Cytotoxic Thiophenes from the Root of *Echinops grijisii* Hance

Peng Zhang, Dong Liang, Wenrong Jin, Haibin Qu, Yiyu Cheng, Xian Li, and Zhongjun Ma

* Institute of Pharmaceutical Informatics, School of Pharmaceutical Sciences, Zhejiang University, Zijingang Campus, No. 388 Yuhangtang Rd., Hangzhou 310058, China. Fax: 86–571–88208428. E-mail: mazj@zju.edu.cn

** Author for correspondence and reprint requests

Z. Naturforsch. 64c, 193–196 (2009); received July 10/Oktobre 23, 2008

A new thiophene, echinothiophenegenol (1), together with seven known thiophenes was isolated from the crude ethanol extract of roots of *Echinops grijisii* Hance. The structure of 1 was elucidated on the basis of spectroscopic data. Compounds 2 and 5, isolated from the plant for the first time, and compounds 1–7 were tested for their cytotoxicity against two human cancer cell lines, HL60 and K562. The thiophenes showed better activity than the bithiophenes.

**Key words:** *Echinops grijisii* Hance, Echinothiophenegenol, Cytotoxic Activity

**Introduction**

The genus *Echinops* belongs to the family Compositae and comprises over 120 species, of which 17 occur in China. *E. grijisii* is mainly distributed in the southeast of the country (Shih, 1987). The root of *E. grijisii* (commercial Chinese name: Yuzhou Loulu) is listed in „Chinese Pharmacopeia“ and is used to clear heat, expel miasma and stimulate milk secretion (National Pharmacopeia Committee, 2005). Previous chemical investigations on the root of *E. grijisii* demonstrated the presence of essential oil (Guo et al., 1994) and thiophenes (Guo et al., 1992; Koike et al., 1999; Lin et al., 1999; Liu et al., 2002), which have been proven to possess several activities, like antitumour (Lambert et al., 1991; Marles et al., 1992), insect (Nivsarkar et al., 1991; Sharma and Goel, 1994), antivirus (Hudson et al., 1993; Marles et al., 1992) and anti-inflammatory (Lin et al., 1992).

The present paper describes the structure elucidation of compound 1 and cytotoxic activity of compounds 1–7. The known compounds, including 5-(4-hydroxybut-1-ynyl)-2,2′-bithiophene (2), 2-(penta-1,3-diynyl)-5-(4-hydroxybut-1-ynyl)-thiophene (3), 5-(3,4-dihydroxybut-1-ynyl)-2,2′-bithiophene (4), 5-(pro-1-ynyl)-2-(5,6-dihydroxy-penta-1,3-diynyl)thiophene (5), arctinol-b (6), 5-(penta-1,3-diynyl)-2-(3,4-dihydroxybut-1-ynyl)-thiophene (7), were identified by comparing their spectroscopic data with published data (Guo et al., 1992; Lin et al., 1999; Lu et al., 1989; Menelaou et al., 1991; Selva et al., 1978). Compounds 2 and 5 were isolated from this plant for the first time; they were tested for different tumour inhibitory effects against two human cancer cell lines.

**Material and Methods**

**Plant material**

The roots of *E. grijisii* were collected in Bozhou, north of Anhui Province, People’s Republic of China, in June 2006. The plant material was identified by the authors, and a voucher specimen (EGH060703) has been deposited in the herbarium of the Institute of Pharmaceutical Informatics, College of Pharmaceutical Sciences of Zhejiang University, Hangzhou, China.

