Antitumour Activities of Sesquiterpene Lactones from Inula helenium and Inula japonica

Article in Zeitschrift fur Naturforschung C · September 2012
DOI: 10.5560/ZNC.2012.67c0375 · Source: PubMed

CITATIONS
13

READS
200

11 authors, including:

Shi Qing
Beijing University of Chemical Technology
240 PUBLICATIONS 10,385 CITATIONS

Changhong Huo
Hebei Medical University
88 PUBLICATIONS 510 CITATIONS

Manli Zhang
Hebei Medical University
160 PUBLICATIONS 2,053 CITATIONS

Hiromasa Kiyota
Okayama University
238 PUBLICATIONS 2,389 CITATIONS

Some of the authors of this publication are also working on these related projects:

Bioremediation (degradation of VOC: organochlorines) View project

Sialidase inhibitors View project
Antitumour Activities of Sesquiterpene Lactones from
Inula helenium and Inula japonica

Yong Li, Zhi-Yu Ni, Meng-Chu Zhu, Mei Dong, Si-Ming Wang, Qing-Wen Shi, Man-Li Zhang, Yu-Fang Wang, Chang-hong Huo, Hiromasa Kiyota, and Bin Cong

Department of Forensic Medicine, Hebei Key Laboratory of Forensic Medicine, Hebei Medical University, Shijiazhuang, 050017, Hebei Province, China. E-mail: hbydcongbin@126.com
Department of Thoracic, Fourth Hospital of Hebei Medical University, Shijiazhuang, 050011, Hebei Province, China
Laboratory Medical Science, School of Medical Science and Laboratory Medicine, Jiangsu University, Zhenjing, 212013, Jiangsu Province, China
Department of Medicinal Natural Product Chemistry, School of Pharmaceutical Sciences, Hebei Medical University, 361 Zhongshan East Road, Shijiazhuang, 050017, Hebei Province, China. E-mail: shiqingwen@hebmu.edu.cn
Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai 981-8555, Japan. Fax: +81-22-717-8785. E-mail: kiyota@biochem.tohoku.ac.jp

* Authors for correspondence and reprint requests

Z. Naturforsch. 67c, 375–380 (2012); received March 22/December 27, 2011

Eight sesquiterpene lactones were isolated from the roots of Inula helenium and flowers of I. japonica. Among them, isoalantolactone (3) and santamarine (6) exhibited significant growth inhibitory activities against gynecologic cancer cell lines, while others weakly inhibited the growth of the cell lines (IC_{50} \leq 100 \mu M). In addition, 3 significantly inhibited the tumour growth of S180 tumour-bearing mice. Compounds 3 and 6 were not toxic to human embryonic lung fibroblast cells in vitro. These results demonstrated that the antitumour activities are closely related to the structures of the compounds, that is, an \( \alpha \)-exomethylene-\( \gamma \)-lactone ring is necessary for these activities.

Key words: Inula sp., Sesquiterpenes, Antitumour Activity

Introduction

It has been well documented that medicinal plants confer considerable anticancer activity against various tumours (Dai and Mumper, 2010). Plants contain abundant compounds which have consistently been shown to be associated with a lower risk of cancers at almost every site, such as lung, colon, rectum, prostate, cervix, stomach, pancreas, breast, and bladder (Steinmetz and Potter, 1991). Efforts, therefore, are being made to identify naturally occurring anticarcinogens which would prevent, slow, and/or reverse the cancer induction and its subsequent development (Chuang et al., 2000). Inula helenium is an important herb traditionally used in the treatment of influenza, fever, tuberculotic enterorrhoea, and chronic enterogastritis in China, Japan, and Europe (Okuda, 1986; Olechnowicz-Stepien and Skurska, 1960). Plants of the genus Inula have been shown to contain high levels of sesquiterpene lactones (Zhao et al., 2006; Trendafilova et al., 2010), which recently have received considerable attention in the pharmacological community due to their antineoplastic and anti-inflammatory effects (Konishi et al., 2002; Won et al., 2004). Many studies indicated that sesquiterpene lactones exhibit antitumour activities in a variety of malignant cells (Ghantous et al., 2010; Chen et al., 2007). In the present study, we investigated the antiproliferative activity of eight sesquiterpene lactones from I. helenium and I. japonica against gynecologic cancer cell lines in vitro. We also explored the antitumour activity against ascite tumour S180 xenografts in mice in vivo.

