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水朝阳旋覆花中新的细胞毒活性麝香草酚类化合物

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摘要：从水朝阳旋覆花（*Inula helianthus-aquatica*）的95%乙醇提取物中分离得到4个麝香草酚类化合物，其中化合物1为新化合物。它们的化学结构通过波谱方法鉴定为：8-hydroxy-9，10-dioxyisopropylidene-thymol (1)，10-hydroxy-8，9-dioxyisopropylidene-thymol (2)，8-hydroxy-9，10-diisobutyryloxy-thymol (3) 和8，10-dihydroxy-9-isobutyryloxy-thymol (4)。肿瘤细胞毒试验结果表明它们在6种肿瘤细胞株上（K562，HT-29，SGC-7901，DU145，MDA-MB-231，U251）显示一定的细胞毒活性，其中化合物2活性最强，它的IC_{50}值为4.20～33.12 μmol L。具有细胞毒活性的麝香草酚类化合物是首次在该种植物中发现。

关键词：水朝阳旋覆花；麝香草酚类化合物；细胞毒活性
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New Cytotoxic Thymol Derivatives from *Inula helianthus-aquatica* (Compositae)*

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Abstract: A new thymol derivative, 8-hydroxy-9, 10-dioxyisopropylidene-thymol (1), together with three known thymol derivatives 10-hydroxy-8, 9-dioxyisopropylidene-thymol (2), 8-hydroxy-9, 10-diisobutyryloxy-thymol (3) and 8, 10-dihydroxy-9-isobutyryloxy-thymol (4), was isolated from 95% EtOH extract of the flowers of *Inula helianthus-aquatica*. Their structures were elucidated on the basis of detailed spectroscopic analysis. These compounds showed cytotoxicities on six cancer cell lines (K562, HT-29, SGC-7901, DU145, MDA-MB-231, U251), and compound 2 is the strongest one with IC_{50s} of 4.20-33.12 μmol L. This is the first time that the cytotoxic thymol derivatives were isolated from this titled plant.

Key words: *Inula helianthus-aquatica*; Thymol derivatives; Cytotoxicity

Inula helianthus-aquatica C. Y. Wu ex Ling (Compositae) has been used commonly to treat various cancers in Yunnan, China (Hu and Xuan, 1982). Some sesquiterpenes, particularly anti-cancer ones in vitro and in vivo such as ergolide and bigelovin, were isolated and determined from this herb (Wang *et al.*,

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1996; Zhang *et al*., 1998; Liang *et al*., 1998; Zeng *et al*., 2008). We describe herein the isolation and structural elucidation of a new thymol derivative (1) and three known thymol derivatives 10-hydroxy-8, 9-dioxyisopropylidene-thymol (2) (Liang *et al*., 2007), 8-hydroxy-9, 10-diisobutyryloxy-thymol (3) (Mossa *et al*., 1997) and 8, 10-dihydroxy-9-isobutyryloxy-thymol (4) (Trang *et al*., 1993) from 95% EtOH extract of the flowers of *I. helianthus-aquatica* (Fig. 1). These compounds were tested on six cancer cell lines (K562, HT-29, SGC-7901, DU145, MDA-MB-231, U251).

Compound 1 was obtained as colorless oil. Its molecular formula was established as $C_{13}H_{18}O_4$ by FAB$^+$-MS and DEPT spectra (Table 1), which was confirmed by HR-ESI-MS *m z* 261.1099 ([M + Na]$^+$ calcd. for $C_{13}H_{18}NaO_4$, 261.1102). The IR spectrum displayed absorption bonds of hydroxyl group (3424 cm^{-1}) and benzene ring (1629 cm^{-1}), the latter was confirmed by the UV spectrum (191, 204, 276, 281 nm). The ^{13}C and ^{1}H NMR spectra revealed the presence of one 1, 3, 4-trisubstituted phenyl and isopropyl moieties. The left four carbons are one methyl, two oxygenated methylenes and one oxygenated quaternary carbon. The HMQC, COSY and HMBC spectra, particularly HMBC correlations of H-2 with C-4, C-6 and C-7, H-5 with C-1, C-3 and C-8, H-6 with C-4 and C-7, H-7 with C-1, H-9 and H-10 with C-4, C-8 and C-1′, H-2′ and H-3′ with C-1′, indicated that 1 is one 8, 9, 10-trioxygenated derivative of thymol (Trang *et al*., 1993; Mossa *et al*., 1997; Liang *et al*., 2007). Therefore the structure of 1 was determined to be 8-hydroxy-9, 10-dioxyisopropylidene-thymol.

