Fitoterapia 145 (2020) 104610

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Flavonoid, stilbene and diarylheptanoid constituents of *Persicaria maculosa* Gray and cytotoxic activity of the isolated compounds

Andrea Vasas[a], Ildikó Lajter[a], Norbert Kúsz[a], Péter Forgó[a], Gusztáv Jakab[b], Csilla Fazakas[c], Imola Wilhelm[c,d], István A. Krizbai[c,d], Judit Hohmann[a,e,*]

[a] Department of Pharmacognosy, University of Szeged, H-6720 Szeged, Hungary
[b] Institute of Environmental Sciences, Faculty of Agricultural Water and Environmental Management, Tessedik Samuel College, H-5540 Szarvas, Hungary
[c] Institute of Biophysics, Biological Research Centre, H-6726 Szeged, Hungary
[d] Institute of Life Sciences, Vasile Goldiş Western University of Arad, RO-310414 Arad, Romania
[e] Interdisciplinary Centre of Natural Products, University of Szeged, H-6720 Szeged, Hungary

ARTICLE INFO

Keywords:
Persicaria maculosa
Flavanones
Chalcones
Stilbene
Diarylheptanoids
Cytotoxicity

ABSTRACT

Persicaria maculosa (Polygonaceae) has been used as edible and as medicinal plant since ancient times. As a result of multistep chromatographic purifications, chalcones [2′-hydroxy-3′,4′,6′-trimethoxychalcone (**1**), pashanone (**2**), pinostrobin chalcone (**3**)], flavanones [6-hydroxy-5,7-dimethoxyflavanone (**4**), pinostrobin (**5**), onysilin (**6**), 5-hydroxy-7,8-dimethoxyflavanone (**7**)], flavonol [3-*O*-methylgalangin (**8**)], stilbene [persilben (**9**)], diarylheptanoids [1,7-diphenylhept-4-en-3-one (**10**), dihydroyashabushiketol (**12**), yashabushidiol B (**13**)] and 3-oxo-α-ionol-glucoside (**11**) were isolated from *P. maculosa*. The present paper reports for the first time the occurrence of diarylheptanoid-type constituents in the family Polygonaceae. Cytotoxicity of **1–5**, **7** and **9–11** on 4 T1 mouse triple negative breast cancer cells was assayed by MTT test. None of the tested compounds reduced the cell viability to less than 80% of the control. On non-tumorigenic D3 human brain endothelial cells the decrease of cell viability was observed in case of **1** and **2**. Further impedance measurements on 4 T1 and D3 cells a concentration-dependent decrease in the cell index of both cell types was demonstrated for **1**, while **2** proved to be toxic only on endothelial cells.

1. Introduction

The genus *Persicaria* (smartweed) (family Polygonaceae) includes about 100 species nearly worldwide [1]. The plants are perennials or annuals [2–3]. *Persicaria maculosa* Gray (syn. *Polygonum persicaria* L., lady's thumb) is an annual plant, native to Europe and widely distributed as a weed throughout temperate and tropical North and South America, Asia, North Africa and Australia [4].The Cherokee, Chippewa, and Iroquois native Americans prepared simple or complex decoctions of *P. maculosa*, which they used as dermatological, urinary, gastrointestinal, and veterinary aids, for cardiac diseases, and as an analgesic [5]. The plant has also been used to treat *e.g.* diarrhoea and infectious diseases, and the leaves and young shoots can be eaten in salads [6]. Previous phytochemical studies revealed the presence of stilbenes, flavonoids, phenolic acids, sesquiterpenes and diterpenes in this species [2,7–8].

In vitro pharmacological studies demonstrated the antibacterial, antifungal and insecticidal activities of the plant [9,10], while in *in vivo* studies the hydroalcoholic extract of the herb exhibited anti-inflammatory effect and decreased locomotion after intraperitoneal administration to rats; therefore, it possessed spasmolytic activity [11]. As concerns the chemical constituents responsible for the observed activities, persilben, a unique naturally occurring *E*-stilbene attracted great interest because of its antimicrobial, antifungal and antioxidant activities and its good penetration through biological membranes in consequence of its high lipophilicity [12,13]. Moreover, flavonoids of the plant have anti-inflammatory and antioxidant activities. Different extracts prepared by our group from *P. maculosa* were investigated on G protein-activated inwardly rectifying K^+ channel (GIRK) using patch clamp method. The $CHCl_3$ extract of the plant exhibited high GIRK channel inhibitory activity at 0.1 mg/mL concentration [9].

In continuation of our work on *P. maculosa*, thirteen compounds (**1**−**13**), among them chalcones, flavanones, flavonol, diarylheptanoids, a stilbene derivative and an α-ionol-glycoside were identified. Cytotoxic activity of the isolated compounds **1–5**, **7** and **9–11** was evaluated against 4T1 and D3 cell lines *in vitro*.

