Xanthanolides with Antitumour Activity from *Xanthium italicum*

Adriána Kovács[a], Andrea Vasas[a], Peter Forgo[a], Borbála Réthy[b], István Zupkó[b], and Judit Hohmann[a,*]

[a] Department of Pharmacognosy, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary. Fax: +36-62-54 57 04. E-mail: hohmann@pharm.u-szeged.hu
[b] Department of Pharmacodynamics and Biopharmacy, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary

* Author for correspondence and reprint requests

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Bioassay-guided fractionation of a $CHCl_3$ extract of the leaves of *Xanthium italicum* Moretti led to the isolation of four xanthanolides: xanthatin (**1**), 4-epixanthanol (**2**), 4-epi-isoxanthanol (**3**), and 2-hydroxyxanthinosin (**4**). Their structures were determined by means of 1D and 2D NMR spectroscopy, including ^{1}H-^{1}H COSY, NOESY, HSQC and HMBC experiments, which resulted in complete and unambiguous ^{1}H and ^{13}C NMR chemical shift assignments. The isolated compounds **1**–**4** were evaluated for their antiproliferative activities, and were demonstrated to exert significant cell growth inhibitory activity against human cervix adenocarcinoma (HeLa), skin carcinoma (A431), and breast adenocarcinoma (MCF7) cells.

Key words: Xanthium italicum, Xanthanolides, Antitumour Activity

Introduction

The genus *Xanthium* (Asteraceae, Heliantheae), represented by 25 species, exhibits a global distribution and is found abundantly throughout Eurasia and America. This genus has been the subject of many chemical studies, which have led to the isolation of a special type of sesquiterpene lactones (xanthanolides) (Saxena and Mondal, 1994), diterpenes (Piacente *et al.*, 1996), thiazinediones (Ma *et al.*, 1998; Qin *et al.*, 2006), sterols and caffeoylquinic acids (Agata *et al.*, 1993) as main secondary metabolites. *Xanthium* species have been used in traditional herbal medicine worldwide; some of them have been applied for the treatment of basal cell carcinoma, different cancers and "cold tumours" (Hartwell, 1968). *X. italicum* Moretti [syn. *X. strumarium* L. subsp. *italicum* (Moretti) D. Löve], a member of the *X. strumarium* group, has been used in folk medicine against cancers in Latin-America (Hartwell, 1968). Previous investigations revealed the presence of xanthanolides and phytosterols in *X. italicum* (Marco *et al.*, 1993; Tsankova *et al.*, 1993, 1994).

In the course of our screening programme for antiproliferative compounds in Central and Eastern European Asteraceae species, extracts of *X. italicum* were investigated earlier (Réthy *et al.*, 2007). High tumour cell growth inhibitory activities on HeLa (cervix adenocarcinoma), A431 (skin carcinoma), and MCF7 (breast adenocarcinoma) human cell lines were recorded *in vitro* for different lipophilic extracts by means of the MTT assay. The present paper reports on investigations of the antiproliferative effects of the extracts of different plant parts of *X. italicum*, collected before and in the flowering stage, with the aim of selecting the most effective extract. The bioassay-guided fractionation of the $CHCl_3$ extract of the leaves was additionally performed in an effort to identify the compounds responsible for the tumour cell growth inhibitory effect. This led to the isolation and identification of four xanthanolides: xanthatin (**1**), 4-epixanthanol (**2**), 4-epi-isoxanthanol (**3**), and 2-hydroxyxanthinosin (**4**), all possessing tumour cell growth inhibitory activities.

Results and Discussion

The dried and powdered plant parts (radix, flower, stem and leaf) collected before and during the flowering period were extracted with MeOH and, after evaporation, the residues from the extracts were subjected to solvent-solvent partitioning. Extraction with lipophilic solvents yielded *n*-hexane-, $CHCl_3$- and H_2O-soluble phases. These fractions, together with the original MeOH extract, were tested for their tumour cell prolifera-

