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**Bioactivity-guided isolation of antiproliferative compounds
from *Centaurea arenaria***

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Keyword:	<i>Centaurea arenaria</i> , Asteraceae, lignans, flavonoids, feruloyl-serotonin, antiproliferative activity



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13 **Bioactivity-guided isolation of antiproliferative compounds from**

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16 ***Centaurea arenaria***

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21 **Antiproliferative compounds from *Centaurea arenaria***

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Abstract

The antiproliferative effects of *n*-hexane, chloroform and aqueous methanol extracts prepared from the whole plant of *Centaurea arenaria* M.B. ex Willd. were investigated against cervix adenocarcinoma (HeLa), breast adenocarcinoma (MCF7) and skin epidermoid carcinoma (A431) cells, using the MTT assay. The chloroform extract displayed high tumour cell proliferation inhibitory activity (higher than 85% at 10 µg/ml concentration), and was therefore subjected to a bioassay-guided multistep separation procedure. Flavonoids (eupatilin, eupatorin, 3'-methyleupatorin, apigenin and isokaempferid), lignans (arctigenin, arctiin and matairesinol), the sesquiterpene cnicin, serotonin conjugates (moschamine and *cis*-moschamine), β-amyrin and β-sitosterin-β-D-glycopyranoside, identified by means of UV, MS and NMR spectroscopy, were obtained for the first time from this species. The isolated compounds were also evaluated for their tumour cell growth inhibitory activities on HeLa, MCF7 and A431 cells, and different types of secondary metabolites were found to be responsible for the antitumour effects of the extracts; in addition to moderately active compounds (isokaempferid and moschamine), especially apigenin, eupatorin, arctigenin, arctiin, matairesinol and cnicin exert marked antitumour effects against these cell lines.

Keywords: *Centaurea arenaria*; Asteraceae; lignans; flavonoids; feruloyl-serotonin; antiproliferative activity

INTRODUCTION

Due to the ever-increasing number of tumourous diseases, there is a great need worldwide to develop new drugs with antitumour activity, either by synthesizing new molecules or through isolation from plants. Since the majority of drugs used in anticancer therapy are isolated from plants, or are modifications of natural molecules, or designed on templates of natural origin, it is crucial to maintain the search for plants with antitumour activity (Cragg *et al.*, 2009).

As part of our ongoing research into antiproliferative compounds from Hungarian species of Asteraceae family, extracts of several *Centaurea* species (*C. biebersteini*, *C. jacea* and *C. spinulosa*) native to Middle and Eastern Europe were earlier tested against human tumour cell lines (Csupor-Löffler *et al.*, 2009). High cell proliferation inhibitory activity was recorded for many extracts, which drew our attention to the investigation of other, not well-known species of the *Centaurea* genus. The aim of the present study was an investigation of the antiproliferative effects of active compounds in *C. arenaria* M.B. ex Willd.

The genus *Centaurea* from the tribe Cynereae comprises about 500 species, and has been the object of numerous chemical studies, leading to the isolation of acetylenes, sesquiterpene lactones, flavonoids and lignans as the main characteristic secondary metabolites (Hegnauer 1989). *C. arenaria* M.B. ex Willd. is a 30-120 cm high biennial or perennial herb, mostly found on sandy ground, indigenous to Middle-eastern and South-eastern Europe. Previous investigations revealed the toxicity of the methanol extract of *C. arenaria* in the brine shrimp lethality test (Janackovic *et al.*, 2008), but without the identification of any compound. With the aim of determining the potency of

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4 the plant extracts against human tumour cells, the *n*-hexane, chloroform and aqueous
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6 MeOH extracts were assayed *in vitro* on cervix epithelial adenocarcinoma (HeLa),
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8 breast epithelial adenocarcinoma (MCF7) and skin epidermoid carcinoma (A431) cell
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10 lines. The most active CHCl₃ extract was subjected to detailed phytochemical study
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12 with guidance of antiproliferative assays. The flavonoids eupatilin (1), eupatorin (2) and
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14 apigenin (4), the sesquiterpene cnicin (6), and the lignans arctigenin (7), arctiin (8) and
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16 matairesinol (9) were obtained as antitumour constituents of the active fractions. The
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18 preparative work also resulted in the isolation of 3'-methyleupatorin (3), isokaempferid
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20 (5), moschamine (11), *cis*-moschamine (12), β-amyrin (13) and β-sitosterin-β-D-
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22 glycopyranoside (14), which proved to be inactive or exerted only a limited effects
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24 against the tested tumour cells.
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33 MATERIALS AND METHODS