* Corresponding author at: Department of Pharmacognosy, University of Szeged, H-6720 Szeged, Hungary.
E-mail address: hohmann@pharm.u-szeged.hu (J. Hohmann).

https://doi.org/10.1016/j.fitote.2020.104610
Received 18 March 2020; Received in revised form 16 April 2020
Available online 17 May 2020

2. Experimental section

2.1. General experimental procedures

Vacuum liquid chromatography (VLC) was carried out on silica gel G (15 μm, Merck); preparative thin-layer chromatography (preparative TLC) was performed on silica gel 60 F_{254} plates (Merck). Medium-pressure liquid chromatography (MPLC) was performed by a Biotage SP1™ Purification System using a KP-C18HS 40 + M column. HPLC was performed on a LiChrospher RP-18 (5 μm, 250 × 4 mm, Merck) column using mixture of acetonitrile–H_2O as mobile phase on a Waters 600 instrument. NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer at 500 MHz (^{1}H) and 125 MHz (^{13}C). The peaks of the residual solvents were taken as reference. Two-dimensional data were acquired and processed with standard Bruker software. In the ^{1}H–^{1}H COSY, HSQC and HMBC experiments, gradient-enhanced versions were used. ESI and APCI mass spectra were recorded on an API 2000 triple quadrupole mass spectrometer equipped with an electrospray and APCI interfaces.

2.2. Plant material

Persicaria maculosa Gray was collected in the flowering period in Homoródalmás (Hungary) in July 2012. Botanical identification was performed by G. J. (Institute of Water Management and Irrigation, Szent István University, H-5540 Szarvas, Hungary). A voucher specimen (No. 811) has been deposited at the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

2.3. Extraction and isolation

The air-dried and ground whole plants of *P. maculosa* (3.15 kg) were extracted with MeOH (20 L) at room temperature. The crude extract was concentrated *in vacuo* and subjected to solvent–solvent partition first with 3 × 500 mL *n*-hexane, then with 3 × 500 mL of $CHCl_3$. After evaporation, the $CHCl_3$ phase (36.8 g) was fractionated by MPLC on reversed-phase silica gel, using a gradient system of MeOH–H_2O (from 3:7 to 8:2). The fractions were combined into twelve subfractions (I–XII) according to the TLC monitoring. Subfraction IV (229.4 mg) was separated by VLC on silica gel, using a gradient system of CH_2Cl_2–acetone (from 99:1 to 8:2) to yield 13 main fractions (IV/1–13). Fraction IV/5 was further purified by preparative TLC with CH_2Cl_2–MeOH (4:1) to yield compound **11** (3.2 mg). Subfraction IX (282.8 mg) was chromatographed by VLC on silica gel with the gradient system of cyclohexane–CH_2Cl_2–acetone (from 5:5:0 to 0:95:5). After TLC monitoring, 12 main fractions (IX/1–12) were obtained. Fraction IX/1 was subjected to preparative TLC on silica gel using cyclohexane–CH_2Cl_2–acetone (5:5:1) as developing system, to yield compounds **10** (2.3 mg) and **12** (1.8 mg). Fraction IX/3 was also purified by prep. TLC with cyclohexane–CH_2Cl_2–MeOH 40:20:1, and compounds **9** (2.8 mg) and **7** (4.5 mg) were afforded. Fraction IX/4 (30.3 mg) was chromatographed by Sephadex LH-20 gel using MeOH as eluent, and thereafter by prep. TLC with cyclohexane–CH_2Cl_2–MeOH 20:30:1 to obtain compounds **6** (5.2 mg) and **13** (4.0 mg). Fraction XI (229.4 mg) was separated by normal phase VLC, which was eluted with the gradient system of CH_2Cl_2–MeOH (from 99:1 to 9:1) to yield 13 main fractions (XI/1–13). Compound **5** (4.8 mg) was obtained from fraction XI/2 (8 mg) by RP-HPLC, using acetonitrile–H_2O (9:1) (isocratic elution, flow: 0.5 mL/min). Fraction XI/5 (76.5 mg) was purified by Sephadex LH-20 gel chromatography, using MeOH as eluent to yield 6 subfractions (XI/5/1–6). From subfraction XI/5/2 compounds **2** (4.5 mg) and **8** (1.1 mg) were separated by prep. TLC, using toluene–ethyl acetate–MeOH (5:4:1). Compound **1** (7.0 mg) was obtained from subfraction XI/5/3 by prep. TLC using CH_2Cl_2–acetone (19:1). Compound **3** (3.1 mg) was crystallized from fraction XI/7, and **4** (2.2 mg) from fraction XI/10.

2.3.1. Characterization of pinostrobin chalcone (3)

Orange crystals, m.p. 149–150 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ ppm 14.1 (1H, s, 2'OH), 7.86 (1H, d, J = 15.6 Hz, H-β), 7.76 (1H, d, J = 15.6 Hz, H-α), 7.59 (2H, m, H-2, H-6), 7.38 (3H, m, H-3–H-5), 6.01 and 5.94 (2 × 1H, 2 × d, J = 1.0 Hz, H-3′, H-5′) 3.91 (3H, s, OCH_3); ESI-MS positive m/z 293 $[M + Na]^+$, 271 $[M + H]^+$, 167 $[C_8H_7O_4]^+$.