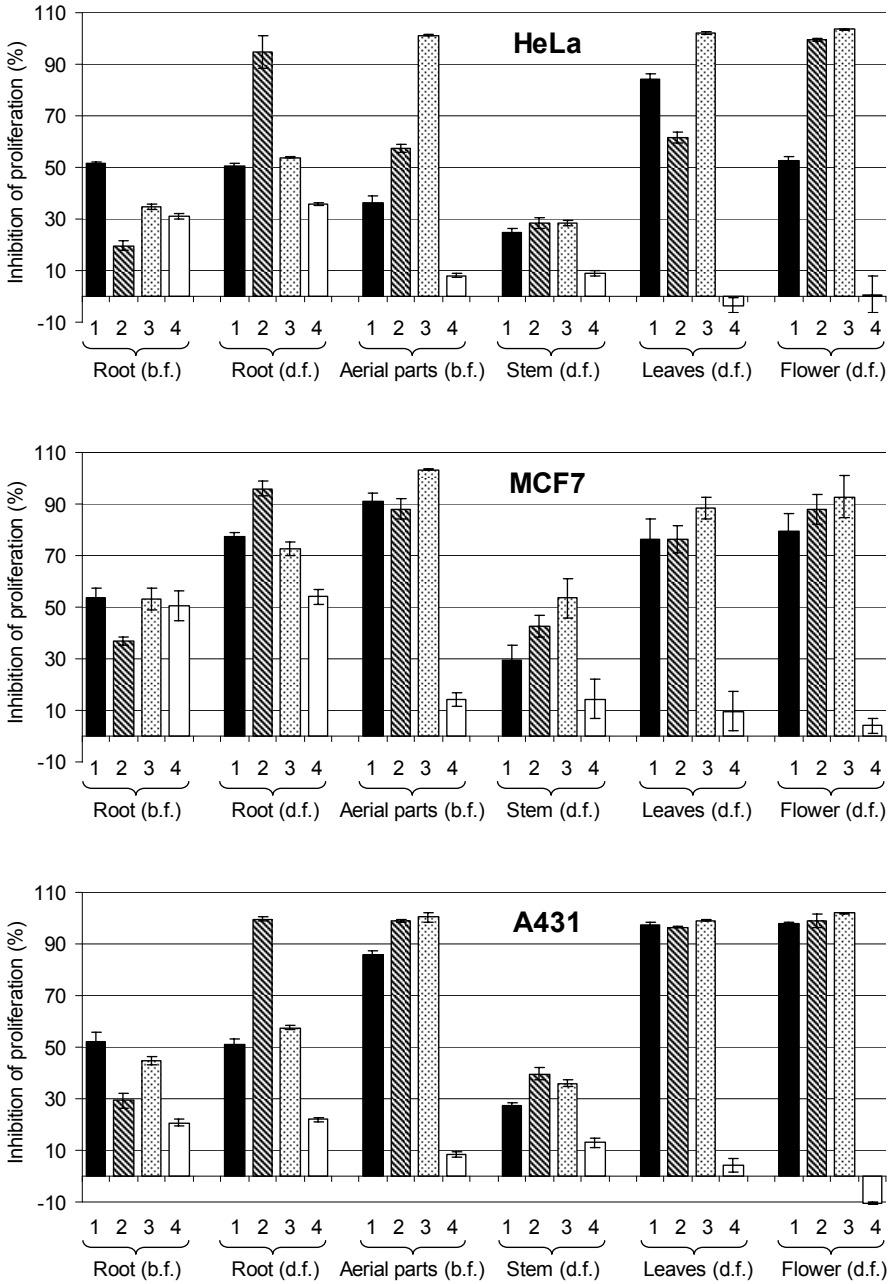
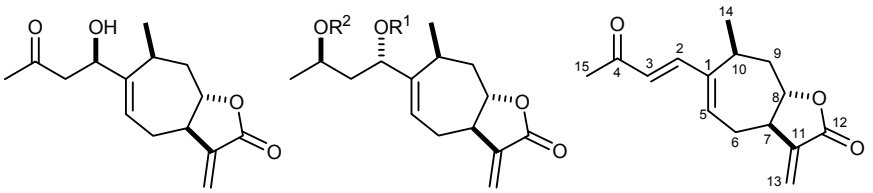


Fig. 1. Antiproliferative activities of extracts from different parts of *X. italicum* on human tumour cell lines (1, MeOH extract; 2, *n*-hexane fraction; 3, $CHCl_3$ fraction; 4, H_2O fraction) at a concentration of 10 μg/ml. b.f., Before flowering; d.f., during flowering.

