BIOACTIVITY-GUIDED ISOLATION OF ANTIPROLIFERATIVE COMPOUNDS FROM *ONOPORDUM ACANTHIUM*

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INTRODUCTION

Antiproliferative screening of 51 Asteraceae species against human tumor cell lines demonstrated high cell proliferation inhibitory activity of *Onopordum acanthium*. The chloroform extract, prepared from herbs and roots, significantly inhibited the growth of HeLa (98.30% and 94.17%), MCF7 (98.08% and 88.74%) and A431 (94.17% and 93.35%) cells *in vitro* at 30 µg/mL concentration (**Fig. 1**) [1-2].

This plant is naturalized in various parts of the world, from the Mediterranean area to Western Asia, its antitumor constituents were not investigated earlier. *O. acanthium* has been used traditionally for its antibacterial, hemostatic, and hypotensive properties. Moreover, the juice has been considered to be effective against cancerous ulcers, carcinoma of the face and other cancers in traditional medicine [3]. Previous studies revealed the presence of lupeol, amyrin, taraxasterol and its acetate, onopordopicrin and arctiopicrin [4-5].

The aim of the present work was the isolation and identification of the antiproliferative compounds of different plant parts using bioactivity guided fractionations.

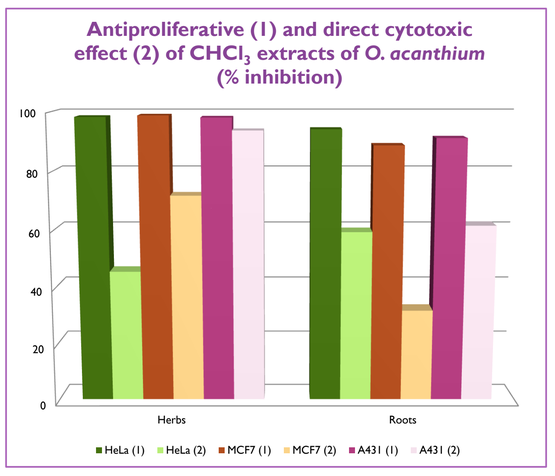

Figure 1. Cell proliferation inhibitory and cytotoxic activity of $CHCl_3$ extracts of *O. acanthium*