2.3.2. Characterization of 6-hydroxy-5,7-dimethoxyflavanone (4)

White crystals, m.p. 148–149 °C; ^{1}H NMR (500 MHz, CD_3OD) δ ppm 7.53 (2H, d, J = 7.3 Hz, H-2′, H-6′), 7.42 (2H, t, J = 7.2 Hz, H-3′, H-5′), 7.37 (1H, t, J = 7.3 Hz, H-4′), 6.17 (1H, s, H-8), 5.49 (1H, dd, J = 12.7, 2.9, H-2), 3.80 (3H, s, 5-OCH_3), 3.76 (3H, s, 7-OCH_3), 3.01 (1H, dd, J = 12.7, 16.7 Hz, H-3a), 2.76 (1H, dd, J = 16.7, 3.0, H-3b); ^{13}C NMR (125 MHz, CD_3OD): δ ppm 192.0 (C-4), 159.7 (C-5), 159.4 (C-6), 140.5 (C-1′), 131.1 (C-7), 129.7 (C-3′, C-5′), 129.6 (C-4′), 127.2 (C-2′, C-6′), 106.3 (C-10), 94.2 (C-8), 80.6 (C-2), 61.4 (7-OCH_3), 56.2 (5-OCH_3), 46.4 (C-3).

2.3.3. Characterization of pinostrobin (5)

Yellow solid; ^{1}H NMR (500 MHz, CD_3OD) δ ppm 12.0 (1H, s, 5-OH), 7.50 (2H, d, J = 7.4 Hz, H-2′, H-6′), 7.42 (2H, t, J = 7.2 Hz, H-3′, H-5′), 7.37 (1H, t, J = 7.1 Hz, H-4′), 6.10 (1H, d, J = 1.8 Hz, H-8), 6.06 ((1H, d, J = 1.8 Hz, H-6), 5.59 (1H, dd, J = 12.5, 2.6 Hz, H-2), 3.82 (3H, s, OCH_3), 3.13 (1H, dd, J = 12.8, 17.1 Hz, H-3a), 2.82 (1H, dd, J = 17.1, 2.9 Hz, H-3b); APCI-MS m/z 271 $[M + H]^+$, 167 $[M–C_8H_8]^+$, 131, 103.

2.3.4. 3-Methylgalangin (8)

Yellow powder; ^{1}H NMR (500 MHz, CD_3OD) δ ppm 8.06 (2H, m, H-2, H-6′), 7.55 (3H, m, H-3'–H-5′), 6.43 (1H, d, J = 2.0 Hz, H-8), 6.23 (1H, d, J = 2.0 Hz, H-6), 3.80 (3H, s, OCH_3); ESI-MS positive m/z 285 $[M + H]^+$, 270 $[M + H\text{-}CH_3]^+$.

2.3.5. Persilben (9)

Yellowish powder; ^{1}H NMR (500 MHz, $CDCl_3$) 13.9 (1H, s, COOH), 8.15 (1H, d, J = 15.6, Hα), 7.86 (1H, d, J = 15.6 Hz, Hβ), 7.65 (2H, m, H-2, H-6), 7.40 (3H, m, H-3–H-5), 6.88 (1H, s, H-6), 6.10 (1H, s, H-4), 3.91, 3.86 (2 × 3H, 2 × s, 2 × OCH_3).

2.4. Cell culture and toxicity tests

4T1 (mouse triple negative breast cancer cells) were cultured in RPMI 1640 medium supplemented with 5% foetal bovine serum (FBS) (both from Thermo Fischer Scientific, Waltham, MA, USA). D3 (hCMEC/D3 human cerebral microvascular endothelial cells) were kept in rat tail collagen coated dishes in EBM-2 medium complemented with 2% FBS and EGM-2MV kit (all of them purchased from Lonza, Basel, Switzerland).

For MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay, cells were plated in 96-well plates (Corning, Corning, NY, USA) in a density of 5000 4 T1 cells/well or 25,000 D3 cells/well. After 24 h, half of the medium was replaced with serum-free medium, containing the compounds in a final concentration of 10, 20 or 50 μmol/L. Control wells received solvent (DMSO) in max. 0.2% concentration. After 48 h, MTT reagent (Sigma-Aldrich, St. Louis, MO, USA) was added to the cells in a final concentration of 2.5 mg/mL. After incubation at 37 °C for one hour, acidified isopropanol solution was added to each well. Absorbance was measured at 595 nm with a FLUOstar OPTIMA microplate reader (BMG LABTECH, Offenburg, Germany).

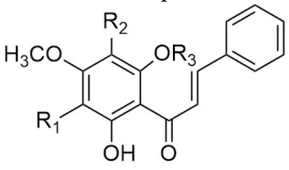
For impedance measurements, cells were plated in 96-well E-plates having micro-electrodes integrated on the bottom (ACEA Biosciences, San Diego, CA, USA), and allowed to attach onto the electrode surface. After 24 h, cells were treated with the test compounds as described above. Electrical impedance was recorded in real-time using an xCELLigence® Real-Time Cell Analysis (RTCA) instrument (ACEA Biosciences). Cell impedance (which depends on cell number, degree of

adhesion, spreading and viability), expressed in arbitrary units (cell index) was automatically calculated by the software of the instrument.

