Original Article

Pellitorine, an extract of Tetradium daniellii, is an antagonist of the ion channel TRPV1

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ABSTRACT

Background: Transient Receptor Potential Vanilloid 1 (TRPV1) confers noxious heat and inflammatory pain signals in the peripheral nervous system. Clinical trial of resiniferatoxin from Euphorbia species is successfully aimed at TRPV1 in cancer pain management and heading toward new selective painkiller status that further validates this target for drug discovery efforts. Evodia species, used in traditional medicine for hundreds of years, are a recognised source of different TRPV1 agonists, but no antagonist has yet been reported.

Hypothesis/Purpose: In a search for painkiller leads, we noted for the first time a TRPV1 antagonist activity in the fresh fruits of Tetradium daniellii (Benn.) T.G. Hartley (syn. Evodia hupehensis Dode).

Methods: Through a combination of extraction and purification methods with functional TRPV1-specific Ca2+ uptake assays (bioactivity-guided fractionation/isolation/purification); we isolated a new painkiller candidate that is a distant structural homologue of capsiate exovanilloids and endovanilloids such as anandamide, but a putative competitive inhibitor of the TRPV1. Four additional inactive compounds (N-isobutyl-4,5-epoxy-2E-decadienamide, geranylpsoralen, 8-(7′,8′-epoxygeranyloxy)psoralen, and xanthotoxol) were also co-purified with pellitorine. Their structures were established by extensive 1D- and 2D-NMR spectroscopic analysis.

Results: 1H- and 13C NMR determination of the chemical structure revealed it to be pellitorine, (2E,4E)-N-(2-methylpropyl)-1,4-decadienamide, which can compete structurally with algesics released in inflammatory pain. In contrast to previous isolates from Evodia species, pellitorine blocked capsaicin-evoked Ca2+ uptake with an IC50 of 154 µg/ml (0.69 mM/l). N-isobutyl-4,5-epoxy-2E-decadienamide and geranylpsoralen, 8-(7′,8′-epoxygeranyloxy)psoralen, and xanthotoxol did not affect the TRPV1.

Conclusion: This is the first evidence that pellitorine, an aliphatic alkylamide analogue of capsaicin, can serve as an antagonist of the TRPV1 and may inhibit exovanilloid-induced pain.

Introduction

Transient Receptor Potential Vanilloid 1 (TRPV1), one of 28 members of the transient receptor potential (TRP) family of ion channels, transduces pain signals in the peripheral nervous system (PNS) of mammals, including human. TRPV1 expressing nerve endings of C- and Aδ-type primary afferent nociceptive neurons are triggered by endo-, and exovanilloids, moderate heat (Tominaga et al., 1998), and acute or chronic inflammatory mediators, either lipid-like eicosanoids (Olah et al., 2001; Zygmunt et al., 1999; Hwang et al., 2000) or sensitized by peptides such as bradykinin (Pan and Chen, 2004; Di Marzo et al., 2002). Moreover, metabolic changes leading to tissue

Abbreviations: ANA, anandamide; CAPS, capsaicin; CapZ, capsazepine; HaCaT, human immortalized keratinocyte cell line; pMTH, plasmid containing metallothionein promoter; PNS, peripheral nervous system; PUFA, polyunsaturated fatty acids; RPC, rotation planar chromatography; RTX, resiniferatoxin; TRP, transient receptor potential; TRPV1, Transient Receptor Potential Vanilloid 1
acidification potentiate the receptor for chronic pain signalling (Caterina et al., 1997; Tominaga et al., 1998).

Pain sensation can be reduced by the inhibition of TRPV1, and therefore identification of potent TRPV1 antagonists have been in the focus of research studies. Capsazepine, the first TRPV1 antagonist, was reported by Bevan et al. (1992). Since the identification of TRPV1, numerous TRPV1 antagonists have been synthesized. An efficient antagonist like SB-705,498 (Gunthorpe et al., 2007) showing IC50 values at nanomolar range has been demonstrated to block the activation of TRPV1 by capsaicin, heat, and decreased pH. However, potent antagonists of TRPV1 have exhibited undesirable chemical, pharmacological or pharmacokinetic properties such as poor solubility causing decreased absorption (Stec et al., 2008), short half-life (Tafesse et al., 2004).

The fruits of T. daniellii were collected in a public park at Hódmezővásárhely (Hungary) in September, and were stored at −15 °C until preparation. A voucher specimen (No. 760) has been deposited in the Herbarium of the Department of Pharmacognosy, University of Szeged, Szeged, Hungary.