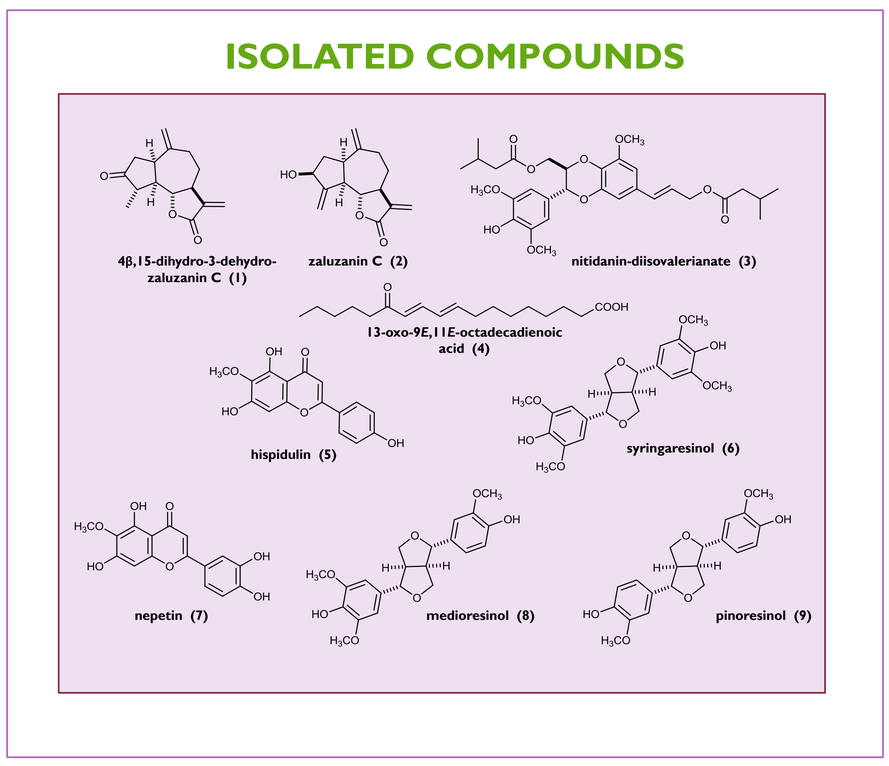

Figure 2. Structures of the isolated compounds 1–9

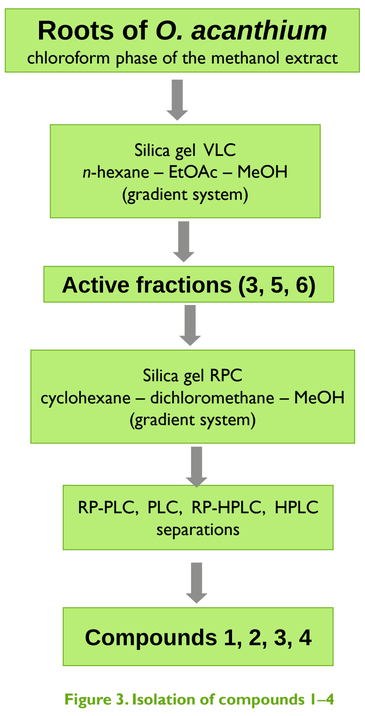

Figure 3. Isolation of compounds 1–4

MATERIALS AND METHODS

Plant material: Herbs and roots of *Onopordum acanthium* were collected in May and November 2008 in South-Lowlands (Hungary).

Extraction and isolation: The fresh and freezed plant material (4.4 and 2.1 kg) was percolated with methanol (6L and 35 L) at room temperature. The methanol extracts were subjected to solvent-solvent partition, affording *n*-hexane, chloroform and the remnant aqueous extracts. The $CHCl_3$ extracts were chromatographed by CC on silica gel and polyamide, and further separated by MPLC, RPC, PLC, GFC, HPLC and RP-HPLC.

Structure determination: NMR spectra were recorded in $CDCl_3$ on a Bruker Avance DRX 500 spectrometer at 500 MHz (^1H) and 125 MHz (^{13}C), with TMS as internal standard. Two dimensional data were acquired and processed with standard Bruker software. MS spectra were recorded API 2000 Triple Quad mass spectrometer at APCI ion source with positive (**1, 2, 3, 5, 7**) and negative (**4, 6, 8, 9**) of polarity.

***Antiproliferative and cytotoxicity assay*:** Antiproliferative effects were measured *in vitro* on HeLa (cervix adenocarcinoma), MCF7 (breast adenocarcinoma) and A431 (skin epidermoid carcinoma) cells using the MTT ([3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide]) assay (Mosmann, 1983). All experiments were carried out in duplicate on 96-well microplates with at least 5 parallel wells. Stock solutions of 10 mg/mL of the tested extracts and compounds were prepared with DMSO. In the assay a cell population of 5000/well and an incubation period of 72 h were used. The IC_{50} values were determined in a concentration range of 10 and 30 µg/mL and dose-response curves were fitted by the means of computer program GraphPad Prism 2.01. In order to differentiate the antiproliferative (cytostatic) and direct cytotoxic effect of the extracts, a parallel set of experiments were performed. The cytotoxic assay was the same as described for antiproliferative assay but a higher cell population (25000/well) was exposed for a shorter period (24 h). The determination of this difference was performed at a concentration of 30 µg/mL. The experimental details are described in ref. 1, 2. Doxorubicin and cisplatin were used as positive controls.

