Bioorganic & Medicinal Chemistry Letters 24 (2014) 5507–5511

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Effect of acyclic monoterpene alcohols and their derivatives on TRP channels

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ARTICLE INFO

Article history:
Received 4 August 2014
Revised 30 September 2014
Accepted 1 October 2014
Available online 12 October 2014

Keywords:
Transient receptor potential channels
TRPA1
TRPM8
TRPV1
Acyclic monoterpene alcohols

ABSTRACT

A series of thirty-six geraniol, nerol, citronellol, geranylamine, and nerylamine derivatives was synthesized and tested on TRPA1, TRPM8, and TRPV1 channels. Most of them acted as strong modulators of TRPA1 channels with EC_{50} and/or IC_{50} values <1 μM. None was able to significantly activate TRPM8 channels, while thirteen of them behaved as 'true' TRPM8 antagonists. Little or no effect was generally observed on TRPV1 channels. Some of the compounds examined, that is, compounds **1d**,**g**,**n**, **2c**,**d**,**h**,**i**,**o**, **3b**,**e** exhibited an appreciable selectivity for TRPA1 subtype.

Mammalian transient receptor potential (TRP) channels constitute a large family of non-selective cation-permeable channels subdivided into six subfamilies on the basis of their amino acid sequence homology: TRPA (ankyrin), TRPC (canonical), TRPM (melastatin), TRPML (mucolipin), TRPP (polycystin), and TRPV (vanilloid).[1] They are cellular sensors involved in a host of physiological functions (such as nociception, taste perception, vision, mechano-, osmo-, and thermosensation). Dysfunctions of TRP channels have been implicated in various disease states (such as chronic pain, overactive bladder, obesity, diabetes, chronic cough, chronic obstructive pulmonary disease, dermatological disorders, cancer).[2] Therefore, the therapeutic potential of targeting TRP channels by agonists or antagonists has been extensively and increasingly explored over the past 15 years. Thermo-TRPs, that is, TRP channels activated by distinct temperature thresholds (TRPA1, TRPM8, TRPV1-4),[2g] appear to be preferred targets for numerous plant-derived chemicals.[3] Thus, for example, TRPA1 responds to cinnamaldehyde, curcumin, thymol, gingerols, eugenol, TRPM8 is the 'menthol receptor', and TRPV1 is activated by capsaicin, resiniferatoxin, piperine, and camphor. Many naturally occurring ligands exhibit however relatively low potencies and poor specificity for individual members of the TRP family.

Among promiscuous TRP modulators are monoterpenic alcohols geraniol, nerol, and citronellol (Fig. 1). In detail, geraniol and nerol were described as weak activators of TRPA1, TRPM8, and TRPV1,[4a,c] while, at higher concentrations, they also inhibited currents thereby induced. The dose-response curves for TRPV1 activation by citronellol and geraniol revealed a rank order potency of citronellol (43 μM) > geraniol (102 μM),[4b] while geranyl acetate, the main constituent of thyme geraniol essential oil, did not affect TRPV1 activity. Geraniol proved to be an extremely weak TRPM8 agonist with a rather low efficacy (28%) and potency (5.9 mM).[4d] TRPM8 and TRPV1, but not TRPA1, were found to be involved in the antinociceptive effects of citronellyl acetate.[5] *N*-Geranyl cyclopropylcarboxamide enhanced Ca^{2+} influx in hTRPV1- and hTRPA1-expressing cells with EC_{50} values of 115 μM and 83.6 μM, respectively.[6] A number of terpenoid analogues, including geraniol and geranylamine derivatives, designed to be used for treating disorders of nerve transmission, was claimed in a patent application to exhibit various degrees of agonist and antagonist activity at TRPV1 channels.[7] Eventually, citronellyl acetate has been included in a list of TRPA1 antagonists intended to reduce the burn associated with hydrogen peroxide in oral care compositions (70% reduction of H_2O_2 TRPA1 activation at 400 μM).[8]

Our previous structure–activity studies on natural product ligands of TRP channels have resulted in the identification of some

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http://dx.doi.org/10.1016/j.bmcl.2014.10.012
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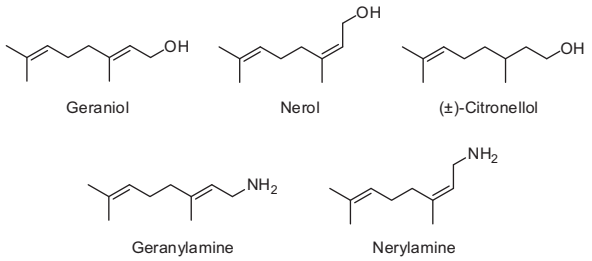


Figure 1. Structures of the acyclic monoterpene alcohols and amines derivatized in this study.

(−)-menthylamine derivatives as potent and selective TRPM8 antagonists,[9] the elucidation of structural determinants for the TRPA1 and TRPV1 agonist properties of gingerols,[10] the identification of thymol-based compounds as modulators of some thermo-TRP channels,[11] and the report of 3-ylidenephthalides as dual modulators of TRPA1 and TRPM8 channels.[12]

In continuation of these studies and with the aim of better defining the TRP modulating properties of geraniol, nerol, citronellol, geranylamine, and nerylamine (Fig. 1) and identifying more potent TRP modulators based on the structure of these monoterpenoids, we have now prepared 36 derivatives and examined their functional activity at TRPA1, TRPM8, and TRPV1 channels (Table 1).