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37 **General.** Melting points are uncorrected. Optical rotation was determined with a
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39 Perkin-Elmer 341 polarimeter. UV spectra were recorded in MeOH on a Shimadzu UV-
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41 2101 PC UV-VIS spectrophotometer. NMR spectra on solutions in CDCl₃ were
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43 recorded on a Bruker Avance DRX 500 spectrometer at 500 MHz (¹H) or 125 MHz
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45 (¹³C); the signals of the deuterated solvent were taken as reference (7.26 ppm in ¹H-
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47 NMR, and 77.0 ppm in ¹³C-NMR). Two-dimensional (2D) experiments (¹H,¹H-COSY,
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49 HSQC, HMBC and NOESY) were set up, performed and processed with the standard
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51 Bruker protocol. Silica gel plates were applied for analytical and preparative TLC
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53 (Merck 5717 and 5715). ESIMS was performed on an Applied Biosystems 3200 QTrap
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55 instrument in ion trap mode. For vacuum liquid chromatography (VLC), SiO₂ (Silica
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4 gel 60G, 15 μm , Merck 11677) was applied. Centrifugal planar chromatography (CPC)
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6 was performed with a Chromatotron instrument (Model 8924, Harrison Research) on
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8 manually prepared SiO_2 (Silica gel 60GF₂₅₄, Merck 7730) plates of 2 and 4 mm
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10 thickness, at a flow rate of 4 and 10 ml/min, respectively. Preparative layer
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12 chromatography (PLC) was carried out on SiO_2 plates (20×20 cm Silica gel 60F₂₅₄,
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14 Merck 5715). Separation was monitored in UV light at 254 nm. HPLC was carried out
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16 on a Waters instrument on a LiChrospher RP-18 (5 μm , 250 x 4 mm, Merck) column.
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23 **Plant material.** *C. arenaria* was gathered in August 2006 in Budapest-Rákosrendező,
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25 Hungary, and was identified by Dr. Tamás Rédei (Hungarian Academy of Sciences,
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27 Institute of Ecology and Botany, Vácrátót, Hungary). A voucher specimen (No. 761)
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29 has been deposited at the Department of Pharmacognosy, University of Szeged.
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35 **Extraction and isolation.** Extracts for the preliminary assay were prepared from 10 g
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37 air-dried, ground plant material with 3x100 ml MeOH, using an ultrasonic bed. After
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39 filtration and evaporation, the residue was dissolved in 50 ml 50% aqueous MeOH, and
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41 subjected to solvent-solvent partition between *n*-hexane (3x50 ml) and CHCl_3 (3x50
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43 ml), affording extracts with the yields presented in Table 1.
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48 Dried and ground *C. arenaria* (3 kg) was extracted with 5 l MeOH for the
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50 isolation of the pure compounds. The concentrated extract was dissolved in 2 l 50%
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52 aqueous MeOH, and extracted first with 3x2 l *n*-hexane, and then with 3x2 l CHCl_3 .
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54 After evaporation, the CHCl_3 phase (33.13 g) was fractionated by CC on silica gel,
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56 using a gradient system of cyclohexane/EtOAc/MeOH [1:0:0 (600 ml), 95:5:0 (5400
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58 ml), 9:1:0 (16800 ml), 85:15:0 (6300 ml), 75:25 (6600 ml), 6:4:0 (2700 ml), 5:5:0 (6000
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60 ml),