tion inhibitory activities on the HeLa, A431 and MCF7 cell lines (Fig. 1). It was observed that the main active components proved to be accumulated in the leaves and flowers, especially in the *n*-hexane and $CHCl_3$ extracts. The extracts from the stems exerted only low effects (< 53%) on all three cell lines. The extracts from the roots in the flowering stage were more active than those from



	R^1	R^2
1		
2	Ac	H
3	H	Ac
4		

Fig. 2. Chemical structures of **1**–**4**.

before the flowering period. The fractions with different polarities revealed that the $CHCl_3$ extracts exerted the highest activity, with exception of the root during the flowering period, where the active compounds accumulated in the *n*-hexane extracts. The $CHCl_3$ extract of the leaves exhibited marked cell growth inhibitory activities of 102.0, 88.5 and 99.1% on the HeLa, MCF7 and A431 cells, respectively, and this extract was therefore selected for a detailed study. In order to identify the active components, the $CHCl_3$ extract was first separated by column chromatography into five main fractions. Of these fractions I and IV (at 10 μg/ml) exhibited cell growth inhibitory activities of 85.0 and 97.2% on the HeLa, 101.2 and 88.2% on the A431, and 93.2 and 86.3% on the MCF7 cell line. Fraction I was subjected to multiple chromatographic purifications, including vacuum liquid chromatography (VLC), centrifugal planar chromatography (CPC) and HPLC, which afforded compounds **1**–**4**.

Compound **1** was obtained as white crystals, and identified as xanthatin by comparison of its ^{13}C NMR spectral data with those published by Marco *et al.* (1993). The detailed NMR studies, including ^1H NMR, JMOD, ^1H-^1H COSY, NOESY, HSQC and HMBC experiments, resulted in complete ^1H chemical shift assignments for **1**, as listed in the experimental section. The NOESY experiment provided information on the stereochemistry of the chiral centres (C-7, C-8 and C-10). The Overhauser effects detected between H-8/H-14, H-8/H-6β and H-9β/H-8, and between H-6α/H-7 and H-7/H-9α proved the *trans* lactone ring junction and the β-oriented 14-methyl group. Xanthatin (**1**), Fig. 2, which was obtained in the highest yield, is one of the main xanthanolides: it has been isolated previously from *X. italicum* (Tsankova *et al.*, 1994), *X. macrocarpum* (Lavault *et al.*, 2005), *X. spinosum* (Omar *et al.*, 1984), *X. sibiricum* (Sato *et al.*, 1997), and *X. strumarium* (McMillan *et al.*, 1975). This compound has attracted considerable attention because of its antileishmanial, antifungal (Lavault *et al.*, 2005), and nitric oxide synthesis and COX-2 inhibitory activities (Yoon *et al.*, 2008).

Compounds **2** and **3** were isolated as colourless oils. On the basis of their ^1H and ^{13}C NMR data, they were identified as 4-epixanthanol (**2**) and 4-epi-isoxanthanol (**3**) (Marco *et al.*, 1993) (Fig. 2).

Compound **4** was isolated as a colourless gum. Its ^1H NMR and JMOD spectra (Table I) revealed the presence of a methylene-substituted lactone ring (δ_H 6.16 d, 5.45 d, 4.30 dt, 2.44 m; δ_C 169.5, 139.4, 118.5, 82.2, 48.2 ppm), and contained signals for a lactone ring condensed with a methyl-substituted seven-membered ring [δ_H 5.86 dd, 2.55 ddd, 2.11 ddd, 2.44 m, 4.30 dt, 2.32 dt, 1.69 dt, 2.81 m], characteristic of a xanthanolide. Additionally, a four carbon atoms containing side chain was suggested by the signals at δ_H 4.50 dd, 2.73 dd, 2.60 dd and 2.21 s, and δ_C 209.1, 73.9, 48.7 and 30.9 ppm. The HSQC and ^1H-^1H COSY spectra led to the identification of two methyl, five methine, and four methylene groups and four quaternary carbon atoms in the molecule. The HMBC correlations

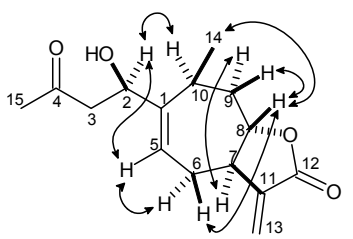


Fig. 3. Diagnostic NOESY correlations of **4**.