**Extraction and isolation**

Air-dried pieces of the roots (14.3 kg) were extracted with 95% ethanol (3 h × 3) to give a crude extract, which was dissolved in distilled water to give a suspension, which was partitioned with dichloromethane (21 × 3) and n-butanol (21 × 3) successively. The n-butanol fraction (60 g) was chromatographed on silica gel, eluting with CH₂Cl₂/MeOH (7:3), to afford a complex mixture, which following RP-HPLC (30% CH₃CN/H₂O) led to the isolation of echinothiophenegen-
nol (1, 21 mg, \( t_R = 34.2 \) min). The dichloromethane fraction (132.2 g) was subjected to column chromatography over silica gel (5 × 50 cm, 300 – 400 mesh, 1.0 kg), eluted with petroleum ether (60–90 °C)/EtOAc to give fractions A–J (1:0, 200:1, 100:1, 50:1, 30:1, 20:1, 10:1, 5:1, 1:1, 0:1, each 3 l). Fractions F and G (total 12.0 g) were combined according to the TLC analysis and separated on a silica gel column (5 × 50 cm, 300 – 400 mesh, 1.0 kg), eluted with petroleum ether/EtOAc (50:1, 40:1, 30:1, 20:1, 10:1, 5:1, 1:1, 0:1, each 1 l) to give fractions 1–8. Then fraction 8 was separated by preparative HPLC using CH3CN/H2O (50:50) as the eluent to obtain two compounds, \( \text{2} (6.7 \text{ mg}, \ t_R = 29.5 \) min) and \( \text{3} (5.9 \text{ mg}, \ t_R = 17.5 \text{ min}) \). Fraction H (5.6 g) was separated on a silica gel column (5 × 50 cm, 300 – 400 mesh, 300 g) eluted with petroleum ether/EtOAc (30:1, 20:1, 15:1, 10:1, 8:1, 5:1, 3:1, 1:1, 0:1, each 1 l) to give 9 subfractions. Then subfraction H-8 was separated by preparative HPLC using CH3CN/H2O (40:60) as the eluent, and compounds \( \text{4} (12 \text{ mg}, \ t_R = 23.5 \text{ min}), \text{5} (11.6 \text{ mg}, \ t_R = 27.5 \text{ min}), \text{6} (8.78 \text{ mg}, \ t_R = 30.4 \text{ min}) \) and \( \text{7} (4.65 \text{ mg}, \ t_R = 33.1 \text{ min}) \) were obtained.

Echinothiophenogenol (1): Pale yellow powder (CHCl3). – IR (KBr): \( \nu_{\text{max}} = 3436, 1697, 1467 \text{ cm}^{-1} \). – 1H NMR (600 MHz, DMSO-\( \text{d}_6 \)) and 13C NMR (125 MHz, DMSO-\( \text{d}_6 \)): see Table I. – ESI-MS: \( m/z = 332 [M+H]^+ \). – HRESI-MS: \( m/z = 331.0643 [M-H]- \) (calcd. 331.0640).

Cytotoxicity assay

In the colorimetric assay, the cytotoxic activity of the isolated thiophenes against HL60 and K562 cells was evaluated by determining the IC50 values using a modification of the sulforhodamine B assay (Chen et al. 1997); the experimental procedures of cell culture and data analysis were performed as published by Jin et al. (2008).

Results

Echinothiophenogenol showed a quasimolecular ion peak at \( m/z \) 332 [M+H]+ in the ESI-mass spectrum. The presence of 17 carbon signals in the 13C NMR spectrum was consistent with the molecular formula \( \text{C}_{17}\text{H}_{16}\text{O}_{5}\text{S} \) that was established by HRESI-MS \( m/z = 331.0643 [M-H]^- \) (calcd. 331.0640), implying ten degrees of unsaturation. The IR absorption bands at 3436, 1697 and 1467 cm\(^{-1} \) suggested the presence of a hydroxy group and an aromatic chromophore. The 1H NMR spectrum (Table I) showed four olefinic protons at \( \delta = 5.66 \) (1H, dd, \( J = 7.6, 14.8 \text{ Hz} \), H-1′), 6.45 (1H, dd, \( J = 10.4, 14.8 \text{ Hz} \), H-2′), 6.12 (1H, dd, \( J = 10.4, 15.2 \text{ Hz} \), H-3′), 5.83 (1H, dt, \( J = 7.0, 15.2 \text{ Hz} \), H-4′), which indicated a linear unsaturated side chain. By COSY and HMBC experiments, the side chain was determined to be (E)-hexa-3,5-dien-1-ol. Except for the aromatic chromophore and the side chain, the remaining signals were identified as one carbonyl group, two olefinic carbon atoms, and a hydroxymethyl group. The above functionalities accounted for eight degrees of unsaturation, revealing a tricyclic structure of the molecule. Thus the parent nucleus of echinothiophenogenol was established to be benzo[b]thiophene fused to a \( \gamma \)-lactone.