The hydroxyl/amino group of the reference compounds has been replaced by lipophilic groups connected to the monoterpene moiety through ester, amide, carbamate, and urea functionalities in the hope that these modifications would enhance efficacy/potency and/or selectivity, in analogy to previous results with some menthol- and thymol-based compounds.[9,11] It is well known that the TRP domain of the different transient potential receptor channels contains various sets of hydrophobic residues, involved in channel assembly and activation.[13]

Esters **1a**–**i**, **2a**–**j**, **3a**–**e** were prepared by acylation of the corresponding alcohols with either acetic anhydride (esters **1a**, **2a**, **3a**) or the appropriate carboxylic acids, using *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) as the carboxylate activator and 4-dimethylaminopyridine (DMAP) as the nucleophilic catalyst (esters **1b**–**i**, **2b**–**j**, **3b**–**e**) (Scheme 1). Amides **1j**,**k** and **2k**,**l** were prepared by condensation of geranylamine (**4a**) and nerylamine (**4b**), respectively, with the appropriate carboxylic acids, using 1-hydroxybenzotriazole (HOBt)/EDC as the carboxylate activator (Scheme 2). Ureas **1l**,**m** and **2m**,**n** were synthesized by condensation of amines **4a**,**b** with the appropriate isocyanates (Scheme 3), while the preparation of carbamates **1n**,**2o** and **1o**,**2p** was accomplished by condensation of **4a**,**b** with 4-*tert*-butylphenyl chloroformate or of 4-*tert*-butylaniline with geranyl/neryl chloroformate, respectively (Scheme 4). Amines **4a**,**b** were prepared from the corresponding alcohols by a Mitsunobu reaction, using potassium phthalimide as the nucleophile, followed by hydrazinolysis of the phthalimide intermediates **5a**,**b** (Scheme 5).[14,15]

The 36 derivatives synthesized here and the reference alcohols were tested for their ability to induce intracellular Ca^{2+} elevation in HEK293 cells stably transfected with either the rat TRPA1, the rat TRPM8, or the human TRPV1 cDNAs (Table 1). The antagonist or desensitizing activity was assessed by adding the test compounds 5 min before stimulation of cells with reference agonists.[16]

Rat TRPA1-HEK293 cells exhibited a sharp increase in intracellular Ca^{2+} upon application of most of the 36 compounds which were 1–3 orders of magnitude more potent than the corresponding alcohols as rTRPA1 activators. Geraniol, nerol, and citronellol proved indeed to be weak rat TRPA1 activators with efficacies <10, 14.1, and 26.1, respectively. Twenty-five of the compounds examined, that is, **1b**,**d**,**f**–**i**,**l**,**n**,**o**, **2b**–**j**,**m**,**o**,**p**, **3b**–**e** produced a robust activation of TRPA1 in transfected cells with EC_{50} values <1 μM. Most of these compounds are non-electrophilic and it is therefore unlikely that they act on TRPA1forming covalent adducts with critical cysteine residues of the channel.[2e] On the other hand, given their electrophilic nature, the possibility that carbamates **1n**,**o**, **2o**,**p** might act via covalent interactions cannot in principle be ruled out. However, the structurally related carbamate URB597, a known potent FAAH covalent inhibitor,[17] was reported to activate TRPA1 channels probably through a non-covalent, direct gating mechanism.[18] Furthermore, our most potent carbamate **2o** gave a very sluggish reaction (43% conversion after 24 h) with the biologically relevant model thiol cysteamine[19] and the *N*-carbamylated cysteamine **6** was isolated as the main product (Scheme 6).[20] These data argue against a covalent binding mechanism with thiol groups of TRPA1 channel for carbamates as well.

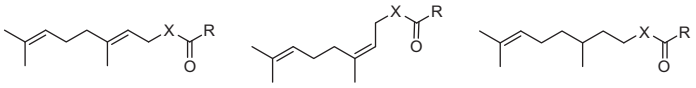
Overall, esters and carbamates exhibited a clear superiority over amides and ureas. Nerol derivatives were better TRPA1 activators than derivatives of the other two alcohols (compare compounds **2b** with **1b** and **3b**, **2e** with **1f** and **3c**, **2m** with **1l**, and **2o** with **1n**). A number of the aforementioned twenty-five compounds was selective (⩾100 fold) versus TRPM8 and TRPV1 activation (compounds **1d**,**g**,**n**, **2c**,**d**,**h**,**i**,**o**, **3b**,**e**). Five-min preincubation of TRPA1-HEK293 cells with each of the twenty-five compounds, and then continued incubation with allyl isothiocyanate caused inhibition of TRPA1 response to this agonist with IC_{50} values ⩽1 μM. Carbamates **1n**, **2o** and 4-chlorobenzyl esters of nerol and citronellol (compounds **2f**, **3d**) appeared to serve as the best functionality for TRPA1 desensitization, while reverse carbamates **1o**, **2p** were ~10 times less potent and amides were generally poor TRPA1 desensitizers. The EC_{50} and IC_{50} values were, with the partial exception of **1o**, **2h**,**p**, comparable and thus, none of compounds acted as 'true' antagonist, in a fashion similar to the effect of capsaicin on the TRPV1 channel.[21]