The frozen plant material (10 kg) was percolated with MeOH (60 l) at room temperature. After concentration to 1.5 l, the MeOH extract was partitioned between cyclohexane (4 × 1.5 l), CHCl3 (4 × 1.5 l) and H2O. The cyclohexane layer was dried in vacuum yielding a dark green oily residue (110 g), which was subjected to VLC (VLC-1) on silica gel, using a gradient system of cyclohexane – EtOAc – EtOH (100:0:0, 19:1:0, 9:1:0, 4:1:0, 7:3:0, 70:30:3, 15:10:1, and 1:1:1). Altogether 83 fractions, each of 250 ml, were collected and successively combined in 18 fractions (Evolo – Evo18) after TLC monitoring. Combined fractions Evo10, eluted with cyclohexane – EtOAc – EtOH (4:1) exhibited capsaicin (CAPS)-induced Ca2+ -uptake inhibitory activity, and were further fractionated by means of VLC (VLC-2) on silica gel with n-hexane – acetone mixtures of increasing polarity (19:1, 9:1, 17:3, 4:1, 3:1, 7:3, and 3:2). The fractions obtained here were combined in nine main fractions (Evo10/1 – Evo10/9) depending on their compositions. Only one fraction (Evo10/1) was found to be effective, and was re-chromatographed by OCC on RP-18 silica gel with elution of 70, 75, 80, 85, 90, 95 and 100% MeOH. The combined fractions (Evo10/1/A – Evo10/1/H) from this separation were assayed for their efficacy, and the active fraction Evo10/1/B was selected for further chromatography. This fraction was purified by preparative TLC on silica gel using CHCl3 – aceton (49:1) as developing system, to afford pellitorine (1) (2.4 mg) (Fig. 4), after three-step chromatographic separations using VLC (silica gel, n-hexane – acetone gradient), RPC (silica gel, toluene – ethyl acetate gradient) and preparative TLC (silica gel, CHCl3 – aceton 19:1), combined fractions 29–33 from the VLC-1 separation yielded N-isobutyl-4,5-epoxy-2E-decaenaemide (3) (1.2 mg), 8-geranyloxypsoralen (5) (150 mg), 8-(7',8'-epoxygeranyloxypsoralen) (7) (2.3 mg) and xanthotoxol (4) (4.3 mg) (Fig. 4).

(2E,4E)-(2-methylpropyl)dec-2,4-dienamide (pellitorine) (1): amorphous solid; 1H NMR (500 MHz, CDCl3, δ ppm): 5.74 (1H, d, J = 15.0 Hz, H-2), 7.18 (1H, dd, J = 15.0, 10.2 Hz, H-3), 6.12 (1H, dd, J = 15.2, 10.4 Hz, H-4), 6.07 (1H, dt, J = 15.1, 6.4 Hz, H-5), 2.13 (2H, dt, J = 7.2, 6.9 Hz, H-6), 1.39 (2H, m, H-7), 1.32–1.32 (4H, m, H-8, H-9), 0.87 (3H, t, J = 7.0 Hz, H-10), 3.15 (1H, t, J = 6.5 Hz, H-11), 1.79 (1H, sept, J = 7.6 Hz, H-2'), 0.92 (6H, d, J = 6.7 Hz, H-3',4'), 5.49 (1H, brs, NH). The data are identical with those published by Ley et al. (2004).

N-isobutyl-4,5-epoxy-2E-decaenaemide (2): amorphous solid; 1H NMR (500 MHz, CDCl3, δ ppm): 6.06 (1H, d, J = 15.2 Hz, H-2), 6.65 (1H, dd, J = 15.2, 6.4 Hz, H-3), 3.19 (1H, brd, J = 6.4 Hz, H-4), 2.85 (1H, dd, J = 6.4, 1.9 Hz, H-5), 1.60 (2H, m, H-6), 1.43 (2H, m, H-7), 1.32 (4H, m, H-8, H-9), 0.90 (3H, t, J = 7.3 Hz, H-10), 1.36 (1H, t, J = 6.5 Hz, H-1'), 1.80 (1H, sept, J = 6.7 Hz, H-2'), 0.93 (6H, d, J = 6.7 Hz, H-3',4'), 5.15 (1H, brs, NH). The data are in good agreement with those published by Wei et al. (2004).