Table I. NMR spectral data of 2-hydroxyxanthinosin (**4**) [500 MHz (^{1}H), 125 MHz (^{13}C), $CDCl_3$, TMS, δ (ppm) (*J* in Hz)].

Position	^{1}H	^{13}C	HMBC (C→H)	NOESY
1	–	148.3	H-2, H-3a, H-3b, H-5, H-6α, H-6β, H-9α, H-9β, H-10, H-14	–
2	4.50 dd (9.3, 1.4)	73.9	H-3a, H-3b, H-5, H-10	H-3b, H-5, H-10
3a	2.73 dd (17.2, 9.6)	48.7	H-15	H-2, H-3b, H-10, H-14
3b	2.60 dd (17.2, 2.7)			H-3a
4	–	209.1	H-2, H-3a, H-3b, H-15	–
5	5.86 dd (9.2, 3.2)	123.7	H-2, H-6α, H-6β, H-10	H-2, H-6α
6α	2.55 ddd (15.1, 9.2, 2.2)	25.2	H-5, H-8	H-5, H-6β, H-7, H-13b
6β	2.11 ddd (15.1, 12.0, 3.2)			H-6α, H-7, H-8, H-14
7	2.44 m	48.2	H-6α, H-6β, H-9α, H-9β, H-13a, H-13b	H-6α, H-6β, H-9α
8	4.30 dt (12.5, 2.8)	82.2	H-6β, H-9α, H-9β	H-6β, H-9β, H-14
9β	2.32 dt (13.1, 3.7)	36.9	H-10, H-14	H-8, H-9α, H-10, H-14
9α	1.69 dt (12.4, 3.4)			H-7, H-9β, H-10
10	2.81 m	29.7	H-2, H-5, H-8, H-9α, H-9β, H-14	H-2, H-3a, H-9β, H-9α, H-14
11	–	139.4	H-6α, H-13a, H-13b	–
12	–	169.5	H-13a, H-13b	–
13a	6.16 d (3.2)	118.5		H-13b
13b	5.45 d (3.2)			H-6α, H-13a
14	1.17 d (7.4)	19.3	H-5, H-9α, H-9β, H-10	H-6β, H-8, H-9β, H-10
15	2.21 s	30.9		H-3a, H-3b

Table II. Antiproliferative activities (IC_{50} in μM) of xanthanolides **1**–**4** on HeLa, A431 and MCF7 cell lines.

Compound	IC_{50} [μM]		
	HeLa	A431	MCF7
Xanthatin (**1**)	8.00 ± 0.27	3.44 ± 0.27	5.19 ± 2.16
4-Epixanthanol (**2**)	15.53 ± 1.46	20.85 ± 0.66	26.81 ± 3.42
4-Epi-isoxanthanol (**3**)	29.83 ± 2.21	37.62 ± 2.09	17.65 ± 1.37
2-Hydroxyxanthinosin (**4**)	7.78 ± 1.21	97.84 ± 4.12	27.94 ± 1.44
Doxorubicin	0.15 ± 0.03	0.15 ± 0.04	0.28 ± 0.01
Cisplatin	12.43 ± 1.05	2.84 ± 0.61	9.63 ± 0.75

of C-1 with H-2, H-3 and H-5, of C-4 with H-2, H-3 and H-15, and of C-2 with H-3, H-5 and H-10 proved the 2-hydroxy-4-oxo-1(5)-ene-substituted xanthanolide structure. The stereochemistry of **4** was investigated by NOESY experiments. The Overhauser effects between H-7/H-9α, H-10/H-2, H-5/H-2 and H-5/H-6α indicated protons below the plane of the molecule, while the NOE interactions between H-8/H-9β, H-8/H-6β and H-8/H-14 demonstrated β-oriented H-8 and H-14 (Fig. 3). All of the above data supported the structure of 2-hydroxyxanthinosin for **4** (Fig. 2), with the first stereochemical characterization of C-2, and determination of the complete ^{13}C NMR assignments as listed in Table I.