Results

Isolation of sesquiterpene lactones

The dichloromethane extract of the roots of I. helenium contained 3\( \beta \),9\( \beta \)-diacetoxyl-1\( \beta \),10\( \alpha \)-epoxy-11\( \alpha \),13-dihydrocostunolide (1) (Milosavljevic et al.,
Inhibition of growth of HeLa and A549 cells

The cytotoxic effect of compounds 1–8 (100 μM) on the gynecologic HeLa cancer cell line was investigated. The MTT (methylthiazolyl tetrazolium) assay revealed that cisplatin, isoalantolactone (3), and santamarine (6) had a strong dose-dependent antiproliferative effect on HeLa cells; the percentages of growth inhibition by these compounds were 66%, 81%, and 86%, respectively, while the other compounds inhibited growth only weakly (Fig. 2). The IC₅₀ values of the proliferation of HeLa cells of cisplatin, isoalantolactone, and santamarine were 20.19, 19.41, and 10.48 μM.

Inhibition of growth of HEC-1, SHIN3, HOC-21, and HAC-2 cells

The MTT assay revealed that cisplatin exhibited the strongest antiproliferative activity against HAC-2 cells (IC₅₀ = 8.95 μM) but did not inhibit the proliferation of HEC-1, SHIN3, and HOC-21 cells, respectively (Table I). Isoalantolactone (3) was strongly antiproliferative against HEC-1, HOC-21, and HAC-2, but not against SHIN3 cells, while the other compounds did not affect growth.

Effect of isoalantolactone (3) on ascites tumours

Cyclophosphamide (cytoxan) and 3 had comparable antitumour growth activities in vivo. Inhibition (see legend to Fig. 2) by 3 at 100 and 10 mg/(kg d) was 64.2% and 43.0%, respectively

![Chemical Structures](image-url)

Fig. 1. Chemical structures of sesquiterpene lactones isolated from *Inula* sp.: 3β,9β-diacetoxy-1β,10α-epoxy-11α,13-dihydrocostunolide (1), 3β,9β-diacetoxy-11α,13-dihydrocostunolide (2), isoalantolactone (3), 2α-hydroxy-11α,13-dihydroisoalantolactone (4), 11α,13-dihydroisoalantolactone (5), santamarine (6), britannilactone (7), and 1-O-acetylbritannilactone (8).
Fig. 2. The effect of the sesquiterpene lactones 1 to 8 on the proliferation of the HeLa tumour cell line. The percentage of growth inhibition by the compounds was calculated by comparing viable cells in the treated group with those in the untreated group. * \( P < 0.05 \), ** \( P < 0.01 \) vs. control.

Table I. The effect of eight sesquiterpene lactones on the proliferation of HEC-1, SHIN3, HOC-21, and HAC-2 tumour cell lines.

<table>
<thead>
<tr>
<th>Compound</th>
<th>HEC-1</th>
<th>SHIN3</th>
<th>HOC-21</th>
<th>HAC-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>8.95</td>
</tr>
<tr>
<td>3b,9b-Diacetoxy-1b,10(\alpha)-epoxy-11(\alpha),13-dihydrocostunolide (1)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3b,9b-Diacetoxy-11(\alpha),13-dihydrocostunolide (2)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Isoalantolactone (3)</td>
<td>32.54</td>
<td>&gt;100</td>
<td>19.65</td>
<td>11.53</td>
</tr>
<tr>
<td>2(\alpha)-Hydroxy-11(\alpha),13-dihydroisoalantolactone (4)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>11(\alpha),13-Dihydroisoalantolactone (5)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Santamarine (6)</td>
<td>&gt;100</td>
<td>12.42</td>
<td>42.62</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Britannilactone (7)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>1-O-Acetylbritannilactone (8)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

(Fig. 4A). The thymus and spleen index, respectively, decreased significantly in the cyclophosphamide group but they were invariable in 3 (Fig. 4B). Furthermore, the data suggested that 3 inhibited tumour growth \textit{in vivo} in a dose-dependent manner.

\textbf{Discussion}

Traditionally, many plants containing high levels of sesquiterpene lactones have been used as folk medicines because of their pharmacological properties. \textit{Inula} species, rich in sesquiterpene lactones, have been widely used as herbal medicines in China, Japan, and Europe to treat a number of diseases, and their pharmacological activities have been confirmed (O’Shea et al., 2009; Cantrell et al., 1999). Some pure compounds have been isolated from \textit{I. helenium} and \textit{I. japonica} to prove their anticancer activity (Konishi et al., 2002; Dorn et al., 2006). In our research, the antitumour activities of eight sesquiterpene lactones, 1–8, extracted from \textit{I. helenium} and \textit{I. japonica} were explored. Our data showed that isoalantolactone (3) and santamarine (6) significantly inhibited the growth of the human gynecologic cancer cell lines HeLa, HEC-1, SHIN3, HOC-21, and HAC-2, but were not cytotoxic to human embryonic lung fibroblast cells (HELF), while compounds 1, 2, 4, 5, 7, and 8 were only weakly growth inhibitory in all cell lines. Furthermore, 3 exhibited \textit{in vivo} antigrowth
activity against ascites tumours in a dose-dependent manner.