8-Geranyloxypsoralen (3): white crystals; mp = 57–59 °C; 1H NMR (500 MHz, CDCl3, δ ppm): 6.35 (1H, d, J = 9.6 Hz, H-3), 7.75 (1H, d, J = 9.6 Hz, H-4), 7.35 (1H, s, H-5), 6.80 (1H, d, J = 2.1 Hz, H-6), 7.68 (1H, d, J = 2.1 Hz, H-7), 5.01 (3H, m, H-1', H-7'), 5.59 (2H, t, J = 7.0 Hz, H-2'), 1.69 (3H, s, H-4'), 2.00 (4H, m, H-5', H-6'), 1.56 (3H, s, H-9'), 1.64 (3H, s, H-10'); 13C NMR (125 MHz, CDCl3, δ ppm): 160.5 (C-2), 114.7 (C-3), 144.3 (C-4), 113.2 (C-5), 106.7 (C-6), 146.6 (C-7), 143.1 (C-8), 144.0 (C-9), 116.5 (C-10), 125.8 (C-11), 149.0 (C-12), 70.1 (C-1'), 119.4 (C-2'), 131.6 (C-3'), 16.5 (C-4'), 39.6 (C-5'), 26.3 (C-6'), 123.8 (C-7'), 131.7 (C-8'), 17.6 (C-9'), 25.6 (C-10'). NMR chemical shifts are in good agreement with the published values (Miyake et al., 1999).

8-(7',8'-Epoxygeranyloxypsoralen) (4): amorphous solid; 1H NMR (500 MHz, CDCl3, δ ppm): 6.37 (1H, d, J = 9.6 Hz, H-3), 7.76 (1H, d, J = 9.6 Hz, H-4), 7.36 (1H, s, H-5), 6.81 (1H, d, J = 2.1 Hz, H-6), 7.69 (1H, d, J = 2.1 Hz, H-7), 5.03 (2H, d, J = 7.1 Hz, H-1'), 5.65 (2H, t, J = 7.1 Hz, H-2'), 1.73 (3H, s, H-4'), 2.17 (1H, m, H-5'), 2.14 (1H, m, H-5'), 1.59 (2H, m, H-6'), 2.64 (1H, J = 6.2 Hz, H-7'), 1.24 (3H, s, H-9'), 1.28 (3H, s, H-10'). The data are in good agreement with those published by Ziegler and Spitterer (1992).

Xanthotoxol (5): white crystal; mp = 249–250 °C. Identification of 5
was on the basis of its TLC co-elution with authentic standard and on its
$^1$H NMR spectrum (Harkar et al., 1984).

CAPS and capsazepine (CapZ), a well characterized antagonist of
TRPV1, were dissolved in DMSO (all from Sigma, St. Louis, MO, USA).
RTX (LC Laboratories, Woburn, MA, USA) was dissolved in ethanol at
2 mg/ml concentration and further diluted in ddH$_2$O.

**Cell culture and preparation of permanent TRPV1-HaCaT cell line**

The spontaneously immortalized human keratinocyte cell line
HaCaT was kindly provided by Dr. Fusseneg, Heidelberg, Germany
(Boukamp et al., 1988), and cultured in MIXMEM medium supple-
mented with 10% FCS (Sigma-Aldrich, St. Louis, MO, USA).

The C-terminally tagged rat TRPV1e DNA (accession number:
NM_031982.1) construct was prepared in the plasmid vector containing
metallothionein promoter (pMTH) as described previously (Ohl et al.,
2001). To avoid the decrease in cell survival that occurs when the
TRPV1 is overexpressed, only the basal activity of the inducible me-
tallothionein promoter was used. To prepare a cell line permanently
expressing the TRPV1, HaCaT cells were transfected with the plasmid
vector using the Exgen 500 reagent according to the recommendations
of the manufacturer. After 24 h cells were transferred to selection
medium, containing 0.8 mg/ml G418 (Sigma, St. Louis, MO, USA)
which was replaced every second day. After about a month, G418-re-
sistant colonies were tested with vanilloid-induced 45Ca$^{2+}$ uptake as-
say and a colony exhibiting 20-fold above the base line uptake was
chosen for further studies.