Consistent with their interaction with a series of environmental irritants and endogenous mediators of inflammation and pain, TRPA1 channels have been validated as a promising target for various potential therapeutic applications including neuropathic and inflammatory pain and airway disorders.[22] Although a large number of TRPA1 modulators has been already reported, many of them tend to be either reactive or of low potency and/or selectivity and therefore they are not optimal tools for pharmacological studies. The identification of easily synthesized, non-reactive, non-volatile, stable compounds **1d**,**g**,**n**, **2c**,**d**,**h**,**i**,**o**, **3b**,**e**, as potent and selective TRPA1 modulators should enable further assessment of the TRPA1 pharmacology and designate them as candidates for future evaluation of in vivo efficacy in rodent models of inflammatory and neuropathic pain.

As concerns TRP assays on TRPM8-HEK293 cells, none of thirty-six derivatives was able to significantly activate this channel, while thirteen of them behaved as 'true' antagonists (that is, inhibition without agonism *per se*, and hence not due to desensitization) with IC_{50} values <1 μM (compounds **2e**,**f**,**j**,**m**, **3d**) or between 1 μM and 10 μM (compounds **1f**,**j**,**k**, **2b**,**g**,**k**,**l**, **3c**). The nerol chain appeared to be particularly effective in this respect, as eight of the thirteen TRPM8 antagonists belong to this series. It is worth noting the marked difference between compounds **2b**,**f**,**j**,**m** and the corresponding geraniol derivatives **1b**,**g**,**i**,**l** which are completely inactive.

Together with TRPA1, TRPM8 is involved in the development and maintenance of cold allodynia arising from traumatic neuropathy, as well as in bladder pain, and TRPM8 antagonists comprising a variety of chemotypes with a potential role in these chronic pain conditions has been recently disclosed.[23] In addition, TRPM8 represents a prostate tumor marker with a potential use in the diagnosis as well as in the therapy of this type of cancer. Indeed,

Table 1
Results of the TRPA1, TRPM8, and TRPV1 assays of monoterpenic alcohols geraniol, nerol, and citronellol and their derivatives/analogues **1**–**3**[a]