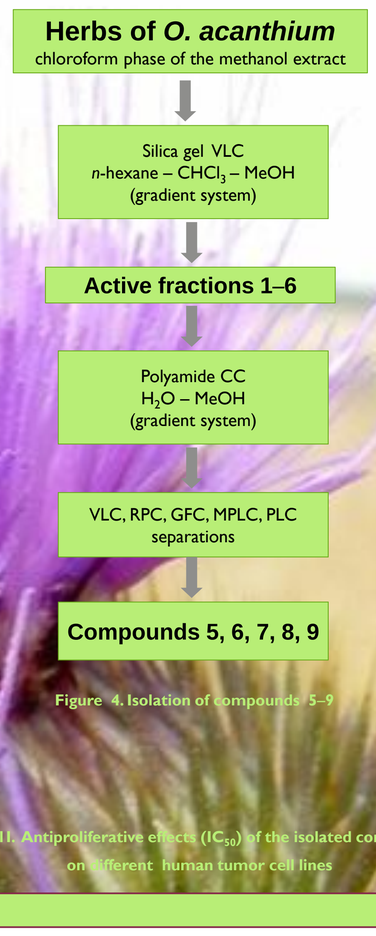

Figure 4. Isolation of compounds 5–9

Table 1. NMR data of compound 1 [500 MHz (^1H), 125 MHz (^{13}C), δ ppm, J = Hz, $CDCl_3$]

Atom	^1H	^{13}C	HMBC
1	3.09 m	39.8	2, 9b, 14a, 14b
2	2.55 m	43.9	1, 5
3	-	219.2	2, 4, 5, 15
4	2.30 m	47.1	6, 5, 15
5	2.30 m	50.7	1, 2, 6, 15
6	3.99 t (9.7)	88.8	5, 7, 8a, 8b
7	3.01 tt (3.0, 10.5)	44.0	8b, 13a, 13b
8a	2.30 m	31.8	6, 9a, 9b, 14a, 14b
8b	1.48 dd		
9a	2.63 m	38.6	8b, 8a, 14a, 14b
9b	2.22 m		
10	-	148.7	2, 5, 8a, 9a, 9b, 14a, 14b
11	-	138.8	7, 13a, 13b
12	-	169.0	13a, 13b
13a	6.29 d (3.4)	121.3	
13b	5.58 d (3.0)		
14a	5.01 s	113.1	9a, 9b
14b	4.68 s		
15	1.28 d (6.5)	14.2	4, 5

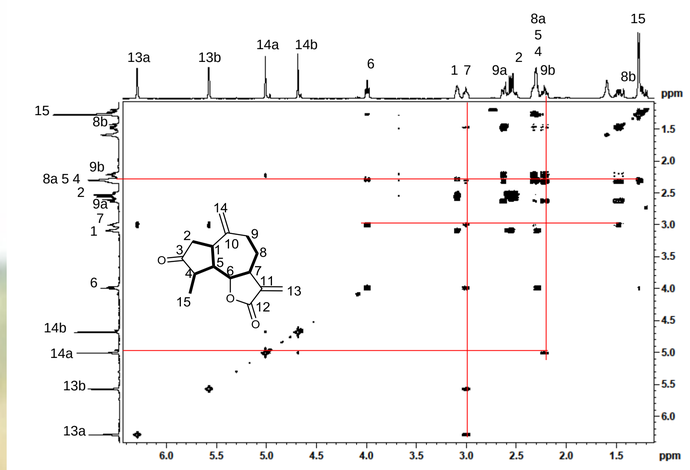

Figure 5. ^1H-^1H COSY spectrum of compound 1

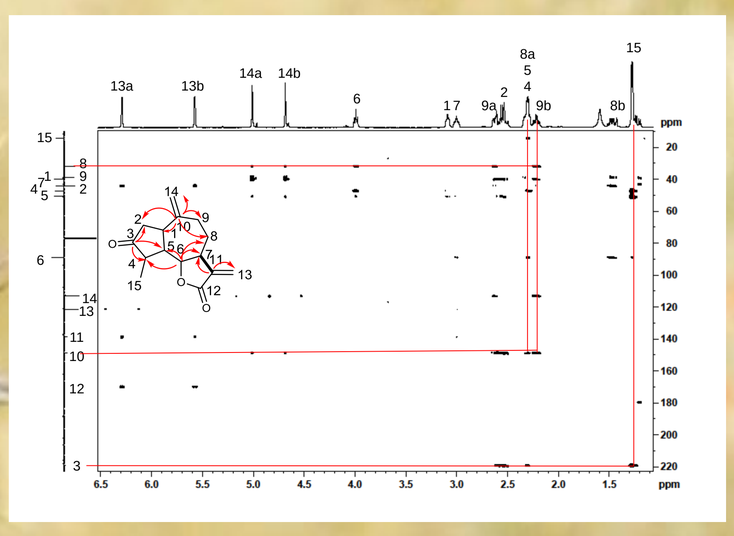

Figure 6. HMBC spectrum of compound 1 (C→H)