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4 ml), 5:5:0.5 (9000 ml), 5:5:1 (10800 ml), 5:5:2 (5400 ml), 1:1:1 (4500 ml), 0:1:1 (2400
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6 ml), 0:1:4 (45000 ml) and 0:0:1 (10200 ml)]. Fractions with similar compositions
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8 according to TLC monitoring were combined, affording fractions F1-F36, which were
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10 tested for their antiproliferative effects. From the marginally active fraction F6, eluted
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12 with cyclohexane/EtOAc 9:1, β -amyrin (**13**) (7.0 mg) was crystallized. Crystallization
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14 of fractions F21 and F22, which were eluted with cyclohexane/EtOAc 75:25, resulted in
15
16 the isolation of apigenin (**2**) (36.2 mg) and 3'-methyleupatorin (**3**) (48.5 mg). In the
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18 bioassay, F21 exhibited a pronounced growth inhibitory effect (67.9%) against HeLa
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20 cells.
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26 Fractions F25, F26 (eluted with *n*-hexane/EtOAc 3:2) and F28 (eluted with *n*-
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28 hexane/EtOAc 1:1) were found to exert marked antiproliferative activity, and were
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30 analysed in detail. All of these fractions were separated by OCC on polyamide, using a
31
32 gradient system of MeOH/H₂O (1:4, 2:3, 3:2, 4:1, 1:0). The material obtained from the
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34 chromatography of F25 [cell proliferation inhibition 37.6% (A431), 4.0% (MCF7) and
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36 55.7% (HeLa)] with MeOH/H₂O 3:2 was further fractionated by preparative TLC on
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38 silica gel, using a mobile phase of CHCl₃/MeOH 24:1, which led to the isolation of
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40 isokaempferid (**5**) (15.2 mg).
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45 Fractions F26/3 and F26/4, obtained by polyamide CC of F26 [cell proliferation
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47 inhibition 61.5% (A431), 41.3% (MCF7) and 76.2% (HeLa)] with MeOH/H₂O 1:4 and
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49 2:3, respectively, were purified by preparative TLC, using *n*-hexane/CHCl₃/MeOH
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51 5:4:2 as developing system on silica gel, resulting in the isolation of arctigenin (**7**) (69.5
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53 mg) and, after preparative TLC purification (silica gel, cyclohexane/CHCl₃/MeOH
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55 5:4:2), matairesinol (**9**) (31.3 mg). From fraction F26/5, eluted with MeOH/H₂O 3:2,
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57 pure eupatilin (**1**) was crystallized (8.1 mg).
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4 The subfraction obtained from the active fraction F28 [cell proliferation
5 inhibition 76.4% (A431), 44.8% (MCF7) and 42.0% (HeLa)] with MeOH/H₂O 3:2 was
6 chromatographed by preparative TLC with the solvent system CHCl₃/MeOH 49:1,
7 resulting in the isolation of eupatorin (**4**) (30.1 mg).
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13 Fraction F30, which was eluted with cyclohexane/EtOAc/MeOH 10:10:1, and
14 which exerted cell proliferation inhibition of 33.2% on A431, 34.5% on MCF7 and
15 26.5% on HeLa cells, was separated by VLC on silica gel with a gradient system of
16 CHCl₃/MeOH into 8 subfractions. Subfraction 2, obtained with CHCl₃ and
17 CHCl₃/MeOH 99:1, was purified on RP-TLC with MeOH/H₂O 7:3 as eluent, yielding
18 (±)-syringaresinol (**10**) (2.3 mg).
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28 Analogous VLC of fraction F31 [cell proliferation inhibition of 23.8% (A431),
29 35.8% (MCF7) and 21.7% (HeLa)] yielded 10 subfractions, of which subfraction 6 was
30 separated by CPC with a gradient system of *n*-hexane/acetone 3:2, 5:5 and 2:3, to give
31 cnicin (**6**) (16.4 mg) and a subfraction, which, after preparative TLC (silica gel, solvent
32 system: CH₂Cl₂/MeOH 95:5), afforded a mixture of isomeric compounds. This mixture
33 was separated by RP-HPLC, using the mobile phase MeOH/H₂O 9:11, to yield
34 moschamine (**11**) (17.9 mg) and *cis*-moschamine (**12**) (4.5 mg). Furthermore,
35 crystallization of fraction F31 before VLC chromatography resulted in the isolation of
36 β-sitosterin-β-D-glucopyranoside (**14**) (12.8 mg).
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49 Fraction F33, eluted with cyclohexane/EtOAc/MeOH 5:5:1, exhibited good
50 activity against HeLa cells [cell proliferation inhibition 64.3% (HeLa), 16.8% (A431)
51 and 26.2% (MCF7)]. It was submitted to CPC, using a gradient system of
52 cyclohexane/EtOAc/MeOH; the subfraction eluted with cyclohexane/EtOAc/MeOH
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5:5:3 was rechromatographed by preparative TLC with CHCl₃/MeOH 9:1, to give pure arctiin (**8**) (42.0 mg).