1 **2** **3**

Compound	X	R	TRPA1 (efficacy)[b]	TRPA1 (EC_{50}, μM)	TRPA1 (IC_{50}, μM)[c]	TRPM8 (efficacy)[d]	TRPM8 (EC_{50}, μM)	TRPM8 (IC_{50}, μM)[e]	TRPV1 (efficacy)[d]	TRPV1 (EC_{50}, μM)	TRPV1 (IC_{50}, μM)[f]
Geraniol			<10	ND	>100	<10	ND	>100	<10	ND	>100
1a	O	R = Me	83.9	25.8	21.2	<10	ND	>100	<10	ND	>100
1b	O	R = Ph-4-Me	75.2	0.33	1.0	<10	ND	>100	17.6	6.0	>100
1c	O	R = Ph-3-Me	69.8	2.4	1.4	<10	ND	>100	<10	ND	>100
1d	O	R = Ph-4-CN	72.6	0.48	0.73	<10	ND	>100	<10	ND	>100
1e	O	R = Ph-3,5-CF_3	18.8	11.9	>100	<10	ND	>100	<10	ND	>100
1f	O	R = CH_2Ph-4-Me	85.5	0.36	0.32	<10	ND	5.8	<10	ND	>100
1g	O	R = CH_2Ph-4-Cl	86.1	0.12	0.13	<10	ND	>100	<10	ND	>100
1h	O	R = CH_2Ph-4-OMe	88.8	0.51	0.38	<10	ND	>100	13.2	8.0	>100
1i	O	R = CH_2CH_2Ph	85.6	0.39	0.17	<10	ND	>100	27.7	4.0	>100
1j	NH	R = CH_2Ph-4-Cl	89.1	1.2	1.4	<10	ND	3.8	57.5	1.1	0.89
1k	NH	R = CH_2CH_2Ph	97.1	7.5	7.4	<10	ND	5.5	10.5	16.2	>100
1l	NH	R = NHPh-3-Cl	123.6	0.61	0.48	<10	ND	>100	21.2	2.5	0.71
1m	NH	R = NHcC_6H_{11}	74.9	4.9	11.8	<10	ND	>100	36.0	4.8	9.9
1n	NH	R = OPh-4-*t*-Bu	107.7	0.045	0.038	<10	ND	86.5	22.4	10.7	92.3
1o	O	R = NHPh-4-*t*-Bu	72.7	0.092	0.57	<10	ND	>100	21.9	8.7	76.8
Nerol			14.1	11.7	>100	<10	ND	>100	<10	ND	>100
2a	O	R = Me	58.5	13.3	20.7	<10	ND	>100	<10	ND	>100
2b	O	R = Ph-4-Me	82.0	0.093	0.11	<10	ND	1.2	<10	ND	>100
2c	O	R = Ph-3-Me	78.0	0.14	0.14	<10	ND	30.8	<10	ND	>100
2d	O	R = Ph-4-CN	78.3	0.26	0.34	<10	ND	>100	<10	ND	>100
2e	O	R = CH_2Ph-4-Me	73.2	0.043	0.041	<10	ND	0.28	<10	ND	>100
2f	O	R = CH_2Ph-4-Cl	81.8	0.016	0.013	<10	ND	0.75	<10	ND	>100
2g	O	R = CH_2Ph-4-OMe	88.0	0.14	0.26	<10	ND	9.7	<10	ND	>100
2h	O	R = CH_2Ph-3-I	61.6	0.10	0.94	<10	ND	>100	<10	ND	>100
2i	O	R = CH_2-2-Naft	73.4	0.081	0.18	<10	ND	>100	<10	ND	>100
2j	O	R = CH_2CH_2Ph	93.5	0.11	0.11	<10	ND	0.29	<10	ND	>100
2k	NH	R = CH_2Ph-4-Cl	99.7	1.0	0.94	<10	ND	2.2	57.9	0.43	0.53
2l	NH	R = CH_2CH_2Ph	72.7	6.6	13.9	<10	ND	2.2	<10	ND	>100
2m	NH	R = NHPh-3-Cl	104.3	0.43	0.41	<10	ND	0.71	20.1	1.7	0.38
2n	NH	R = NHcC_6H_{11}	79.4	2.65	4.4	<10	ND	30.0	50.1	8.9	15.7
2o	NH	R = OPh-4-*t*-Bu	100.3	0.018	0.029	<10	ND	>100	15.8	6.5	>100
2p	O	R = NHPh-4-*t*-Bu	73.6	0.041	0.29	<10	ND	>100	24.0	7.8	74.6
Citronellol			26.1	>100	>100	<10	ND	>100	<10	ND	>100
3a	O	R = Me	32.2	12.2	>100	<10	ND	>100	<10	ND	>100
3b	O	R = Ph-4-Me	55.2	0.28	0.87	<10	ND	>100	<10	ND	>100
3c	O	R = CH_2Ph-4-Me	88.9	0.090	0.12	<10	ND	2.4	<10	ND	>100
3d	O	R = CH_2Ph-4-Cl	88.0	0.053	0.048	<10	ND	0.83	<10	ND	>100
3e	O	R = CH_2CH_2Ph	77.1	0.16	0.34	<10	ND	>100	<10	ND	>100

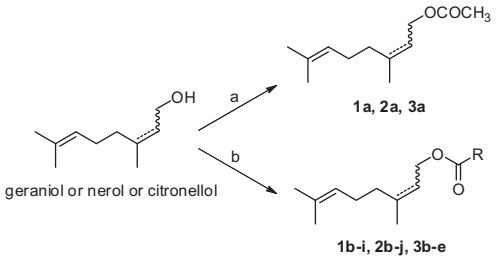
[a] Data are means of at least 3 separate determinations. Standard errors are not shown for the sake of clarity and were never higher than 10% of the means.
[b] As percent of allyl isothiocyanate (100 μM).
[c] Determined against the effect of allyl isothiocyanate (100 μM).
[d] As percent of ionomycin (4 μM).
[e] Determined against the effect of icilin (0.25 μM).
[f] Determined against the effect of capsaicin (0.1 μM). ND, not determined when efficacy is lower than 10%.

both activation by monoterpenoid menthol[24] or blockade by a menthylamine derivative[9] of TRPM8 channels induce apoptosis of TRPM8-expressing LNCaP prostate carcinoma cells.

Eleven compounds belonging to the geraniol and nerol series, that is, **1b**,**h**–**j**,**l**,**m**,**o**, **2m**–**p**, modestly elevated intracellular Ca^{2+} in hTRPV1-HEK293 cells with EC_{50} values ranging from 1 to 10 μM. The sole TRPV1 activator endowed with submicromolar potency was the amide **2k**. Five-min exposure of hTRPV1-HEK293 cells to compounds **1j**,**l**,**m**, **2k**,**m** before stimulation with capsaicin induced desensitization of this channel with IC_{50} values between 0.38 μM and 9.9 μM. Amides and ureas appeared to be superior to the other derivatives as far as TRPV1 desensitization was concerned.

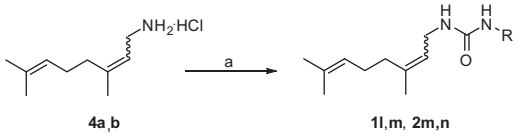
Contrary to what observed on TRPA1 channels, the effects of the thirty-six derivatives on TRPM8 and TRPV1 channels appear to be specific and not to represent a general property of the class, as just a few members were able to effectively modulate these channels, while the majority of the compounds were significantly less potent or completely inactive.