**45Ca$^{2+}$ uptake assay**

One day before the assay, cells were seeded in 96-well flat bottom
plates (Orange Scientific, Braine-l’Alleud, Belgium) at a density of
20,000 cells/well and left for duplication O/N. The assays were per-
formed with a BioMek 1000 robotized liquid handler (Beckman
Instruments Inc., Fullerton, CA, USA). The plates were washed three
times with assay medium (Ca$^{2+}$- and Mg$^{2+}$-free Hanks
balanced salt
solution supplemented 0.8 mM MgCl$_2$ and with 25 mM Tris-HCl,
$\text{pH} = 7.4$). CAPS dilutions in the presence of45Ca$^{2+}$ were prepared by
the robot. The Ca$^{2+}$ uptake assay was performed for 10 min at 24 °C
using 0.07 µCi of 45Ca$^{2+}$ in 100 µl final volume/well. To terminate
45Ca$^{2+}$ uptake and remove the free isotope, cells were washed three
additional times and then lysed in 100 µl/well lysis buffer (50 mM Tris-
HCl, $\text{pH} 7.5$, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 5 mM EDTA)
for 30 min. Eighty µl aliquots of the solubilized cell extracts were mixed
with 120 µl aliquots of Optiphase Supermix scintillation cocktail
(Perkin Elmer, Wellesley, MA, USA) and counted in a Perkin Elmer li-
quid scintillation counter.

**Eye wipe test**

Hundred µM of CAPS solution in 0.05% of methylcellulose or
100 µM of CAPS solution supplemented with 3.75 mM of pellitorine in
0.05% of methylcellulose was dropped into the eyes of the BALB/c mice
and the number of defensive wiping movements was counted for 2 min.
Two-tailed t-test was used for statistical analysis. The group number
was 7 or higher.

**Results and discussion**

The extraction and the purification of cyclohexane phase of fruits of
*Tetradium danielli* (Benn.) T.G. Hartley (syn. *Evodia hupehensis* Dode)
resulted in the isolation of five compounds. The concentrated MeOH
extracts were partitioned between cyclohexane, CHCl$_3$ and H$_2$O. VLC
separation of the active component containing cyclohexane fraction
(Fig. 1A) on silica gel, using a gradient system of cyclohexane/EtOAc/
EtOH, resulted 18 fractions with different composition (Evo1 – Evo18).

Fraction Evo10 exhibited antagonist activity on CAPS-induced 45Ca$^{2+}$
uptake in the TRPV1-HaCaT keratinocyte cell line (Fig. 1B). To test the
antagonist activity of the extracts, the TRPV1-HaCaT cells were acti-
vated for maximal 45Ca$^{2+}$ uptake with 2 µM CAPS, a sub-maximum
dose that was stringent enough, but did not cause cell damage due to
Ca$^{2+}$-excitotoxicity. Fractions to be tested were then co-incubated for
10 min with a 45Ca$^{2+}$ uptake solution, and the accumulation of the
radioactive tracer in the adhesive TRPV1-HaCaT cell line was de-
termined. For agonist activity tests, increasing concentrations of the
extracts were used instead of CAPS in the same assay. Pellitorin had no
effect on the Ca$^{2+}$ uptake of the non-transfected HaCaT cell line that
does not express a functional TRPV1 (data is not shown).

In contrast with expectations from previous studies, our extraction
procedure from *T. danielli* fruits revealed potent antagonist activity.
The dose-response curves generated with the fraction Evo10 demon-
strated full antagonist effect on the TRPV1 (Fig. 1B) with an efficacy of
IC$_{50}$ = 215 µg/ml. CapZ, a bona fide inhibitor of the TRPV1 compared
side-by-side in a 96 well plate assay (Fig. 1B), gave an IC$_{50}$ = 5.3 µg/ml
showing an only 40 times greater potency of inhibition than that of the
crude, but biologically active fraction Evo10.

A VLC procedure with the active fraction Evo10 resulted in the se-
paration of a set of apolar compounds. The fraction Evo10/1 preserved
the antagonistic activity, the TRPV1-HaCaT cell-based bioassay resulting
in IC$_{50}$ = 57 µg/ml (Fig. 1C).

To attain complete homogeneity, fraction Evo10/1 was subjected to
OCC on the RP-18 stationary phase. This led to the separation of eight
fractions (Evo10/1A – Evo10/1H) of which Evo10/1B was the most
effective as concerns CAPS-induced Ca$^{2+}$ uptake (IC$_{50}$ = 32 µg/ml),
determined with the TRPV1-HaCaT cell line (Fig. 1D).

The final step of Evo10/1B purification by preparative TLC yielded a
pure compound. Structure analysis identified this TRPV1 inhibitor as
pellitorine (1). As a structural analogue of CAPS (2), it inhibited TRPV1
in a concentration-dependent manner with IC$_{50}$ = 154 µg/ml
(0.69 mM/l) (Fig. 2). The higher IC$_{50}$ value of pellitorine compared that
of the Evo10/1B fraction might be caused by the potential loss of other
active components during the purification procedure.