NOESY cross-peak correlations of H-4 (7.48)/H-3 (7.32) and H-3/H-1″ (4.70) confirmed the fusion way and the relationship of the three groups. The position of the hydroxy group was verified by its long-range correlations with C-4 (114.4), C-5 (150.6) and C-5a (135.1) in the HMBC spec-

<table>
<thead>
<tr>
<th>No.</th>
<th>( \delta_c )</th>
<th>( \delta_h )</th>
<th>No.</th>
<th>( \delta_c )</th>
<th>( \delta_h )</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>143.3</td>
<td></td>
<td>8b</td>
<td>150.4*</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>119.4</td>
<td>7.32 (s)</td>
<td>1′</td>
<td>125.2</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>125.2</td>
<td></td>
<td>2′</td>
<td>134.7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>114.4</td>
<td>7.48 (s)</td>
<td>3′</td>
<td>130.5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>150.6*</td>
<td></td>
<td>4′</td>
<td>134.7</td>
<td></td>
</tr>
<tr>
<td>5a</td>
<td>135.1</td>
<td></td>
<td>5′</td>
<td>36.3</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>81.2</td>
<td>6.16 (d, ( J = 7.6 \text{ Hz} ))</td>
<td>6′</td>
<td>60.7</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>169.5</td>
<td></td>
<td>1″</td>
<td>59.2</td>
<td></td>
</tr>
<tr>
<td>8a</td>
<td>120.2</td>
<td></td>
<td>5-OH</td>
<td>10.11 (s)</td>
<td></td>
</tr>
</tbody>
</table>

* Signals can be exchanged.
The coupling between H-6 (6.16) and H-1' (5.66) in the NOESY spectrum, together with the cross-peaks of H-6 with C-8 (169.5), C-8a (120.2), C-1' (125.2) and C-2' (134.7), and H-2' with C-6 (81.2) led to the conclusion that the side chain is substituted at C-6. Therefore, the structure of echinothiophenegenol was established as 5-hydroxy-6[(1E,3E)-6-hydroxy-1,3-hexadienyl]-2-hydroxymethylthieno[2,3-e]-isobenzofuran-8(6H)-one (Fig. 1). The 1H and 13C NMR signals (see Table I) were assigned based on COSY, HSQC, HMBC (Fig. 1).

The absolute configuration of C-6 was discussed in a previous paper (Koike et al., 1999). Because of the equilibrium between the 6R and 6S isomer via an enol intermediate in solution, it was not possible to separate them. According to the reference, the 6R isomer is the more stable one based on molecular mechanics and dynamic calculations. Thus, it is believed that the 6R isomer is most probably the one that crystallized out and for which NMR data were obtained.

The in vitro cytotoxic activity of compounds 1–7 was tested against different human cell lines. The 50% inhibitory concentrations (IC50) are listed in the Table II. All seven compounds exhibited cytotoxic activity against HL60 and K562 cells, with the IC50 values ranging from 0.23 to 30.6 μg/ml.

### Discussion

All the isolated compounds were found to be highly hydroxylated thiophenes. However, there were no reports on the cytotoxicities of these types of thiophenes from *E. grijisii*. The cytotoxicity of the monothiophenes 3 and 7 was extremely higher than that of the other thiophenes in the cytotoxicity tests. The substituted alkyne groups on both sides of the monothiophenes might be a key factor in enhancing the cytotoxic activity. Besides, the cytotoxicity of all seven compounds against the HL60 cell line was much higher than that against the K562 cell line.

### Acknowledgements

The research was supported by the Program for New Century Excellent Talents in University (NCET-06–0515) and National Natural Science Foundation of China (No. 30701048).

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 [μg/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HL60a</td>
</tr>
<tr>
<td>1</td>
<td>12.7</td>
</tr>
<tr>
<td>2</td>
<td>13.5</td>
</tr>
<tr>
<td>3</td>
<td>0.23</td>
</tr>
<tr>
<td>4</td>
<td>17.4</td>
</tr>
<tr>
<td>5</td>
<td>15.2</td>
</tr>
<tr>
<td>6</td>
<td>14.1</td>
</tr>
<tr>
<td>7</td>
<td>0.27</td>
</tr>
</tbody>
</table>

a For HL60 cells, cell inhibitory rate of the positive control (platinol) was 87% at 4 μg/ml.
b For K562 cells, cell inhibitory rate of the positive control (adriamycin) was 80% at 4 μg/ml.