In conclusion, we have disclosed here a series of derivatives of the acyclic monoterpene alcohols geraniol, nerol, and citronellol and of the related amines geranylamine and nerylamine that act as potent and selective TRPA1 channel modulators with EC_{50} and IC_{50} values distinctly lower than those of reference alcohols, compare favourably in terms of potency and/or selectivity with most of other chemotypes presented as TRPA1 modulators and published recently,[22,25] and qualify therefore as useful pharmacological tools in studies on TRP channel modulation using natural products as lead compounds.

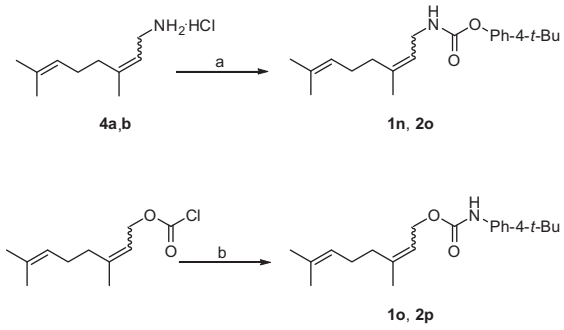


Scheme 1. Synthesis of compounds **1a**–**i**, **2a**–**j**, **3a**–**e**. Reagents and conditions: (a) Ac_2O, Et_3N, CH_2Cl_2, rt, 16 h. (b) RCO_2H, EDC/DMAP, CH_2Cl_2, rt, 16 h.

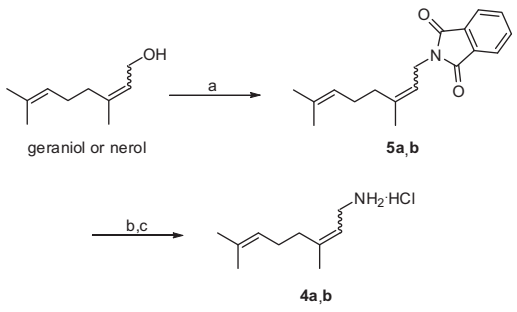
Scheme 2. Synthesis of compounds **1j**,**k**, **2k**,**l**. Reagents and conditions: (a) RCO_2H, HOBt/EDC, rt, 1 h, then **4a** or **4b**, Et_3N, DMF, rt, 16 h.



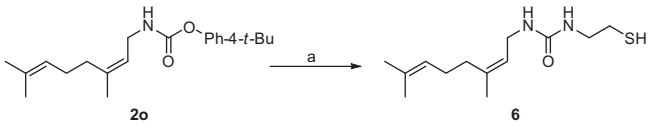
Scheme 3. Synthesis of compounds **1l**,**m**, **2m**,**n**. Reagents and conditions: (a) RNCO, Et_3N, DMF, rt, 16 h.



Scheme 4. Synthesis of compounds **1n**, **2o**, **1o**, **2p**. Reagents and conditions: (a) 4-*t*-BuPhOCOCl, Et_3N, DMF, rt, 16 h. (b) 4-*t*-BuPhNH$_2$, Et_3N, DMF, rt, 16 h.



Scheme 5. Synthesis of compounds **4a**,**b**. Reagents and conditions: (a) potassium phthalimide, PPh_3, DIAD, THF, rt, 16 h. (b) $NH_2NH_2{\cdot}H_2O$, MeOH, reflux, 2.5 h. (c) HCl, Et_2O, 0 °C.



Scheme 6. Reaction of **2o** with cysteamine. Reagents and conditions: (a) cysteamine, DMSO, rt, 72 h.