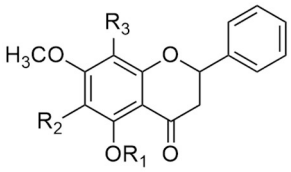
3. Results and discussion

3.1. Isolation and structure elucidation of the compounds

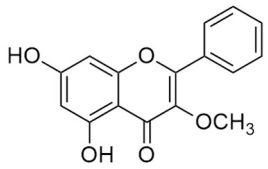
MeOH extract was prepared from dried whole plant of *P. maculosa* and subjected to solvent-solvent partition, yielding *n*-hexane, $CHCl_3$ and remaining aqueous extracts. The $CHCl_3$ extract was fractionated by medium pressure liquid chromatography on reversed phase silica gel resulting twelve fractions. Further purification of the fractions with combination of different chromatographic techniques (VLC, prep TLC, Sephadex LH-20 gel chromatography and HPLC) resulted in the isolation of thirteen compounds (**1**–**13**).



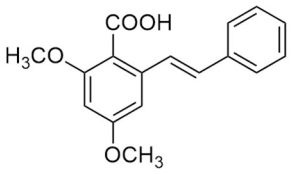
	R_1	R_2	R_3
1	OCH_3	H	CH_3
2	H	OCH_3	H
3	H	H	H



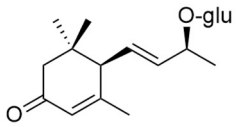
	R_1	R_2	R_3
4	CH_3	OH	H
5	H	H	H
6	H	OCH_3	H
7	H	H	OCH_3



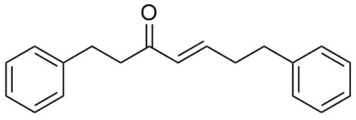
8



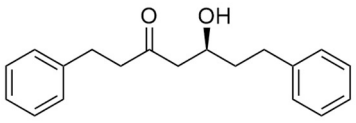
9



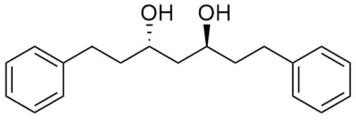
11



10

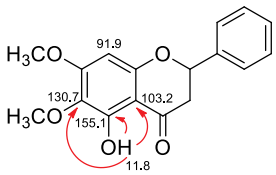


12

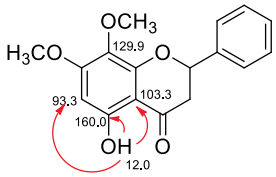


13

Eight compounds (**1**–**8**) are belonging to the group of flavonoids, interestingly all of them have an unsubstituted ring B. Three chalcones were identified as 2′-hydroxy-3′,4′,6′-trimethoxychalcone (**1**), 2′,6′-dihydroxy-3′,4′-dimethoxychalcone (pashanone = polygochalcone, **2**), and 2′,6′-dihydroxy-4′-methoxychalcone (pinostrobin chalcone, **3**) by comparison with reference data [14,15]. Pinostrobin (**5**) [16,17], and three isomeric rare flavanones, 6-hydroxy-5,7-dimethoxyflavanone (**4**) [18], 5-hydroxy-6,7-dimethoxyflavanone (onysilin, **6**) [19], and 5-hydroxy-7,8-dimethoxyflavanone (**7**) [22] were identified by analysis of their 1D and 2D NMR spectra and comparison with the data published in the literature [22]. In previous studies, onysilin (**6**) and 5-hydroxy-7,8-dimethoxyflavanone (**7**) were differentiated on the basis of minor difference in ^{13}C NMR data, UV spectra with shift reagents, and melting points [22,20]; however, in our HMBC investigations clear arguments were found for structural assignment of **6** and **7** (Fig. 1).



6



7

Fig. 1. Diagnostic HMBC correlations (H→C) of compounds **6** and **7** between 5-OH and C-5, C-6 and C-10 (chemical shifts shown in δ ppm).

In the present experiment, the only isolated flavonol is 3-*O*-methylgalangin (**8**) [21]. Compound **9** was identified as the known persilben [15], and compound **11** as (6*R*,9*S*)-3-oxo-α-ionol-glucoside based on the NMR and optical rotation data [α_D^{28} + 43° (*c* 0.19, MeOH)] [22]. Compound **10** revealed to be 1,7-diphenyl-4-en-3-heptanone based on 1D and 2D NMR and MS data, which were in agreement with published data [23].

Close analogues of **10**, (5*S*)-1,7-diphenylhept-5-ol-3-one (=dihydroyashabushiketol) (**12**) [measured $[\alpha]_D$ = +14 (*c* 0.1, $CHCl_3$)] [24], and (3*S*,5*S*)-1,7-diphenylhept-3,5-diol (=yashabushidiol B) (**13**) [measured $[\alpha]_D$ = −5 (*c* 0.1, $CHCl_3$)] [25] were also identified from *P. maculosa*. Our NMR measurements allowed previously unpublished ^1H and ^{13}C NMR assignments for compounds **3**, **4**, **5**, **8**, and **9**, these data are listed in Materials and Methods section.

3.2. Chemotaxonomic significance

A variety of diarylheptanoids have been isolated previously from plant families Aceraceae, Actinidiaceae, Betulaceae, Burseraceae, Casuarinaceae, Juglandaceae, Leguminosae, Myricaceae, and Zingiberaceae, but the present paper reports for the first time the occurrence of diarylheptanoid-type constituents in the family Polygonaceae [26]. The isolation of compounds **10**, **12**, and **13** provided new chemotaxonomic information, the presence of diarylheptanoids might serve as a chemotaxonomic marker for *Persicaria* species. 1,7-Diphenylhept-4-en-3-one (**10**) was reported previously only as the metabolite of *Alpinia officinarum* (Zingiberaceae) with potent PAF receptor binding inhibitory and inducible NO synthase protein and mRNA expression suppressing activities [27,28]. Dihydroyashabushiketol (**12**), and yashabushidiol B (**13**) were isolated formerly from *Alpinia*, *Acorus*, *Ammomum* and *Alnus* species, with cytotoxic activity against IMR-32 human neuroblastoma cells [29]. These compounds were previously obtained by chemical synthesis, too [30,31].