Results from cell growth inhibition assay indicated that these four compounds showed cytotoxicities on a panel of six cancer cell lines (K562, HT-29, SGC-7901, DU145, MDA-MB-231, U251) (Table 2). Particularly compound 2 inhibited cell proliferation of all tested cancer cell lines, in which K562 was the most sensitive one with IC_{50} of 4.20 μmol L, while compound 1, 3, 4 selectively affected K562 with IC_{50s} of 92.89, 20.69, 30.38 μmol L. That means thymol derivatives

Table 1　^{1}H and ^{13}C NMR spectral data of compound 1 in $CDCl_3$ (^{1}H: 400 MHz, ^{13}C: 100 MHz)

position	δ_C	δ_H (J)	HMBC
1	140.1s		
2	118.7d	6.73s	C-4, C-6, C-7
3	156.6s		
4	118.4s		
5	124.9d	6.84d (7.9)	C-1, C-3, C-8
6	120.5d	6.66br d (7.9)	C-4, C-7
7	21.0q	2.28s	C-1, C-2, C-6
8	72.1s		
9	67.9t	3.84d (11.9), 4.22 (11.9)	C-4, C-8, C-1′
10	67.9t	3.84d (11.9), 4.22 (11.9)	C-4, C-8, C-1′
1′	98.7s		
2′	17.7q	1.57s	C-1′
3′	29.1q	1.52s	C-1′

Table 2　Cytotoxicities of compounds 1-4 against cancer cell lines

Cell lines	IC_{50} (μmol L)			
	1	2	3	4
K562	92.89	4.20	20.69	30.38
HT-29	—	19.12	—	—
SGC-7901	—	4.43	—	—
DU145	—	15.74	—	—
MDA-MB-231	—	19.41	—	—
U251	—	33.12	—	—

“—”, no activity.