References and notes

1. (a) Li, M.; Yu, Y.; Yang, J. *Adv. Exp. Med. Biol.* **2011**, *704*, 1; (b) Liu, Y.; Qin, N. *Adv. Exp. Med. Biol.* **2011**, *704*, 185.
2. (a) Kaneko, Y.; Szallasi, A. *Br. J. Pharmacol.* **2014**, *171*, 2474; (b) Bishnoi, M.; Premkumar, L. S. *Open Pain J.* **2013**, *6*, 10; (c) Szallasi, A.; Sheta, M. *Expert Opin. Investig. Drugs* **2012**, *21*, 1351; (d) Moran, M. M.; McAlexander, M. A.; Bíró, T.; Szallasi, A. *Nat. Rev. Drug Disc.* **2011**, *10*, 601; (e) Baraldi, P. G.; Preti, D.; Materazzi, S.; Geppetti, P. *J. Med. Chem.* **2010**, *53*, 5085; (f) Di Marzo, V.; De Petrocellis, L. *Curr. Med. Chem.* **2010**, *17*, 1430; (g) Vay, L.; Gu, C.; McNaughton, P. A. *Expert Rev. Clin. Pharmacol.* **2010**, *3*, 687; (h) Trevisani, M.; Szallasi, A. *Open Drug Discov. J.* **2010**, *2*, 37; (i) Cortright, D. N.; Szallasi, A. *Curr. Pharm. Des.* **2009**, *15*, 1736; (j) Patapoutian, A.; Tate, S.; Woolf, C. J. *Nat. Rev. Drug Disc.* **2009**, *8*, 55; (k) Gunthorpe, M. J.; Szallasi, A. *Curr. Pharm. Des.* **2008**, *14*, 32; (l) Vennekens, R.; Owsianik, G.; Nilius, B. *Curr. Pharm. Des.* **2008**, *14*, 18; (m) Westaway, S. M. *J. Med. Chem.* **2007**, *50*, 2589; (n) Gharat, L.; Szallasi, A. *Drug Dev. Res.* **2007**, *68*, 477; (o) Venkatachalam, K.; Montell, C. *Annu. Rev. Biochem.* **2007**, *76*, 387.
3. (a) Premkumar, L. S. *ACS Chem. Neurosci.* **2014**. article ASAP; (b) Vetter, I.; Lewis, R. J. *Adv. Exp. Med. Biol.* **2011**, *704*, 41; (c) Appendino, G.; Minassi, A.; Pagani, A.; Ech-Chahad, A. *Curr. Pharm. Des.* **2008**, *14*, 2.
4. (a) Lübbert, M.; Kyereme, J.; Schöbel, N.; Beltrán, L.; Horst Wetzel, C.; Hatt, H. *PLoS ONE* **2013**, *8*, e77998; (b) Ohkawara, S.; Tanaka-Kagawa, T.; Furukawa, Y.; Nishimura, T.; Jinno, H. *Biol. Pharm. Bull.* **2010**, *33*, 1434; (c) Stotz, S. C.; Vriens, J.; Martyn, D.; Clardy, J.; Clapham, D. E. *PLoS ONE* **2008**, *3*, e2082; (d) Behrendt, H.-J.; Germann, T.; Gillen, C.; Hatt, H.; Jostock, R. *Br. J. Pharmacol.* **2004**, *141*, 737.
5. Rios, E. R.; Rocha, N. F.; Carvalho, A. M.; Vasconcelos, L. F.; Dias, M. L.; de Sousa, D. P.; de Sousa, F. C.; Fonteles, M. M. *Chem.-Biol. Interact.* **2013**, *203*, 573.
6. Kim, M. J.; Son, H. J.; Kim, Y.; Kweon, H.-J.; Suh, B.-C.; Lyall, V.; Rhyu, M.-R. *PLoS ONE* **2014**, *9*, e89062.
7. Reed, M. A.; Weaver, D.; Sun, S., McLellan, A.; Lu, E. PCT Int. App. WO034232, 2012.
8. Haught, J. C.; Sreekrishna, K. T.; Das, S.; Hoke, S. H. H.; Coffindaffer, T. W.; Bakes, K. A.; Glandorf, W. M. PCT Int. App. WO176897, 2013.
9. Ortar, G.; De Petrocellis, L.; Morera, L.; Schiano Moriello, A.; Orlando, P.; Morera, E.; Nalli, M.; Di Marzo, V. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2729.
10. Morera, E.; De Petrocellis, L.; Morera, L.; Schiano Moriello, A.; Nalli, M.; Di Marzo, V.; Ortar, G. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1674.
11. Ortar, G.; Morera, L.; Schiano Moriello, A.; Morera, E.; Nalli, M.; Di Marzo, V.; De Petrocellis, L. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3535.
12. Ortar, G.; Schiano Moriello, A.; Morera, E.; Nalli, M.; Di Marzo, V.; De Petrocellis, L. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 561.
13. Bonache, M. Á.; Balsera, B.; López-Mendéz, B.; Millet, O.; Brancaccio, D.; Gómez-Monterrey, I.; Carotenuto, A.; Pavone, L. M.; Reille-Seroussi, M.; Gagey-Eilstein, N.; Vidal, M.; de la Torre-Martínez, R.; Fernández-Carvajal, A.; Ferrer-Montiel, A.; García-López, M. T.; Martín-Martínez, M.; Pérez de Vega, M. J.; González-Muñiz, R. *ACS Comb. Sci.* **2014**, *16*, 250.
14. Więcek, M.; Kottke, T.; Ligneau, X.; Schunack, W.; Seifert, R.; Stark, H.; Handzlik, J.; Kieć-Kononowicz, K. *Bioorg. Med. Chem.* **2011**, *19*, 2850.
15. *General procedure for the synthesis of esters* ***1b–i***, ***2b–j***, ***3b–e***. A solution of the appropriate carboxylic acid (0.55 mmol), geraniol or nerol or citronellol (0.50 mmol), EDC (0.90 mmol), and DMAP (0.82 mmol) in dry CH_2Cl_2 (3 mL) was stirred at room temperature overnight. The mixture was diluted with 2 N HCl and extracted with AcOEt. The organic phase was washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by column chromatography. *General procedure for the synthesis of amides* ***1j***, ***k***, ***2k,l***. To a stirred solution of the appropriate carboxylic acid (0.50 mmol) in DMF (4 mL), HOBt (0.53 mmol) and EDC (0.53 mmol) were added at 0 °C. The mixture was stirred at 0 °C for 15 min and at room temperature for 1 h. Then, **4a** or **4b** (0.60 mmol) and Et_3N (0.75 mmol) were added, and the mixture was stirred at room temperature overnight. The mixture was diluted with brine and extracted with AcOEt. The organic phase was washed with 2 N HCl solution, saturated $NaHCO_3$, and brine, dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by column chromatography. *General procedure for the synthesis of ureas* ***1l,m***, ***2m,n***. A solution of the appropriate isocyanate (0.48 mmol), **4a** or **4b** (0.48 mmol), and Et_3N (0.72 mmol) in dry DMF (2 mL) was stirred at room temperature overnight. The mixture was diluted with brine and extracted with AcOEt. The organic phase was washed twice with brine, dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by column chromatography. *General procedure for the synthesis of carbamates* ***1n,o***, ***2o***, ***p***. To a stirred 20% phosgene solution in toluene (1.20 mL, 3.30 mmol) a solution of 4-*tert*-butylphenol or of geraniol or nerol (0.58 mmol) and Et_3N (0.72 mmol) in dry toluene (5.8 mL) was added dropwise at 0 °C. The reaction mixture was