The isolated xanthanolides were tested against the HeLa, MCF7 and A431 cell lines, and all were found to exert significant cell growth inhibitory effects (Table II). Compound **1** demonstrated the highest activity with IC_{50} values of 8.00, 3.44 and 5.19 μM on the HeLa, A431 and MCF7 cell lines, respectively; its potency was close to that of the positive control, cisplatin. 2-Hydroxyxanthinosin (**4**) displayed significant activity (IC_{50} 7.78 μM) against HeLa cells, and **2** and **3** exerted moderate effects (IC_{50} 15.53–37.62 μM) against all three tumour cell lines.

Compounds **1**–**4** contain an α-methylene-γ-lactone ring, which is generally regarded as a structural requirement of sesquiterpenes for cytotoxic activity. The most potent, xanthatin (**1**), has an additional α,β-unsaturated carbonyl group in the side chain; this structural feature presumably enhances the antitumour activity. In previous pharmacological studies, this side chain was similarly found to be responsible for pronounced

biological activities, *e.g.* iNOS and COX-2 expression suppressive effects (Yoon *et al.*, 2008).

In conclusion, the strong inhibitory effect of the leaves of *X. italicum* on the proliferation of cultured human tumour cell lines (HeLa, MCF7 and A431) may be attributed to xanthanolides. Predominantly the content of the most active main compound, xanthatin (**1**), determines the antitumour activity of the extracts, with the minor xanthanolides playing additional roles in this effect.

Material and Methods

General experimental procedures

The melting point is uncorrected. NMR spectra were recorded in $CDCl_3$ on a Bruker Avance DRX 500 spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C); the signals of the deuterated solvent were taken as the reference (7.26 ppm in ^1H NMR, and 77.0 ppm in JMOD). Two-dimensional experiments (^1H-^1H COSY, HSQC, HMBC and NOESY) were set up, performed and processed with the standard Bruker protocol. For VLC, silica gel (Kieselgel GF_{254}, 15 μm, Merck) was used. Preparative TLC was carried out on silica gel (Kieselgel $60F_{254}$, Merck). The chromatograms were visualized under UV light at 254 nm, and by spraying with concentrated vanillin/sulfuric acid, followed by heating at 110 °C for 10 min. CPC was carried out on a Chromatotron (Harrison Research Inc., Palo Alto, CA, USA) apparatus, using manually coated silica gel (60 GF_{254}, Merck) plates with 2 or 4 mm thickness. For column chromatography, polyamide (ICN Polyamide 6, Germany, mean particle size 50 – 160 μm) was used. RP-HPLC was carried out on a LiChrospher 100 RP-18 (10 μm, 250 × 4 mm) reversed-phase (RP) column (Merck), and NP-HPLC on LiChrospher Si 100 (5 μm, 250 × 4 mm), using a Waters instrument: Pump 600E, Dual λ Absorbance Detector 2487, Injector Rheodyne 7725i, with detection at 254 and 280 nm.

Plant material

Plant materials were collected in August 2006 in Budaörs and in September 2006 on the banks of the River Tisza in Szentes, Hungary, and identified by Dr. Anikó Böszörményi (Department of Plant Biology, University of Szeged, Hungary). Voucher specimens (765 and 766) are deposited in the herbarium of the Department of Pharmacognosy, University of Szeged, Hungary. The plant materials were dried at room temperature, yielding 1070 g of aerial parts and 310 g of roots from the first gathering, and 1020 g of leaves, 680 g of roots, 150 g of flowers and 870 g of stems from the second gathering.

Preparation of the extract for antiproliferative screening

10 g of the dried and powdered plant parts (radix, flower, leaves, and stem) were extracted with 3 × 100 ml MeOH. The extracts were concentrated *in vacuo*, and then subjected to liquid-liquid partition with *n*-hexane (3 × 50 ml) and $CHCl_3$ (3 × 50 ml). The fractions were concentrated and tested for antiproliferative activity.