The present experiment afforded the first isolation of chalcones **1**, **3** and flavanone **4** from the family Polygonaceae. 2'-Hydroxy-3′,4′,6′-trimethoxychalcone (**1**) was obtained formerly from Annonaceae, Piperaceae and Rosaceae species [32], while pinostrobin chalcone (**3**) from *Alpinia* species [18]. 6-Hydroxy-5,7-dimethoxyflavanone (**4**) was isolated previously only from *Piper hispidum* [21]. Onysilin (**6**), 5-hydroxy-7,8-dimethoxyflavanone (**7**), 3-*O*-methylgalangin (**8**), 3-oxo-α-ionol-glucoside (**11**) were isolated previously from different species of family Polygonaceae [33].

Flavonoids have generally been used as chemotaxonomic marker in genus *Polygonum*. The most common feature of *Polygonum* and *Persicaria* genus was hold the flavonoid spectrum with glycosylated and/or methoxylated derivatives of kaempferol, quercetin, myricetin, apigenin and luteolin, glycosylated at C-3 [34]. Interestingly in our study, with except of 3-*O*-methylgalangin (**8**), chalcones and flavanones were isolated, among them biogenetically related chalcone–flavanone pairs, such as pinostrobin chalcone (**3**) and pinostrobin (**5**), and pashanone (**2**) and 5-hydroxy-7,8-dimethoxyflavanone (**7**). This finding serves as a chemotaxonomic marker; the common occurrence of chalcone–flavanone pairs is regarded as taxonomic characteristic only to *Polygonum* and *Persicaria* genus in Polygonaceae family.

3.3. Evaluation of biological effects of compounds isolated from P. maculosa

In order to test the possible toxic effects of the isolated compounds on tumor cells, at first an MTT assay was performed on proliferating mouse breast cancer cells. At 10 μM concentration, none of the tested compounds (**1–5**, **7** and **9–11**) reduced viability of 4 T1 cells to less than 80% of the control after 48 h of treatment (not shown). As a model of non-tumorigenic cells, D3 human brain endothelial cells were used. Confluent D3 monolayers were treated with the test compounds at concentrations of 10 and 20 μM, and real-time impedance measurements were performed, which revealed that only 2′-hydroxy-3′,4′,6′-trimethoxy chalcone (**1**) and pashanone (**2**) decreased cell index (Fig. 2). MTT assay data were in line with this result, showing a concentration-dependent decrease in the viability of D3 cells in response to 2′-hydroxy-3′,4′,6′-trimethoxy chalcone (**1**) and pashanone (**2**) (Fig. 3).

As a next step, we aimed to characterize in detail the effects of compounds **1** and **2** on tumor and normal cells. Impedance measurements did not show tumor cell selectivity; a concentration-dependent decrease in the cell index of both cell types was found in response to 2′-hydroxy-3′,4′,6′-trimethoxy chalcone (**1**), while pashanone (**2**) proved to be toxic only on endothelial cells (Figs. 4 and 5).

MTT data confirmed the results obtained with impedance measurements (Fig. 6).

In the course of our studies, chalcone (**1–3**), flavanone (**4–7**), flavonol (**8**), diarylheptanoid (**10**,**12**,**13**), stilbene (**9**), and ionol (**11**) derivatives were isolated from *P. maculosa*, most of them having chemotaxonomic significance. The cytotoxicity assays of selected compounds (**1–5**, **7** and **9–11**) on tumorigenic 4 T1 mouse triple negative breast cancer and non-tumorigenic D3 human brain endothelial cells by impedance measurements and MTT assay revealed moderate activity of two chalcones, 2′-hydroxy-3′,4′,6′-trimethoxy chalcone (**1**) and pashanone (**2**), the other compounds did not show any potency. A concentration-dependent decrease in the cell index of both cell types was demonstrated for 2′-hydroxy-3′,4′,6′-trimethoxy chalcone (**1**), while pashanone (**2**) proved to be toxic only on endothelial cells.

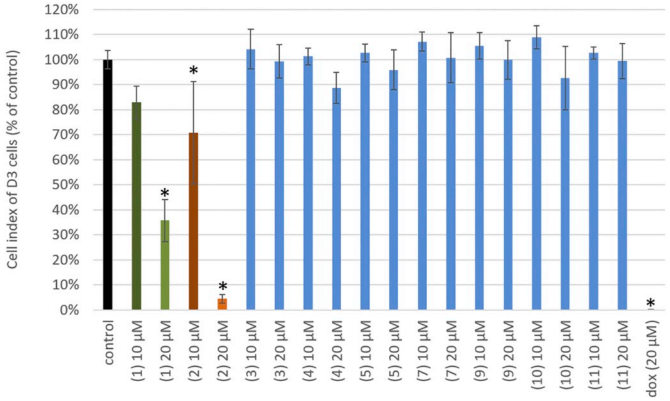


Fig. 2. Impedance of D3 human brain endothelial cells treated for 48 h with compounds **1–5**, **7**, and **9–11** isolated from *Persicaria maculosa*. Doxorubicin was used as a positive control of toxicity. * $P < .01$ compared to control (ANOVA and Bonferroni's post-hoc test).