The extensive purification procedures yielded fractions containing
other compounds that were purified either completely or nearly to
homogeneity; these were determined by 1H NMR spectrometry as:
N-isobutyl-4,5-epoxy-2-decaenamide (3), one of the closest, though
surprisingly, completely inactive epoxy-derivatives of pellitorine; and
several coumarins such as 8-geranyloxyypsoralen (4), 8-(4′-isobutyl-4,
5-epoxygeranyloxy)ypsoralen (7) and xanthotoxol (4), were also co-purified
(Fig. 4). None of these distant, xanthotoxol-derivatised homologues
were proved active in the functional TRPV1 bioassay. On the contrary,
a previous study investigated imperatorin, a furocoumarin that showed
weak agonist activity on TRPV1 (Chen et al., 2014).

Following *in vitro* tests, pellitorine was challenged *in vivo* tests of eye
wiping in response to pungent vanilliods. 3.75 mM pellitorine applied
together with 100 µM CAPS significantly (p = 0016) decreased the
frequency of vanilloid-evoked defending movements (Fig. 3). The number
of eye wiping movements evoked by 100 µM CAPS, however,
did not change significantly following either a 1 h or 15 min pre-
treatment with 3.75 mM or 4.5 mM pellitorine. Consequently, pelli-
torine inhibited the pain-evoked defensive movements of BALB/c mice
in response to pungent vanilliods.

To date, only TRPV1 agonists have been described from extracts of
*Evodia* species, compounds that are also known to induce the release of
inflammatory CGRP and substance P, both of them peptide agonists of
CGRP receptors and neurokinin receptors, respectively, and are in-
volved in the transmission of pain signals in second-order neurons of
the PNS (Kobayashi et al., 2001). However, in our research study the
coumarins isolated from *T. danielli* did not change signi-
ificantly (p = 0016) decreased the
frequency of vanilloid-evoked defending movements (Fig. 3). The number
of eye wiping movements evoked by 100 µM CAPS, however,
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treatment with 3.75 mM or 4.5 mM pellitorine. Consequently, pelli-
torine inhibited the pain-evoked defensive movements of BALB/c mice
in response to pungent vanilliods.

The “inflammatory soup” is rich in potent endogenous agonists of
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TRPV1, such as (but not confined to) eicosanoids and prostaglandin analogues. Anandamide, 15-(S)-hydroperoxyeicosatetraenoic acid (6), 5-, and 15-(S)-hydroxyeicosatetraenoic acids, leukotriene B(4), N-oleoyldopamine, and N-oleoylethanolamine (8) are the best-known endogenous algesics in inflammation (Chu et al., 2003; Olah et al., 2001; Hwang et al., 2000). Similarly to skin-irritating phorbol esters, certain polyunsaturated fatty acids (PUFAs), such as omega-3, have been demonstrated to upregulate the TRPV1 via the protein kinase mediated phosphorylation of Ser502 and Ser800 of TRPV1 (Matta et al., 2007). Moreover, the activation of cyclooxygenase isozymes leads to the generation of numbers of agonists showing structural similarity to pellitorine, an aliphatic alkylamide analogue of CAPS, inhibits TRPV1. Pellitorine, one of the natural substances of Evodia species are recognised sources of bioactive substances, but previous studies indicated only agonist activity on TRPV1 (Beak et al., 2004; Pearce et al., 2004). 8-geranyloxypsoralen (5) and other coumarins, such as 8-(7′,8′-epoxygeranyloxy)psoralen (7) and xanthotoxol (4) (Fig. 4), proved inactive in our functional TRPV1 bioassay.

**Conclusion**

Pellitorine may also be considered as a structural homologue of exovanilloids, however, it was found to be an antagonist rather than agonist of TRPV1. Pellitorine, one of the natural substances of

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Fig. 1. The effect of fractions from Tetradium daniellii fruits on the TRPV1

A - Antagonist activity of the cyclohexane phase. Increasing concentrations of this fraction were added to TRPV1 expressing HaCaT cells in the presence of 2 µM CAPS in 96 well plates for 10 min with robotic liquid handling. Co-incubation of extracts reduced significantly the vanilloid-induced \( \text{\textsuperscript{45}Ca}^{2+} \) uptake. The data are the means triplicate measurements with either TRPV1-HaCaT or TRPV1-NIH3T3 permanent cell lines.