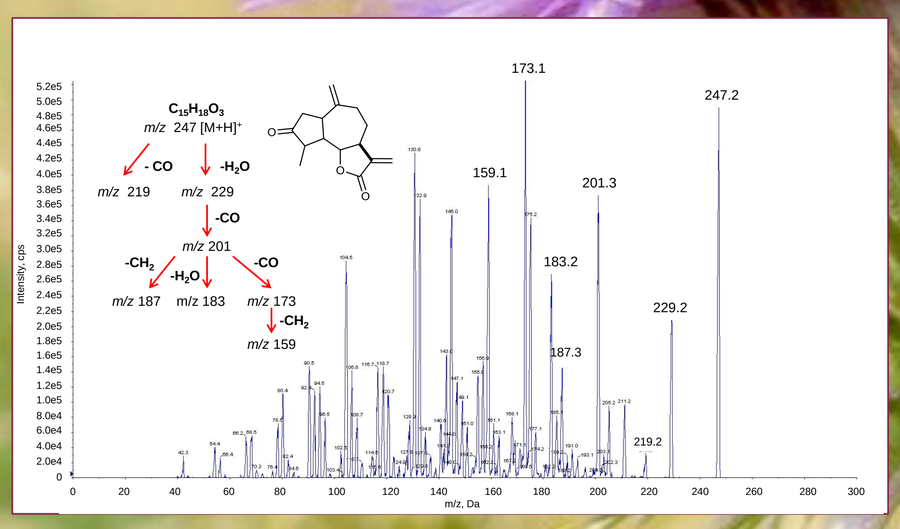

Figure 7. APCI pos mass spektrum of compound 1

Table 11. Antiproliferative effects (IC_{50}) of the isolated compounds on different human tumor cell lines

Compound	IC_{50} (µM) HeLa	MCF7	A431
4β,15-dihydro-3-dehydro-zaluzanin C (**1**)	15,06	4.191	2.68
zaluzanin C (**2**)	inactive	29.51	inactive
hispidulin (**5**)	5.68	37.97	80.97
syringaresinol (**6**)	inactive	inactive	inactive

RESULTS AND DISCUSSION

Herbs and roots of *O. acanthium* were extracted with methanol. The methanol extracts were subjected to solvent-solvent partition, affording *n*-hexane, chloroform and the remnant aqueous extracts. The $CHCl_3$ extracts were chromatographed by CC on silica gel and polyamide, and further separated by MPLC, RPC, preparative TLC and RP-HPLC (**Fig. 3-4**).

Compounds **1–9** were isolated from the active fractions and identified by means of MS and NMR spectroscopy (^1H NMR, JMOD, ^1H-^1H COSY, NOESY, HSQC and HMBC) and comparison of the spectral data with literature values (**Table 1**, **Fig. 5-7**). The flavonoids hispidulin (**5**) and nepetin (**7**), other phenolic compound nitidanin-diisovalerianate (**3**), the lignanes syringaresinol (**6**), medioresinol (**8**) and pinoresinol (**9**), the sesquiterpene lactones 4β,15-dihydro-3-dehydro-zaluzanin C (**1**), zaluzanin C (**2**), and the fatty acid 13-oxo-9*E*,11*E*-octadecadienoic acid (**4**) were obtained for the first time from this species (**Fig. 2**).

Hispidulin (**5**), 4β,15-dihydro-3-dehydro-zaluzanin C (**1**), zaluzanin C (**2**) and syringaresinol (**6**) were evaluated for their tumor cell inhibitory activity on HeLa, MCF7 and A431 cells and found that 4β,15-dihydro-3-dehydro-zaluzanin C (**1**) exhibited high activity on all cell lines (IC_{50} 15,06 µM on HeLa, 4.191 µM on MCF7 and 2.68 µM on A431), while hispidulin (**5**) was the most active on HeLa cell line (5.68 µM) (**Table 2**).

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ACKNOWLEDGEMENTS

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