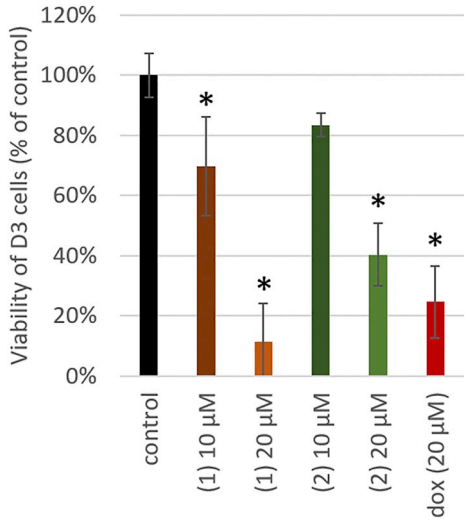


Fig. 3. Viability of D3 cells treated for 48 h with 2′-hydroxy-3′,4′,6′-trimethoxy chalcone (**1**) and pashanone (**2**), as assessed by MTT assay. * $P < .01$ compared to control (ANOVA and Bonferroni's post-hoc test).

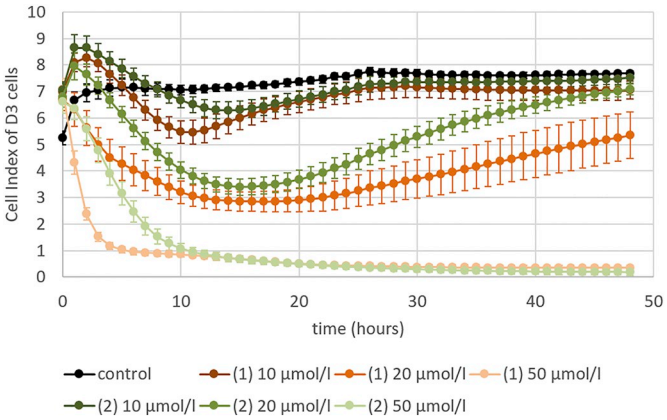


Fig. 4. Impedance changes of D3 cells treated with 2′-hydroxy-3′,4′,6′-trimethoxy chalcone (**1**) and pashanone (**2**) in concentrations of 10, 20 or 50 μmol/L. * $P < .01$ [**1** and **2** in 20 and 50 μmol/L concentrations] compared to control, as assessed by comparing areas under curves with ANOVA and Bonferroni's post-hoc test.

Previous studies have been proved that chalcones are promising antitumor lead compounds due to their antioxidant, cytotoxic, and apoptosis inducing activities. The cytotoxicity of chalcones against tumor cell lines may be the result of disruption of the cell cycle, inhibition of angiogenesis, mitochondrial uncoupling, apoptosis induction, antiproliferation, and antimetastasis. For antimitotic activity, the α,β-unsaturated carbonyl part, the planar structure geometry, and presence of methoxy and 2′ oxygenated substituents are favourable features [35,36]. 2′-Hydroxy-3′,4′,6′-trimethoxychalcone (**1**) and pashanone (**2**) fulfil these requirements.

Although the tumor specificity of chalcones has been reported several times, *e.g.* in comparing sensitivity of HepG2 cells to normal liver cells; osteosarcoma to bone marrow and small intestinal epithelial cells; murine leukemia cells to normal human lymphocytes; and human prostate cancer cells to normal human prostate epithelial cells [37], the

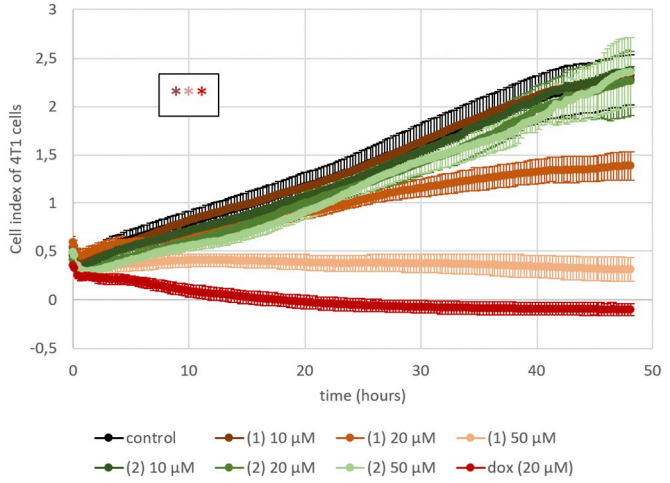


Fig. 5. Impedance changes of 4 T1 mouse triple negative breast cancer cells treated with 2′-hydroxy-3′,4′,6′-trimethoxy chalcone (**1**) and pashanone (**2**) in concentrations of 10, 20 or 50 μmol/L. * $P < .01$ [**1** in 20 and 50 μmol/L concentrations and doxorubicin] compared to control, as assessed by comparing areas under curves with ANOVA and Bonferroni's post-hoc test.