Eupatilin (**1**): a yellow powder; mp. 231-233 °C; UV and ¹H-NMR: data identical with those published by Gonzalez *et al.* (1988). ¹³C-NMR: Table 1.

Eupatorin (**2**): yellow crystals; mp. 184-190 °C; UV, ¹H- and ¹³C-NMR data identical with those published by Nagao *et al.* (2002).

3'-Methyleupatorin (**3**): yellow crystals; mp. 191-193 °C; UV and ¹H-NMR: data identical with those published by Nacer *et al.* (2006). ¹³C-NMR: Table 1.

Apigenin (**4**): mp. 332-335 °C; identified by co-TLC with authentic sample, and via ¹H- and ¹³C-NMR spectroscopy.

Isokaempferid (**5**): yellow crystals; Mp. 259-266 °C; ¹H-NMR data identical with published data (Nakatani *et al.*, 1991); ¹³C-NMR: Table 2.

Cnicin (**6**): white crystals; mp. 144-148 °C; ¹H-NMR (500 MHz, CD₃OD, δ ppm): 5.10 (1H, m, H-1), 2.33 (1H, m, H-2a), 2.20 (1H, brd, *J* = 14.1 Hz, H-2b), 2.04 (1H, m, H-3a), 2.63 (1H, m, H-3b), 4.95 (1H, d, *J* = 9.5 Hz, H-5), 5.21 (1H, t, *J* = 9.5 Hz, H-6), 3.28 (1H, m, H-7), 5.10 (1H, m, H-8), 2.57 (1H, m, H-9a), 2.50 (1H, dd, *J* = 12.0 and 6.6 Hz, H-9b), 6.19 (1H, d, *J* = 2.7 Hz, H-13a), 5.81 (1H, d, *J* = 2.7 Hz, H-13b), 1.54 (3H, s, H-14), 4.26 (1H, d, *J* = 13.6 Hz, H-15a), 4.02 (1H, d, *J* = 13.6 Hz, H-15b), 4.52 dd (1H, dd, *J* = 5.9 and 3.7 Hz, H-3'), 3.72 (1H, dd, *J* = 11.3, 3.7 Hz, H-4'a), 3.49 (1H, dd, *J* = 11.3 and 6.1 Hz, H-4'b), 6.39 (1H, s, H-5'a), 6.09 (1H, s, H-5'b), 4.57 (1H, s, 4'-OH); ¹³C-NMR (125 MHz, CD₃OD, δ ppm): 130.9 (C-1), 26.9 (C-2), 35.2 (C-3), 145.5 (C-4), 129.7 (C-5), 78.7 (C-6), 54.1 (C-7), 74.5 (C-8), 49.0 (C-9), 133.2 (C-10), 137.4 (C-11), 172.1 (C-12), 125.3 (C-13), 17.0 (C-14), 60.7 (C-15), 166.6 (C-1'), 142.3 (C-2'), 71.9 (C-3'), 66.7 (C-4'), 127.0 (C-5').

Arctigenin (**7**): an amorphous solid; ^1H - and ^{13}C -NMR data identical with those published by Rahman *et al.* (1990).