Isolation of the compounds

The dried leaves (1010 g) of the plant were crushed and percolated with 10 l MeOH at room temperature. The MeOH extract was concentrated to 500 ml; then 500 ml H_2O were added. The extract was subjected to solvent-solvent partition using 5 × 1000 ml *n*-hexane and 5 × 1000 ml $CHCl_3$. The $CHCl_3$ fraction (31.32 g) was chromatographed on a polyamide column with mixtures of MeOH/H_2O (1:4, 2:3, 3:2, and 4:1). A total of 50 fractions were collected and combined on the basis of TLC monitoring, affording 5 main fractions (I–V). Sesquiterpenes were detected in fraction I. Upon standing, fraction I afforded a considerable amount of a crystalline material, which was recrystallized from MeOH to yield compound **1** (1.12 g). The mother liquor that remained (10.22 g) was subjected to VLC on silica gel, using a gradient system of *n*-hexane/acetone (9:1, 4:1, 7:3, and 1:1, 100 ml each) and $CHCl_3$/MeOH (9:1, 7:3, and 1:1, 100 ml each). This separation resulted in 11 sub-fractions, I/1 – I/11, which were tested on the HeLa, MCF7 and A431 cell lines. Fractions I/4 and I/5 exhibited significant activity, and were further purified. Fraction I/4 (3.39 g), obtained with *n*-hexane/acetone (7:3), was subjected repeatedly to silica gel VLC, using toluene/EtOAc mixtures of increasing polarity. From fraction I/4.1, 60.3 mg of **2** was crystallized. The remaining part of this fraction was fractionated first by CPC, using a gradient system of toluene/EtOAc (9:1, 4:1, 7:3, 3:2, and 1:1), and then purified by NP-HPLC, using cyclohexane/EtOAc/EtOH (20:10:1) as mobile

phase (flow rate 1 ml/min), affording **3** (42 mg). Fraction I/5 (1.7 g), obtained with *n*-hexane/acetone (1:1), was further purified by RPC on silica gel, using a gradient system of toluene/EtOAc. Selected fractions from this chromatography were subjected to RP-HPLC, using MeOH/H_2O (3:2) (flow rate 1 ml/min), to yield compound **4** (10.8 mg) (t_R = 3.36 min).

Xanthatin (**1**): White crystals; m.p. 114–115 °C. – ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.03 (1H, d, J = 15.2 Hz, H-2), 6.17 (1H, d, J = 15.2 Hz, H-3), 6.26 (1H, dd, J = 9.1, 3.3 Hz, H-5), 2.77 (1H, ddd, J = 16.7, 9.1, 2.6 Hz, H-6α), 2.20 (1H, ddd, J = 16.7, 12.2, 3.3 Hz, H-6β), 2.52 (1H, dt, J = 12.2, 2.6 Hz, H-7), 4.26 (1H, dt, J = 12.2, 2.6, H-8), 2.34 (1H, ddd, J = 12.8, 3.8, 2.6 Hz, H-9β), 1.82 (1H, dt, J = 12.8, 3.8 Hz, H-9α), 3.04 (1H, m, H-10), 6.15 (1H, d, J = 3.3 Hz, H-13a), 5.46 (1H, d, J = 3.3 Hz, H-13b), 1.12 (3H, d, J = 7.5 Hz, H-14), 2.26 (3H, s, H-15). – ^{13}C NMR ($CDCl_3$, 125 MHz): data identical with those published by Marco *et al.* (1993).

4-Epixanthanol (**2**): Colourless oil. – $[\alpha]_D$ –60° (c 3). – ^{1}H and ^{13}C NMR: data identical with those published by Marco *et al.* (1993).

4-Epi-isoxanthanol (**3**): Colourless oil. – $[\alpha]_D$ –20° (c 1). – ^{1}H and ^{13}C NMR: data identical with those published by Marco *et al.* (1993).

2-Hydroxyxanthinosin (**4**): Colourless gum. – ^{1}H and ^{13}C NMR, HMBC and NOESY data: see Table I.