B - Antagonist activity of fraction Evo10. This fraction was added in increasing concentrations to TRPV1 expressing HaCaT cells in the presence of 2 µM CAPS in 96 well plates for 10 min with robotic liquid handling. Co-incubation of extracts or CapZ reduced significantly the vanilloid-induced \( \text{\textsuperscript{45}Ca}^{2+} \) uptake. The data are the means of triplicate measurements with either TRPV1-HaCaT or TRPV1-NIH3T3 permanent cell lines.

C - Dose-dependent inhibition of the TRPV1 by VLC fractions Evo 10/1 – Evo 10/9. Evo10 was further separated into nine fractions (Evo 10/1 – Evo 10/9) differing in composition by VLC on silica gel with n-hexane – acetone mixtures of increasing polarity. The concentrated fractions were co-incubated with CAPS for 10 min in the cell-based TRPV1 inhibition assay and their IC\textsubscript{50} values were determined. Fraction Evo 10/1 fully inhibited the activation of the TRPV1 with an IC\textsubscript{50} of 57 µg/ml. The experiments were repeated in triplicate two additional times with similar results.

D - Fractionation of VLC fraction Evo 10/1 by column chromatography was carried out on RP-18 silica gel with elution with MeOH/H\textsubscript{2}O mixtures. The eluted fractions were lyophilized and then redissolved in EtOH and their activities were tested in CAPS-induced TRPV1-HaCaT cells. RP-CC fraction Evo10/1B inhibited the \( \text{\textsuperscript{45}Ca}^{2+} \)-uptake. Each experiment was repeated in triplicate at least two additional times with similar results.

Fig. 2. Profiling of inhibitory activity of pellitorine (1). The membrane transport of \( \text{\textsuperscript{45}Ca}^{2+} \) was characterized in the presence of progressively increasing concentrations of pellitorine. Kinetics similar to that represented here was observed in two additional experiments, carried out in duplicate samples.

TRPV1, such as (but not confined to) eicosanoids and prostaglandin analogues. Anandamide, 15-(S)-hydroperoxyeicosatetraenoic acid (6), 5-, and 15-(S)-hydroxyeicosatetraenoic acids, leukotriene B(4), N-oleoyldopamine, and N-oleoylethanolamine (8) are the best-known endogenous algesics in inflammation (Chu et al., 2003; Olah et al., 2001; Hwang et al., 2000). Similarly to skin-irritating phorbol esters, certain polyunsaturated fatty acids (PUFAs), such as omega-3, have been demonstrated to upregulate the TRPV1 via the protein kinase mediated phosphorylation of Ser502 and Ser800 of TRPV1 (Matta et al., 2007). Moreover, the activation of cyclooxygenase isozymes leads to the generation of numbers of agonists showing structural similarity to pellitorine, an aliphatic alkylamide analogue of CAPS, inhibits TRPV1. Evodia species are recognised sources of bioactive substances, but previous studies indicated only agonist activity on TRPV1 (Beak et al., 2004; Pearce et al., 2004). 8-geranyloxypsoralen (5) and other coumarins, such as 8-(7′,8′-epoxygeranyloxy)psoralen (7) and xanthotoxol (4) (Fig. 4), proved inactive in our functional TRPV1 bioassay.

**Conclusion**

Pellitorine may also be considered as a structural homologue of exovanilloids, however, it was found to be an antagonist rather than agonist of TRPV1. Pellitorine, one of the natural substances of
Tetradium daniellii, and Piper longum L. (Liu et al., 2015), a new antagonist of TRPV1 showed a completely different chemical structure than that of previously described potent antagonists of TRPV1. Co-purified homologous compounds, that are either active or inactive on TRPV1 Ca\(^{2+}\)-channel activation, can serve as valuable lead structures in medicinal chemistry in directing these pharmacophores to the therapeutic target with improved specificity and efficacy.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

ZO was supported by MC-IRG030854-PAINKILLER; GVOP-3.3.1-05/1.-2005-05-0057/3.0. CV was supported by grants the National Office for Research and Technology (OM-00051/2005 and OMFB-01575/2006) and the Hungarian Ministry of Health (552/2006). Financial support from the Hungarian Scientific Research Fund (OTKA K109846) is gratefully acknowledged. DR is a grantee of the János Bolyai Research Fellowship of the http://dx.doi.org/10.13039/501100003825. The authors thank Professor Kálmán Szendrei (Department of Pharmacognosy, University of Szeged) for his inspiration initiating this study.

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