Arctiin (**8**): an amorphous solid; ^1H -NMR (500 MHz, CDCl_3 , δ ppm): 6.48 (1H, s, H-2), 6.59 (1H, s, H-2'), 6.74 (1H, d, $J = 8.1$ Hz, H-5), 6.90 (1H, d, $J = 8.1$ Hz, H-5'), 6.54 (1H, d, $J = 8.2$ Hz, H-6), 6.57 (1H, d, $J = 8.2$ Hz, H-6'), 2.81 (2H, m, H-7'), 4.10 (1H, t, $J = 7.9$ Hz, H-9'a), 3.69 (1H, dd, $J = 8.9$ and 2.0 Hz, H-9'b), 4.82 (1H, d, $J = 6.3$ Hz, H-1''), 3.82 – 3.38 (overlapped signals, H-2''–H-6''), 3.80 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.65 (3H, s, OCH_3); ^{13}C -NMR: data identical with literature data (Rahman *et al.*, 1990).

Matairesinol (**9**): an amorphous solid; ^1H - and ^{13}C -NMR data identical with those published by Rahman *et al.* (1990).

(\pm)-Syringaresinol (**10**): colourless needles; mp. 172-4 °C; $[\alpha]_{\text{D}}^{28} = -5$ (c 0.1, CHCl_3); ^1H - and ^{13}C -NMR data identical with those previously published (Zhuang *et al.*, 1982; Min *et al.*, 2006). ESIMS: m/z 418.

Moschamine (**11**): an amorphous solid; ^1H - and ^{13}C -NMR data in good agreement with those published by Zhang *et al.* (1997).

cis-Moschamine (**12**): an amorphous solid; ^1H - and ^{13}C -NMR spectral data identical to the published values (Sarker *et al.*, 1997).

β -Amyrin (**13**): identified by co-TLC with an authentic sample, and via the ^1H - and ^{13}C -NMR spectra.

β -Sitosterol- β -D-glucopyranoside (**14**): mp. 288-290 °C, identified by co-TLC with an authentic sample, and via the ^1H and ^{13}C -NMR spectra.

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4 **Antiproliferative assays.** Antiproliferative effects were measured *in vitro* on three
5 human cell lines (HeLa, MCF-7 and A431) with the MTT ([3-(4,5-dimethylthiazol-2-
6 yl)-2,5-diphenyltetrazolium bromide]) assay (Mosmann, 1983). Briefly, a limited
7 number of human cancer cells (5000/well) were seeded onto a 96-well microplate and
8 became attached to the bottom of the well overnight. On the second day of the
9 procedure, the original medium was removed and 200 μ l new medium containing the
10 test substances was added. After an incubation period of 72 hours, the living cells were
11 assayed by the addition of 20 μ l 5 mg/ml MTT solution. MTT was converted by intact
12 mitochondrial reductase and precipitated as blue formazan crystals during a 4-hour
13 contact period. The medium was then removed and the precipitated crystals were
14 dissolved in 100 μ l DMSO during a 60-minute period of shaking. Finally, the reduced
15 MTT was assayed at 545 nm using a microplate reader, wells with untreated cells being
16 taken as control. All *in vitro* experiments were carried out on two microplates with at
17 least 5 parallel wells. Doxorubicin and cisplatin were used as positive controls. Stock
18 solutions of 10 mg/ml of the tested compounds and extracts were prepared with DMSO.
19 The highest DMSO concentration (0.3%) of the medium did not have any significant
20 effect on cell proliferation. The dose-response curves of the compounds were fitted by
21 means of the computer program GraphPad Prism 4.0 (GraphPad Software, San Diego,
22 CA, USA), and IC₅₀ values (the concentration at which the cell proliferation is 50% of
23 the untreated control) were calculated.
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51 52 53 **RESULTS AND DISCUSSION** 54 55 56 57 58 59 60