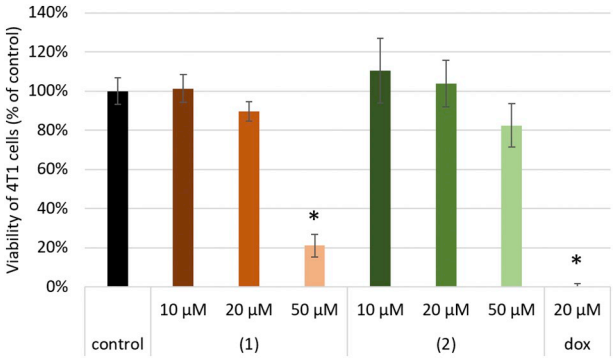


Fig. 6. Viability 4 T1 cells treated for 48 h with different concentrations 2′-hydroxy-3′,4′,6′-trimethoxy chalcone (**1**) and pashanone (**2**), as assessed with MTT assay. * $P < .01$ compared to control (ANOVA and Bonferroni's post-hoc test).

tumor specificity could not be presented in our experiment when chalcones **1** and **2** on 4T1 mouse triple negative breast cancer and non-tumorigenic D3 human brain endothelial cells were tested.

Declaration of Competing Interest

The authors declare no competing financial interest.

Acknowledgments

Financial support for this research was provided by the Economic Development and Innovation Operative Program GINOP-2.3.2-15-2016-00012 and GINOP-2.3.2-15-2016-00020. Grant 20391-3/2018/FEKUSTRAT awarded by the Ministry of Human Capacities, Hungary, is acknowledged. C.F. is recipient of BO/00213/19/8 and UNKP-19-4-SZTE-18 fellowships and support from NKFIH (PD-121130). Work of I.W. is supported by the NKFIH FK-124114 and the UEFISCDI PN-III-P1-1.1-TE-2016-1352 projects.