Of the eight sesquiterpene lactones isolated from *Inula* species, 1 and 2 belong to germacrane sesquiterpenes with a 10-membered ring, 3 – 6 belong to eudesmane sesquiterpenes with a trans-decalin (6/6-membered) ring, while 7 and 8 are britannilane sesquiterpenes with a 6-membered ring. Among the four eudesmanes, the exomethylene group at the γ-lactone ring of 3 and 6 is saturated in 4 and 5.

The experimental results indicate that the antitumour activity against various human lung cancer cells in vitro and xenograft ascites tumours in vivo. These findings indicate that 3 and 6 have significant therapeutic potential and might serve as powerful novel antitumour lead compounds.

**Experimental**

**Material**

The roots of *Inula helenium* (3 kg dry weight) and dried flowers of *I. japonica* (10 kg) were purchased at the Anguo medicinal herbs market, Hebei province, China, in April 2008. Male Kunming mice (10-week-old, 18 – 22 g) were purchased from the Hebei Medical University, Hebei, China,
and were fed a standard pellet diet and drinking water ad libitum. The gynecologic cell lines HeLa, HEC-1, SHIN3, HOC-21, and HAC-2 were obtained from the Department of Environmental Biochemistry, Graduate School of Medicine, Chiba University, Chiba, Japan. Human embryonic lung fibroblast (HELF) cells were obtained from the Biology Laboratory, Hebei Medical University, Hebei, China. Methylthiazolyl tetrazolium (MTT) and cisplatin were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

**Extraction of sesquiterpene lactones and structure determination**

The dichloromethane extract of the roots of *Inula helenium* was applied to a silica gel column eluted with CH₂Cl₂/acetone step gradients (30:1 to 1:1) to yield 78 major fractions designated FrDA-1 to FrDA-78. FrDA-1 – FrDA-5 were submitted to a 1:1) to yield 78 major fractions designated FrDA-1 to FrDA-78. FrDA-1 – FrDA-5 were submitted to a 1:1 reduction assay performed in triplicate. Cells containing various concentrations of the compounds was determined by the MTT determination assay. After treatment with the compounds for 48 h, 10 μl of 5 mg/ml MTT was added into each well, respectively, for another 4 h. Finally, 150 μl of stop solution [10 ml of 10% sodium dodecylsulfate (SDS), 6 μl of 12 M HCl] were added into each well, and the plate was placed in an incubator with 5% CO₂ at 37 °C for 12 h. Absorbance at 570 nm was measured with a microplate reader using wells without cells as blanks (reference wavelength 490 nm). Cell survival was calculated from the absorbance and presented as percentage of the surviving cells. The growth inhibition was calculated using the following formula: growth inhibition (%) = (1 – T/C) · 100%, where T and C are the mean absorbance values of the treated and control groups, respectively.

**Effects of isoalantolactone (3) on ascites tumours**

Ascite tumour cells (S180) aspirated from the peritoneal cavity of mice were washed with saline, and 10⁶ tumour cells were implanted into the right mediadorsal flask. One day later, animals were divided into five groups (ten mice per group). Animals in group I were kept with water (0.02 ml/g); animals in group II received intraperitoneal administration of cyclophosphamide [Sigma; C0768; 30 mg/(kg d)]; animals in groups III – V were given intragastric administration of isoalantolactone at concentrations of 1, 10, 100 mg/(kg d), respectively. Daily administration of cyclophosphamide and isoalantolactone was carried out from day 1 to day 10. Animals were observed for the development of ascite tumours. At the termination of the experiment all animals were sacrificed, and tumours, thymuses, and spleens were dissected and weighed. Tumour inhibition was calculated using the following formula: tumour inhibition (in % of control) = (C – T)/C · 100%, where T and C are the mean tumour weights of treated and control mice, respectively. Spleen indexes were calculated by the formula: spleen index = mean spleen weight (mg)/mean body weight (g) · 100%.

**Statistical analysis**

Statistical analysis was performed by ANOVA with Bonferroni for multiple comparisons. The data are given as mean ± SD. A value of *P* > 0.05 was considered statistically significant.
Acknowledgements

We are grateful for the financial support from the National Natural Science Foundation of China (81072551), the Key Project of Science & Technology of Hebei Province (11276103D-89), the Scientific Research Foundation for Returned Overseas Chinese Scholars of Hebei Province (2006–02), the Scientific Research Foundation of Hebei Province (08B032 and C2010000489), the Medical Research Foundation of the Health Department of Hebei Province (20090151), and a grant-in-aid from the Japan Society for the Promotion of Science (Nos. 19580120 and 22580112).