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5 In a preliminary study, the antiproliferative effects of the extracts prepared from the
6 whole plant of *C. arenaria* were evaluated on the HeLa, A431 and MCF7 cell lines,
7 using the MTT assay. The dried plant material was extracted with MeOH, and this
8 extract was subjected to solvent–solvent partitioning, yielding *n*-hexane-, CHCl₃- and
9 aqueous MeOH-soluble phases. The CHCl₃ extract exerted substantial tumour cell
10 growth inhibitory activity at 10 µg/ml concentration against all three cell lines (Table
11 1), and accordingly it was selected for a detailed phytochemical-pharmacological
12 analysis in order to identify the antiproliferative constituents.
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24 In the preparative experiment, *C. arenaria* was extracted by the same method,
25 and the effective CHCl₃ extract was subjected to OCC using gradient elution. Fourteen
26 compounds (**1-14**) were isolated from the highly and moderate active fractions through
27 the use of VLC, CPC, preparative TLC and HPLC techniques. The compounds were
28 identified by means of UV, ESIMS and NMR spectroscopy (¹H-NMR and JMOD) and
29 comparison of the spectral data with literature values. For compounds **1, 3, 5, 6** and **8**,
30 2D NMR measurements (¹H-¹H COSY, NOESY, HSQC and HMBC) were also carried
31 out, which allowed complete ¹H- and ¹³C-NMR assignments, and therefore the
32 supplementation of previously published data (see Table 2 and in the Materials and
33 Methods).
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47 All isolated compounds were identified for the first time from *C. arenaria*, but
48 the compounds represent mainly the characteristic secondary metabolites of the genus.
49 Methoxylated flavones, such as eupatilin (**1**), eupatorin (**2**), 3'-methyleupatorin (**3**) and
50 apigenin (**4**) and a flavonol [isokaempferid (**5**)] were described previously from
51 different *Centaurea* species (Hegnauer 1989). Similarly, the germacranolide cnicin (**6**)
52 was detected in some *Centaurea* species (Tesevic *et al.*, 2007). The
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dibenzylbutyrolactone-type lignans, such as arctigenin (7), arctiin (8) and matairesinol (9), often occur in *Centaurea* species (Ferguson *et al.*, 2003; Janackovic *et al.*, 2004, 2008; Marco *et al.*, 2005), but sesamin-type lignans [such as (+)-syringaresinol (10)] were previously unknown in this genus. *N*-Feruloylserotonins (11 and 12) had been found earlier only in two species of the genus (*C. moschata* and *C. nigra*) (Sarker *et al.* 1997; Kumarasamy *et al.*, 2003); the serotonin conjugates were reported to accumulate in the seeds. In agreement with Sarker's group, we obtained a 1:2 mixture of *cis* and *trans* isomers, and observed that, after successful RP-HPLC separation, the compounds readily formed the mixture again.

The isolated compounds were evaluated for their inhibitory activity on HeLa, MCF7 and A431 cells (Table 3), and it emerged that flavone, sesquiterpene and lignan-type compounds and a serotonin conjugate exerted pronounced concentration-dependent effects. The highest activity was demonstrated by the lignan arctigenin (7), with IC₅₀ values of 0.73 μM (HeLa), 3.47 μM (MCF7) and 4.47 μM (A431). Apigenin (4), cnicin (6) and matairesinol (9) inhibited the proliferation of all three tumour cell lines significantly. Arctiin (8) and eupatorin (2) were also active against the HeLa and A431 tumour cells. Eupatilin (1) and moschamine (11) exerted high or moderate potency selectively against the A431 cell line.

The lignans exhibited an interesting character of activity, involving relatively low calculated IC₅₀ values, which were comparable to those of reference compounds [arctigenin (7): HeLa 0.271 μM, MCF7 3.47 μM and A431 4.47 μM; matairesinol (9): HeLa 7.51 μM, MCF7 36.23 μM and A431 19.92 μM], and a limited maximal effect, which was typically around 75% inhibition of the cell proliferation (Fig 1). Isokaempferid (5) displayed unique activity: it caused a concentration-dependent