Bioassays

Cytotoxic effects were measured *in vitro* on the HeLa (human cervix adenocarcinoma), A431 (skin carcinoma), and MCF7 (breast adenocarcinoma) cell lines, using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay. A limited amount of human cancer cells (5000/well) were seeded onto a 96-well microplate, and allowed to adhere overnight before the drugs were introduced. The original medium was then removed, 200 μl culture medium containing the compound of interest were added, and the cells were incubated for 72 h. The tested extracts and compounds were dissolved in DMSO. The final content of DMSO never exceeded 0.3%, and therefore had no essential effect on the cell growth. Next the living cells were assayed: aliquots (20 μl at 5 mg/ml) of the MTT stock solution were pipetted into each well and reduced by viable cells to an insoluble formazan product during a further 4 h. After this contact period, the medium was removed, and the formazan crystals were dissolved in 100 μl DMSO by gentle shaking for 60 min. Finally, the absorbance was measured at 545 nm with a microplate reader (Mosmann, 1983). In this way, the cell growth or drug toxicity was determined. The 50% inhibitory concentration (IC_{50}) was derived from the concentration-response curves fitted to the measured points by GraphPad Prism 2.01. All *in vitro* experiments were carried out on 2 microplates with at least 5 parallel wells. Cisplatin and doxorubicin were used as positive controls.

Acknowledgements

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Agata I., Goto S., Hatano T., Nishibe S., and Okuda T. (1993), 1,3,5-Tri-*O*-caffeoylquinic acid from *Xanthium strumarium*. Phytochemistry **33**, 508–509.

Hartwell J. L. (1968), Plants used against cancer. J. Nat. Prod. **31**, 71–170.

Lavault M., Landreau A., Larcher G., Bouchara J.-P., Pagniez F., Le Pape P., and Richomme P. (2005), Antileishmanial and antifungal activities of xanthanolides isolated from *Xanthium macrocarpum*. Fitoterapia **76**, 363–366.

Ma Y.-T., Huang M.-C., Hsu F.-L., and Chang H.-F. (1998), Thiazinedione from *Xanthium strumarium*. Phytochemistry **48**, 1083–1085.

Marco J. A., Sanz-Cervera J. F., Corral J., Carda M., and Jakupovic J. (1993), Xanthanolides from *Xanthium*: Absolute configuration of xanthanol, isoxanthanol and their C-4 epimers. Phytochemistry **34**, 1569–1576.

McMillan C., Chavez P. I., and Mabry T. J. (1975), Sesquiterpene lactones of *Xanthium strumarium* in a Texas population and in experimental hybrids. Biochem. Syst. Ecol. **3**, 137–141.

Mosmann T. (1983), Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods **65**, 55–63.

Omar A. A., Elrashidy E. M., Ghazy N. A., Metwally A. M., Ziesche J., and Bohlmann F. (1984), Xanthanolides from *Xanthium spinosum*. Phytochemistry **23**, 915–916.

Piacente S., Pizza C., De Tommasi N., and De Simone F. (1996), Sesquiterpene and diterpene glycosides from *Xanthium spinosum*. Phytochemistry **41**, 1357–1360.

Qin L., Han T., Li H., Zhang Q., and Zheng H. (2006), A new thiazinedione from *Xanthium strumarium*. Fitoterapia **77**, 245–246.

Réthy B., Csupor-Löffler B., Zupkó I., Hajdú Zs., Máthé I., Hohmann J., Rédei T., and Falkay G. (2007), Antiproliferative activity of Hungarian Asteraceae species against human cancer cell lines. Part I. Phytother. Res. **21**, 1200–1208.

Sato Y., Oketani H., Yamada T., Singyouchi K.-I., Ohtsubo T., Kihara M., Shibata H., and Higuti T. (1997), A xanthanolide with potent antibacterial activity against methicillin-resistant *Staphylococcus aureus*. J. Pharm. Pharmacol. **49**, 1042–1044.

Saxena V. K. and Mondal S. K. (1994), A xanthanolide from *Xanthium strumarium*. Phytochemistry **35**, 1080–1082.

Tsankova E. T., Todorova M., Trendafilova A., and Robeva P. (1993), Constituents of *Xanthium italicum* roots. Fitoterapia **64**, 470–471.

Tsankova E. T., Trendafilova A. B., Kujumgiev A. I., Galabov A. S., and Robeva P. R. (1994), Xanthanolides of *Xanthium italicum* Moretti and their biological activity. Z. Naturforsch. **49c**, 154–155.

Yoon J. H., Lim H. J., Lee H. J., Kim H.-D., Jeon R., and Ryu J.-H. (2008), Inhibition of lipopolysaccharide-induced inducible nitric oxide synthase and cyclooxygenase-2 expression by xanthanolides isolated from *Xanthium strumarium*. Bioorg. Med. Chem. Lett. **18**, 2179–2182.