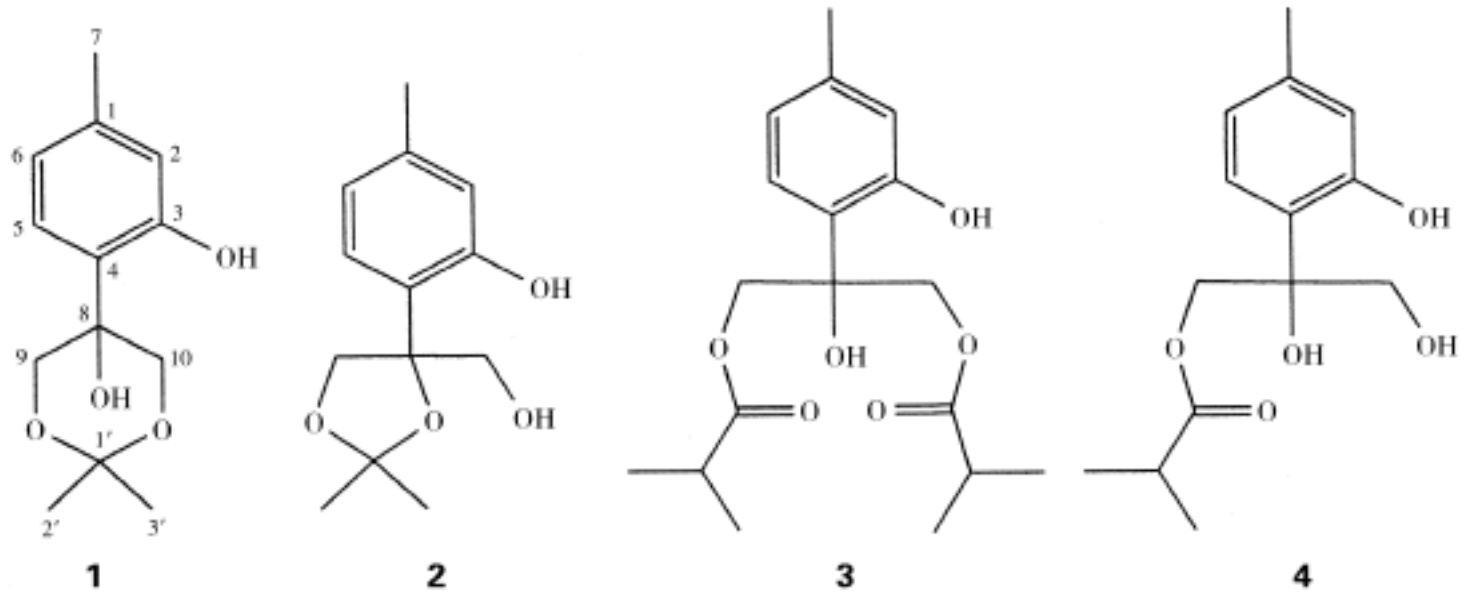

Fig. 1　Structures of compounds 1-4

are another kind of anti-cancer active constituents for this herb . This is the first time that the cytotoxic thymol derivatives were isolated from this titled plant .

Experimental

General IR spectrum was recorded using a Bruker Tensor27 spectrometer . UV spectrum was taken on a Shimadzu UV-2401PC spectrometer . MS was carried out on a VG Autospec-3000 mass spectrometer and API QSTAR Pulsar i spectrometer . NMR spectrum was run in $CDCl_3$ on a Bruker AM-400 and DRX-500 spectrometer with TMS as the internal standard . Column chromatographic silica gel and TLC silica gel plates were from Qingdao Marine Chemical Group Corporation, Qingdao, P . R . China . Fractions were monitored by TLC, in which spots were detected with 5% H_2SO_4 in ethanol .

Material The flowers of *I. helianthus-aquatica* were collected in 2004 in Dali of Yunnan province, P . R . China . It was identified by Professor Li Xi-Wen from Kunming Institute of Botany, Chinese Academy of Sciences . Human cancer cell lines K562 (leukemia cell line), HT-29 (colon cancer cell line), SGC-7901 (gastric cancer cell line), DU-145 (prostate cancer cell line), MDA-MB-231 (breast cancer cell line) and U-251 (glioma) were purchased from the Cell Culture Centre of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Perking, China) .

Extraction and Isolation Dried flowers of *I. helianthus-aquatica* (20.0 kg) were powdered and extracted with 95% EtOH under reflux for three times . The 95% EtOH extract (1.0 kg) was dissolved in water and partitioned with ethyl acetate and *n*-butanol successively . The ethyl acetate extracts (300.0 g) were subjected to column chromatography over silica gel eluted with petroleum ether acetone (15∶1 to 0∶1) to afford four fractions (1 - 4) . Fraction 1 (100.0 g) was chromatographed over silica gel (200 - 300 mesh), eluted with petroleum ether acetone (20∶1 to 1∶1) to afford fractions A-F . Fraction C (6.5 g) was isolated by silica gel column (400 - 600 mesh), eluted with petroleum ether acetone (10∶1 to 3∶1) to give compound 3 (110.0 mg) . Fraction 4 (40.0 g) was chromatographed over silica gel (200 - 300 mesh), eluted with chloroform acetone (10∶1 to 0∶1) to afford 4 fractions G-J . Fraction H (6.3 g) was isolated by silica gel column chromatography [400 - 600 mesh, chloroform acetone (10∶1 to 2∶1)] to give compounds 1 (4.0 mg), 2 (23.0 mg), and 4 (4.0 mg) .