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4 inhibition of the cytotoxicity of the HeLa cells, but increases in proliferation of the A431
5 and MCF7 cells (Fig 1). These results relate to cancerous cell lines and therefore do not
6 permit direct conclusions concerning normal cells. However, extracts prepared from
7 related *Centaurea* species have been reported to exert wound-healing activity, which
8 can be explained by a stimulation of normal epidermic tissue proliferation (Csupor *et*
9 *al.*, 2009; Koca *et al.*, 2009). The results allow the speculation that isokaempferid (**5**)
10 may play a crucial role in the advantageous action exerted by similar plants on tissue
11 regeneration.
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23 In summary, our bioactivity-guided isolations led to the conclusion that the
24 strong inhibitory effect of the CHCl₃ extract of *C. arenaria* on the proliferation of
25 cultured human tumour cell lines (HeLa, MCF7 and A431) may be attributed mainly to
26 flavones, lignans and sesquiterpenoids, other compounds [isokaempferid (**5**) and
27 moschamine (**11**)] exerting additional effects.
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43 material.
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Table 1. Antiproliferative effects and yields of the extracts from *Centaurea arenaria*

Extract	Yield (%)	Inhibition of cell proliferation at 10 µg/ml		
		HeLa	MCF-7	A-431
<i>n</i> -Hexane extract	0.24	24.97	8.33	-4.82
Chloroform extract	1.64	94.08	86.29	98.85
Aqueous methanolic extract	1.00	33.24	25.89	8.69

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Table 2. ¹³C-NMR data on eupatilin (1), 3'-methyleupatorin (3) and isokaempferid (5) (125 MHz, δ ppm)

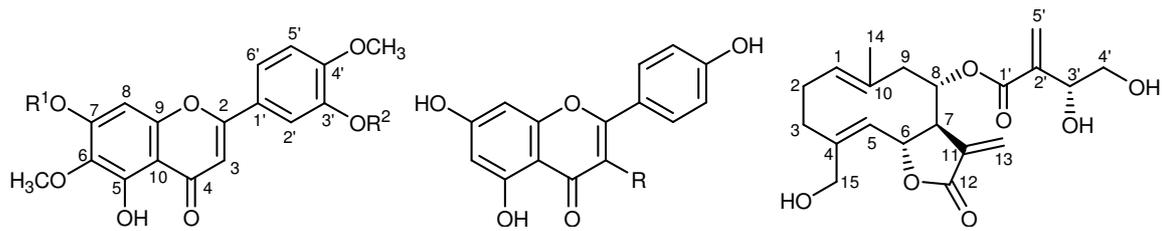
C	1 ^a	3 ^b	5 ^c
2	163.4	164.0	158.5
3	103.3	104.5	139.4
4	182.2	182.6	<i>n.d.</i>
5	152.4 ⁺	153.2	163.0
6	131.3	132.0	99.9
7	157.3	158.7	166.3
8	94.4	90.6	94.9
9	152.7 ⁺	153.1	158.0
10	104.1	106.2	105.8
1'	122.9	123.8	122.6
2'	109.3	108.8	131.4
3'	149.0	149.4	116.6
4'	152.0	152.3	161.7
5'	111.6	111.2	116.6
6'	120.0	120.1	131.4
OCH ₃	60.0	60.9	60.5
	55.8	56.3	
	55.7	56.1	
		56.1	

^a DMSO-*d*₆; ^c CDCl₃; ^b MeOH-*d*₄; ⁺ interchangeable assignments; *n.d.* not detected

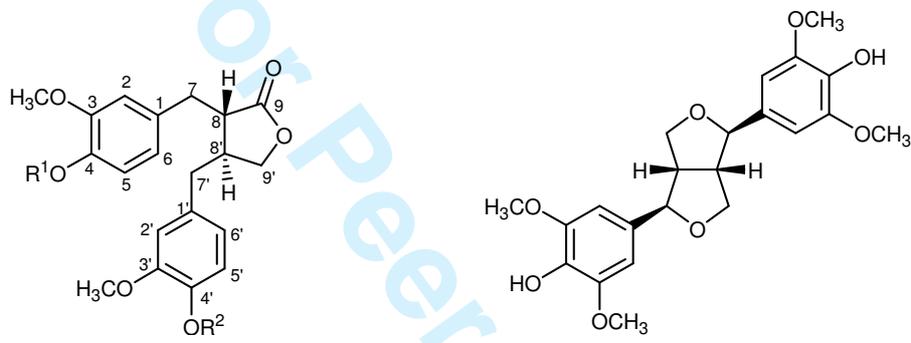
Table 3. Antiproliferative effects (IC₅₀) of the isolated compounds on different human tumour cell lines