References

[1] http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=124629.
[2] M. Derita, S. Zacchino, Chemotaxonomic importance of sesquiterpenes and flavonoids in five Argentinian species of *Polygonum* genus, J. Essent. Oil Res. 23 (2011) 11–14.
[3] M.G. Derita, S.J. Gattuso, S.A. Zacchino, Occurrence of polygodial in species of *Polygonum* genus belonging to *Persicaria* section, Biochem. Syst. Ecol. 36 (2008) 55–58.
[4] http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=220010181.
[5] D.F. Austin, Baboquivari Mountain Plants: Identification, Ecology, and Ethnobotany, The University of Arizona Press, Tucson, Arizona, 2010, pp. 222–223.
[6] https://bie.ala.org.au/species/http://id.biodiversity.org.au/node/apni/2894047.
[7] H.D. Smolarz, Chromatographical analysis of phenolic acids in some species of *Polygonum* L genus. Part 2 – Quantitative determination of the major components by high performance liquid chromatography (HPLC), Acta Soc. Bot. Pol. 69 (2000) 21–23.
[8] N. Prota, R. Mumma, H.J. Bouwmeester, M.A. Jongsma, Comparison of the chemical composition of three species of smartweed (genus *Persicaria*) with a focus on drimane sesquiterpenoids, Phytochemistry 108 (2014) 129–136.
[9] F. Hussain, B. Ahmad, I. Hameed, D. Dastagir, P. Sanaullah, S. Azam, Antibacterial, antifungal and insecticidal activities of some selected medicinal plants of Polygonaceae, Afr. J. Biotechnol. 9 (2010) 5032–5036.
[10] M. Derita, S. Zacchino, Validation of the ethnopharmacological use of *Polygonum persicaria* for its antifungal properties, Nat. Prod. Commun. 6 (2011) 931–933.
[11] H.M. Yano, E.M. Bacchi, L.S.S. Hayashi, R. De Lucia, Anti-inflammatory activity and effect on locomotion of *Polygonum persicaria* L. (Polygonaceae) extract in rats, Lat. Am. J. Pharm. 30 (2011) 1635–1638.
[12] H.D. Smolarz, M.J. Potrzebowski, Persilben, a new carboxystilbene from *Polygonum persicaria*, J. Mol. Struct. 605 (2002) 151–156.
[13] H.D. Smolarz, U. Kosikowska, B. Baraniak, A. Malm, A. Persona, Lipophilicity, antifungal and antioxidant properties of persilben, Acta Pol. Pharm. 62 (2005) 457–460.
[14] K. Ichino, H. Tanaka, K. Ito, Synthesis of helilandin B, pashanone, and their isomers, J. Nat. Prod. 51 (1988) 906–914.
[15] S.N. Malek, C.W. Phang, H. Ibrahim, A.W. Norhanom, K.S. Sim, Phytochemical and cytotoxic investigations of *Alpinia mutica* rhizomes, Molecules 16 (2011) 583.
[16] V.I. Yamovoi, E.A. Kulmagambetova, A.T. Kulyyasov, K.M. Turdybekov, S.M. Adekenov, Molecular structure of a novel polymorphic modification of pinostrobin, Chem. Nat. Compd. 37 (2001) 424–427.
[17] M. Tanjung, T.S. Tjahjandarie, M.H. Sentosa, Antioxidant and cytotoxic agent from the rhizomes of *Kaempferia pandurata*, Asian Pac. J. Trop. Dis. 3 (2013) 401–404.
[18] P.C. Vieira, M.A. De Alvarenga, O.R. Gottlieb, H.E. Gottlieb, 4-Hexadecenylphenol and flavonoids from *Piper hispidum*, Planta Med. 39 (1980) 153–156.
[19] C. Kamperdick, N.H. Van, T.V. Sung, Constituents from *Miliusa balansae* (Annonaceae), Phytochemistry 61 (2002) 991–994.
[20] E. Wollenweber, The occurrence of flavanones in the farinose exudate of the fern *Onychium siliculosum*, Phytochemistry 21 (1982) 1462–1464.
[21] M. Xin, S. Guo, W. Zhang, Z. Geng, J. Liang, S. Du, Z. Deng, Y. Wang, Chemical constituents of supercritical extracts from *Alpinia officinarum* and the feeding deterrent activity against *Tribolium castaneum*, Molecules 22 (2017) 647.
[22] A. Pabst, D. Barron, E. Sémon, P. Schreier, Two diastereomeric 3-oxo-α-ionol β-D-glucosides from raspberry fruit, Phytochemistry 31 (1992) 1649–1652.
[23] Z. Liu, S. Sang, T.G. Hartman, C.T. Ho, R.T. Rosen, Determination of diarylheptanoids from *Alpinia officinarum* (Lesser Galangal) by HPLC with photodiode array and electrochemical detection, Phytochem. Anal. 16 (2005) 252–256.
[24] C.H. Park, K.H. Kim, I.K. Lee, S.Y. Lee, S.U. Choi, J.H. Lee, K.R. Lee, Phenolic constituents of *Acorus gramineus*, Arch. Pharm. Res. 34 (2011) 1289–1296.
[25] V.U. Pawar, V.S. Shinde, Chiron approach to the synthesis of yashabushidiol B, (3*S*,5*S*)-1-(4′-hydroxyphenyl)-7-phenylheptane-3,5-diol, and its 4′-methoxy analogue, Tetrahedron-Asymmetry 22 (2011) 8–11.
[26] Y. Jahng, J.G. Park, Recent studies on cyclic 1,7-diarylheptanoids: their isolation, structures, biological activities, and chemical synthesis, Molecules 23 (2018) 3107/1–3107/42.
[27] G.J. Fan, Y.H. Kang, Y.N. Han, B.H. Han, Platelet-activating factor (PAF) receptor binding antagonists from *Alpinia officinarum*, Bioorg. Med. Chem. Lett. 17 (2007) 6720–6722.
[28] H.J. Lee, J.S. Kim, J.H. Ryu, Suppression of inducible nitric oxide synthase expression by diarylheptanoids from *Alpinia officinarum*, Planta Med. 72 (2006) 68–71.
[29] Y. Sun, K. Tabata, H. Matsubara, S. Kitanaka, T. Suzuki, K. Yasukawa, New cytotoxic diarylheptanoids from the rhizomes of *Alpinia officinarum*, Planta Med. 74 (2008) 427–431.
[30] J. Romanski, P. Nowak, C. Chapuis, J. Jurczak, Total synthesis of (5*S*)-dihydroyashabushiketol, Tetrahedron-Asymmetry 22 (2011) 787–790.
[31] M. Narasimhulu, T. Srikanth Reddy, K. Chinni Mahesh, A. Sai Krishna, J. Venkateswara Rao, Y. Venkateswarlu, Synthesis of yashabushidiol and its analogues and their cytotoxic activity against cancer cell lines, Bioorg. Med. Chem. Lett. 19 (2009) 3125–3127.

[32] T.P. Lien, A. Porzel, J. Schmidt, T.V. Sung, G. Adam, Chalconoids from *Fissistigma bracteolatum*, Phytochemistry 53 (2000) 991–995.
[33] B.K. Datta, S.K. Datta, M.A. Rashid, S.D. Sarker, Flavonoids from *Polygonum stagninum*, Biochem. Syst. Ecol. 30 (2002) 693–969.
[34] C.W. Park, Flavonoid chemistry of *Polygonum* sect. *Echinocaulon*: a systematic survey, Syst. Bot. 12 (1987) 167–179.
[35] M.L. Go, X. Wu, X.L. Liu, Chalcones: an update on cytotoxic and chemoprotective properties, Curr. Med. Chem. 12 (2005) 481–499.
[36] S. Marquina, M. Maldonado-Santiago, J.N. Sánchez-Carranza, M. Antúnez-Mojica, L. González-Maya, R.S. Razo-Hernández, L. Alvarez, Design, synthesis and QSAR study of 2′-hydroxy-4′-alkoxy chalcone derivatives that exert cytotoxic activity by the mitochondrial apoptotic pathway, Bioorg. Med. Chem. 27 (2019) 43–54.
[37] H. Sakagami, Y. Masuda, M. Tomomura, S. Yokose, Y. Uesawa, N. Ikezoe, D. Asahara, K. Takao, T. Kanamoto, S. Terakubo, H. Kagaya, H. Nakashima, Y. Sugita, Quantitative structure–cytotoxicity relationship of chalcones, Anticancer Res. 37 (2017) 1091–1098.