1.9</td>
<td>2.3 ± 0.6</td>
<td>10.3 ± 1.7</td>
</tr>
<tr>
<td>2</td>
<td>6 h</td>
<td>85.4 ± 2.6</td>
<td>4.5 ± 2.0</td>
<td>1.4 ± 1.1</td>
<td>8.8 ± 0.6*</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>77.4 ± 2.9</td>
<td>4.3 ± 1.7</td>
<td>7.7 ± 2.7*</td>
<td>10.6 ± 2.2</td>
</tr>
</tbody>
</table>

Note: Experiments were performed in triplicate and values (percentage of cells) are expressed as mean ± SE. *P < 0.05 (ANOVA followed by Dunnett’s test).

**References**

1 Sasaki DT, Dumas SE, Engleman EG. Discrimination of viable and non-viable cells using propidium iodide in two color immunofluorescence. Cytometry 1987; 8: 413-420