Compound	HeLa		MCF7		A431	
	μM	μg/ml	μM	μg/ml	μM	μg/ml
eupatilin (1)	inactive		inactive		24.16	8.31
eupatorin (2)	29.79	9.89	inactive		52.50	17.43
3'-methyleupatorin (3)	inactive		inactive		inactive	
apigenin (4)	10.64	2.87	13.88	3.75	12.34	3.33
isokaempferid (5)	15.05	4.52	negative		negative	
cnicin (6)	34.50	13.04	16.84	6.37	17.86	6.75
arctigenin (7)	0.73	0.27	3.47	1.29	4.47	1.66
arctiin (8)	1.80	0.96	inactive		19.53	10.43
matairesinol (9)	7.51	2.69	36.23	12.97	19.92	7.13
(±)-syringaresinol (10)	inactive		inactive		inactive	
moschamine (11)	inactive		inactive		58.54	20.61
<i>cis</i> -moschamine (12)	inactive		inactive		inactive	
β-amyrin (13)	inactive		inactive		inactive	
β-sitosterin-glucoside (14)	inactive		inactive		inactive	
doxorubicin	0.15	0.089	0.28	0.158	0.15	0.086
cisplatin	12.43	3.73	9.63	2.89	2.84	0.852

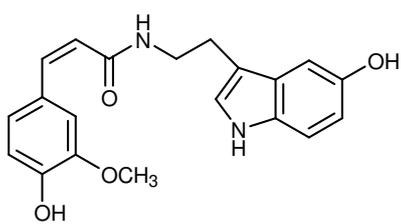
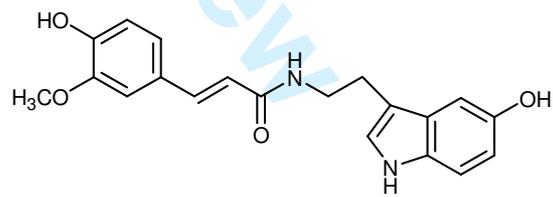
*Inactive indicates < 50% inhibition of cell proliferation at 75 μM.



1	R ¹	R ²	4	R	6
2	H	CH ₃	5	H	
3	CH ₃	H		OCH ₃	
	CH ₃	CH ₃			



7	R ¹	R ²	10
8	CH ₃	H	
9	CH ₃	glu	
	H	H	

**11****12**

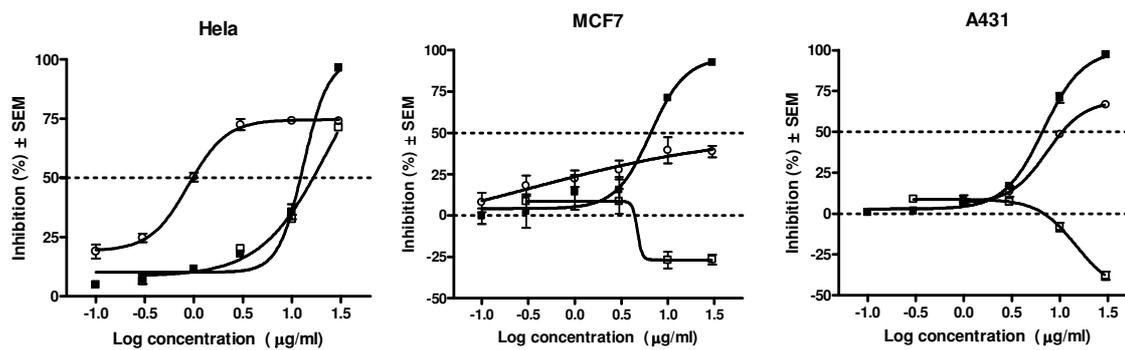


Figure 1. Concentration – inhibitory response curves of representative constituents of *C. arenaria* on the utilized cell lines.

■: cnicin (6); □: isokaempferid (5); ○: arctiin (8)