Cytotoxic Thiophenes from the Root of *Echinops grijisii* Hance

Peng Zhang[a], Dong Liang[b], Wenrong Jin[a], Haibin Qu[a], Yiyu Cheng[a], Xian Li[b], and Zhongjun Ma[a,*]

[a] 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

[b] Department of Natural Medicines, Shenyang Pharmaceutical University, No. 103 Wenhua Road, Shenyang 110016, China

* Author for correspondence and reprint requests

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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 Pharmacopoeia 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 know 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-dihydroxypenta-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 (2 l × 3) and *n*-butanol (2 l × 3) successively. The *n*-butanol fraction (60 g) was chromatographed on silica gel, eluting with CH_2Cl_2/MeOH (7:3), to afford a complex mixture, which following RP-HPLC (30% CH_3CN/H_2O) led to the isolation of echinothiophenege-

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, 300 g) 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 CH_3CN/H_2O (50:50) as the eluent to obtain two compounds, **2** (6.7 mg, t_R = 29.5 min) and **3** (5.9 mg, t_R = 17.50 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 CH_3CN/H_2O (40:60) as the eluent, and compounds **4** (12 mg, t_R = 23.5 min), **5** (11.6 mg, t_R = 27.5 min), **6** (8.78 mg, t_R = 30.4 min) and 7 (4.65 mg, t_R = 33.1 min) were obtained.

*Echinothiophenegenol (***1***):* Pale yellow powder ($CHCl_3$). – IR (KBr): ν_{max} = 3436, 1697, 1467 cm^{-1}. – ^1H NMR (600 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-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 IC_{50} 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

Echinothiophenegenol showed a quasimolecular ion peak at *m/z* 332 $[M+H]^+$ in the ESI-mass spectrum. The presence of 17 carbon signals in the ^{13}C NMR spectrum was consistent with the molecular formula $C_{17}H_{16}O_5S$ 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 δ 5.66 (1H, dd, *J* = 7.6, 14.8 Hz, H-1′), 6.45 (1H, dd, *J* = 10.4, 14.8 Hz, H-2′), 6.12 (1H, dd, *J* = 10.4, 15.2 Hz, H-3′), 5.83 (1H, dt, *J* = 7.0, 15.2 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 echinothiophenegenol was established to be benzo[*b*]thiophene fused to a γ-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 I. The ^1H (DMSO-d_6, 600 MHz) and ^{13}C (DMSO-d_6, 125 MHz) NMR data of compound **1**.

No.	δ_C	δ_H	No.	δ_C	δ_H
2	143.3		8b	150.4*	
3	119.4	7.32 (s)	1′	125.2	5.66 (dd, *J* = 14.8, 7.6 Hz)
3a	125.2		2′	134.7	6.45 (dd, *J* = 14.8, 10.4 Hz)
4	114.4	7.48 (s)	3′	130.5	6.12 (dd, *J* = 15.2, 10.4 Hz)
5	150.6*		4′	134.7	5.83 (dt, *J* = 15.2, 7.0 Hz)
5a	135.1		5′	36.3	2.18 – 2.22 (m)
6	81.2	6.16 (d, *J* = 7.6 Hz)	6′	60.7	3.09 – 3.13 (m)
8	169.5		1″	59.2	4.7 (s)
8a	120.2		5-OH		10.11 (s)

* Signals can be exchanged.

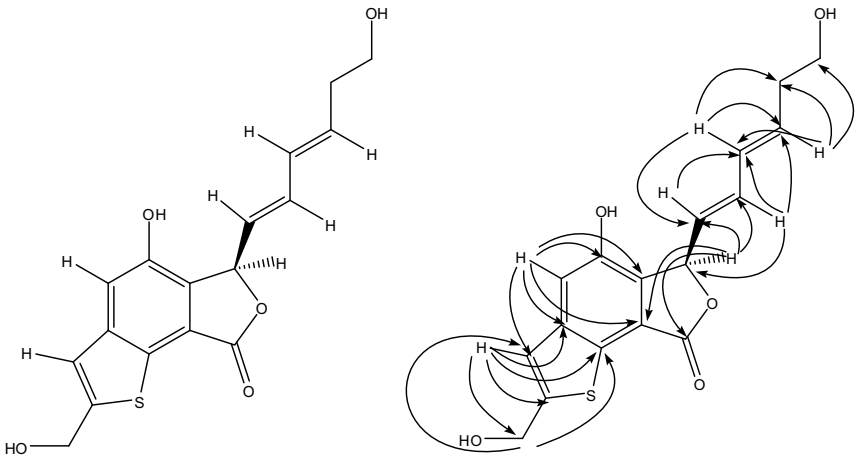

Fig. 1. Chemical structure and key HMBC correlations of compound **1**.

trum. 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[(1*E*,3*E*)-6-hydroxy-1,3-hexadienyl]-2-hydroxymethylthieno[2,3-e]-isobenzofuran-8(6*H*)-one (Fig. 1). The ^{1}H and ^{13}C 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 6*R* and 6*S* isomer *via* an enol intermediate in solution, it was not possible to separate them. According to the reference, the 6*R* isomer is the more stable one based on molecular mechanics and dynamic calculations. Thus, it is believed that the 6*R* isomer is most probably the one that crystallized out and for which NMR data were obtained.

Table II. IC_{50} values of the isolated thiophenes **1**–**7** against different cell lines.

Compound	IC_{50} [μg/ml]	
	HL60[a]	K562[b]
1	12.7	30.6
2	13.5	14.5
3	0.23	0.47
4	17.4	29.5
5	15.2	19.3
6	14.1	21.2
7	0.27	0.43

[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.

The *in vitro* cytotoxic activity of compounds **1**–**7** was tested against different human cell lines. The 50% inhibitory concentrations (IC_{50}) are listed in the Table II. All seven compounds exhibited cytotoxic activity against HL60 and K562 cells, with the IC_{50} 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.

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