stirred at room temperature for 3 h and evaporated under vacuum. The residue of the crude chloroformate was dissolved in dry CH_2Cl_2 (4 mL) and a solution of **4a** or **4b** or of 4-*tert*-butylaniline (0.48 mmol) and Et_3N (0.96 mmol) in dry DMF (2.1 mL) was added dropwise at room temperature with stirring. The reaction mixture was stirred at room temperature overnight, diluted with water, and extracted with AcOEt. The organic phase was washed twice with brine, dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by column chromatography. *Data for selected compounds*: compound **1g**: yield 78%; oil; IR (ATR) 2918, 1733, 1492, 1242, 1150, 970 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.60 (3H, s), 1.68 (6H, s), 2.01–2.10 (4H, m), 3.59 (2H, s), 4.61 (2H, d, J = 7.2 Hz), 5.07 (1H, m), 5.32 (1H, t, J = 7.2 Hz), 7.21 (2H, d, J = 8.1 Hz), 7.29 (2H, d, J = 8.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.48, 17.69, 25.69, 26.28, 39.51, 40.66, 61.95, 118.00, 123.68, 128.66, 130.64, 131.85, 132.56, 133.00, 142.65, 171.17. Compound **1n**: yield 46%; oil; IR (ATR) 3327, 2964, 1714, 1504, 1209, 1175 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (9H, s), 1.61 (3H, s), 1.69 (6H, s), 2.03–2.14 (4H, m), 3.86 (2H, t, J = 6.0 Hz), 4.96 (1H, br s), 5.09 (1H, m), 5.26 (1H, t, J = 6.9 Hz), 7.04 (2H, d, J = 8.7 Hz), 7.35 (2H, d, J = 8.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.28, 17.70, 25.68, 26.44, 31.45, 34.42, 39.10, 39.51, 119.98, 120.96, 123.86, 126.14, 131.78, 140.02, 147.98, 148.82, 154.73. Compound **2e**: yield 81%.; oil; IR (ATR) 2922, 1733, 1445, 1244, 1144, 972 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.59 (3H, s), 1.67 (3H, s), 1.75 (3H, s), 2.02–2.13 (4H, m), 2.32 (3H, s), 3.57 (2H, s), 4.57 (2H, d, J = 7.2 Hz), 5.09 (1H, m), 5.35 (1H, t, J = 7.2 Hz), 7.12 (2H, d, J = 8.4 Hz), 7.17 (2H, d, J = 8.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.65, 21.06, 23.51, 25.69, 26.65, 32.19, 40.96, 61.50, 119.13, 123.59, 129.12, 129.22, 131.06, 132.12, 136.59, 142.60, 171.79. Compound **2f**: yield 62%; oil; IR (ATR) 2916, 1733, 1493, 1244, 1151, 971 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.59 (3H, s), 1.67 (3H, s), 1.76 (3H, s), 2.04–2.09 (4H, m), 3.59 (2H, s), 4.58 (2H, d, J = 7.2 Hz), 5.07 (1H, m), 5.34 (1H, t, J = 7.2 Hz), 7.21 (2H, d, J = 8.4 Hz), 7.29 (2H, d, J = 8.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.65, 23.52, 25.69, 26.66, 32.20, 40.68, 61.70, 118.90, 123.54, 128.67, 130.65, 130.71, 132.20, 132.55, 142.96, 171.16. Compound **2o**: yield 57%; oil; IR (ATR) 3329, 2964, 1715, 1504, 1210, 1175 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (9H, s), 1.61 (3H, s), 1.70 (3H, s), 1.74 (3H, s), 2.04–2.15 (4H, m), 3.83 (2H, t, J = 6.3 Hz), 4.91 (1H, br s), 5.11 (1H, m), 5.28 (1H, t, J = 7.2 Hz), 7.03 (2H, d, J = 8.4 Hz), 7.35 (2H, d, J = 8.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.68, 23.32, 25.70, 26.43, 31.45, 31.96, 34.41, 38.79, 120.85, 120.96, 123.75, 126.13, 132.35, 140.20, 147.96, 148.83, 154.67. Compound **3d**: yield 82%; oil; IR (ATR) 2914, 1735, 1492, 1250, 1156, 1091, 806 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.88 (3H, d, J = 6.3 Hz), 1.09–1.53 (4H, m), 1.60 (3H, s), 1.61–1.71 (1H, m), 1.68 (3H, s), 1.88–2.10 (2H, m), 3.57 (2H, s), 4.12 (2H, m), 5.07 (1H, t, J = 7.2 Hz), 7.21 (2H, d, J = 8.4 Hz), 7.29 (2H, d, J = 8.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.66, 19.36, 25.39, 25.73, 29.46, 35.37, 36.93, 40.77, 63.60, 124.50, 128.66, 130.62, 131.36, 132.57, 133.00, 171.21.