8-Hydroxy-9, 10-dioxyisopropylidene-thymol (1), colorless oil, $C_{13}H_{18}O_4$; UV $\lambda_{max}^{CH_3OH}$ nm (log ε): 191 (3.70), 204 (4.31), 276 (3.35), 281 (3.35); IR ν_{max}^{KBr} cm^{-1}: 3424, 2926, 1629, 1057; ^{1}H- and ^{13}C-NMR: see Table 1; FAB-MS (pos .) *m z*: 238 $[M]^+$ (14), 221 (12), 207 (100); HR-ESI-MS (pos .): 261.1099 ($[M + Na]^+$, $C_{13}H_{18}NaO_4$, calcd . 261.1102) .

Cell Growth Inhibition Assay All cancer cells were cultured in RPMI-1640 containing 10% fetal bovine serum and 5 CC Pen-Strep . Compound cytotoxicity was assessed by the sulfurhodamine B (SRB) assay described before (Skehan *et al.*, 1990) . Firstly, 3000 - 7000 cells well were plated in 96-well plates . Twenty-four hours later, compounds were added to a final concentration of 10 μg mL . After incubated for 48 h, cells were fixed by the addition of 50% (or 80% for K562 cells) ice-cold trichloroacetic acid and left at 4℃ for 1 h . Then Plates were washed 5 times in water, air-dried, and stained for 15 min with 100 μL 0.4% SRB in 1% glacial acetic acid . Excessive dye was removed by washing 5 times in 1% glacial acetic acid . After plates were air-dried, SRB was resuspended in 100 μL 10 mmol L Tris and the OD values were read at 560 nm on a 96-well plate reader (Molecular Devices, SPECTRA MAX 340) . Inhibition data were expressed as IC_{50} values, which were calculated by dose-response curves with at least four concentrations (dilution ratio = 1 10) .

References:

胡月英 (Hu YY), 宣明盛 (Xuan MS), 1982 . 云南抗癌中草药 [M] . 昆明: 云南人民出版社, 226

Liang HX, Bao FK, Dong XP *et al.*, 2007 . Antibacterial thymol derivatives isolated from *Centipeda minima* [J] . *Molecules*, 12: 1606—1613

Liang MD (梁明达), Liang H (梁红), Jia W (贾伟) *et al.*, 1998 . The studies on the anti-cancer effect and toxicity of *Inula helianthus-aquatica* compositae [J] . *Chinese Journal of Ethnomedicine and Ethnopharmacy* (中国民族民间医药杂志), 35: 23—28

Mossa JS, Ei-Feraly FS, Muhammad I *et al.*, 1997 . Sesquiterpene lactones and thymol esters from *Vicoa pentanema* [J] . *Journal of Natural Products*, 60: 550—555

Skehan P, Storeng R, Scudiero D *et al.*, 1990 . New colorimetric cytotoxicity assay for anticancer-drug screening [J] . *Journal of the National Cancer Institute*, 82: 1107—1112

Trang ND, Wanner MJ, Koomen GJ *et al.*, 1993 . New acetophenone and thymol derivatives from *Eupatorium stoechadosmum* [J] . *Planta Medica*, 59: 480—481

Wang Q, Zhou BN, Zhang RW *et al.*, 1996 . Cytotoxicity and NMR spectral assignments of ergolide and bigelovin [J] . *Planta Medica*, 62: 166—168

Zeng GZ, Tan NH, Ji CJ *et al.*, 2009 . Apoptosis inducement of bigelovin from *Inula helianthus-aquatica* on human leukemia U937 cellls [J] . *Phytotherapy Research*, 23: in press

Zhang RW (张人伟), Lin YY (林咏月), Qi YF (戚育芳) *et al.*, 1998 . Studies on the anticancer constituents of *Inula helianthus-aquatica* C . Y . Wu ex Ling [J] . *Natural Products Research and Development* (天然产物研究与开发), 10: 31—33