16. *TRPA1, TRPM8, and TRPV1 channel assays*. HEK293 (human embryonic kidney) cells stably over-expressing recombinant rat TRPA1, rat TRPM8 or human TRPV1 were grown on 100 mm diameter Petri dishes as mono-layers in minimum essential medium (EMEM) supplemented with non-essential amino acids, 10% foetal bovine serum, and 2 mM glutamine, and maintained at 5% CO_2 at 37 °C. Stable expression of each channel was checked by quantitative PCR (data not shown). The effect of the substances on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was determined by using Fluo-4, a selective intracellular fluorescent probe for Ca^{2+}. On the day of the experiment, cells were loaded for 1 h at room temperature with the methyl ester Fluo-4-AM (4 μM in dimethyl sulfoxide containing 0.02% Pluronic F-127, invitrogen) in EMEM without foetal bovine serum, then were washed twice in Tyrode's buffer (145 mM NaCl, 2.5 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM D-glucose, and 10 mM HEPES, pH 7.4), resuspended in the same buffer, and transferred (about 100,000 cells) to the quartz cuvette of the spectrofluorimeter (Perkin-Elmer LS50B equipped with PTP-1 Fluorescence Peltier System; PerkinElmer Life and Analytical Sciences, Waltham, MA, USA) under continuous stirring. The changes in $[Ca^{2+}]_i$ were determined before and after the addition of various concentrations of test compounds by measuring cell fluorescence (λ_{EX} = 488 nm, λ_{EM} = 516 nm) at 25 °C. Curve fitting (sigmoidal dose–response variable slope) and parameter estimation were performed with GraphPad Prism® (GraphPad Software Inc., San Diego, CA). Potency was expressed as the concentration of test substances exerting a half-maximal agonist effect (i.e., half-maximal increases in $[Ca^{2+}]_i$) (EC_{50}). The effects of TRPA1 agonists are expressed as a percentage of the effect obtained with 100 μM allyl isothiocyanate (AITC). In the case of TRPM8 assays, the efficacy of the agonists was first determined by normalizing their effect to the maximum Ca^{2+} influx effect on $[Ca^{2+}]_i$ observed with application of 4 μM ionomycin (Alexis). In the case of TRPV1 assays, the efficacy of the agonists was first determined by normalizing their effect to the maximum Ca^{2+} influx effect on $[Ca^{2+}]_i$ observed with application of 4 μM ionomycin (Alexis). When significant, the values of the effect on $[Ca^{2+}]_i$ in wild-type (i.e., not transfected with any construct) HEK293 cells were taken as baseline and subtracted from the values obtained from transfected cells. Antagonist/desensitizing behaviour was evaluated against AITC (100 μM) for TRPA1, icilin (0.25 μM) for TRPM8, and capsaicin (0.1 μM) for TRPV1, by adding the test compounds in the quartz cuvette 5 min before stimulation of cells with agonists. Data are expressed as the concentration exerting a half-maximal inhibition of agonist-induced $[Ca^{2+}]_i$ elevation (IC_{50}), which was calculated again using GraphPad Prism® software. The effect on $[Ca^{2+}]_i$ exerted by agonist alone was taken as 100%. Dose response curves were fitted by a sigmoidal regression with variable slope. All determinations were performed at least in triplicate. Statistical analysis of the data was performed by analysis of variance at each point using ANOVA followed by the Bonferroni's test.

17. (a) Otrubova, K.; Ezzili, C.; Boger, D. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4674; (b) Seierstad, M.; Breitenbucher, J. G. *J. Med. Chem.* **2008**, *51*, 7327.
18. Niforatos, W.; Zhang, X.-F.; Lake, M. R.; Walter, K. A.; Neelands, T.; Holzman, T. F.; Scott, V. E.; Faltynek, C. R.; Moreland, R. B.; Chen, J. *Mol. Pharmacol.* **2007**, *71*, 1209.
19. Avonto, C.; Tagliatela-Scafati, O.; Pollastro, F.; Minassi, A.; Di Marzo, V.; De Petrocellis, L.; Appendino, G. *Angew. Chem., Int. Ed.* **2011**, *50*, 467.
20. The reaction between carbamate **2o** (49 mg, 0.15 mmol) and cysteamine (19 mg, 0.25 mmol) in DMSO-d_6 (0.75 mL) in a standard NMR tube was monitored by ^1H NMR. After completion of the reaction (~72 h), the solution was diluted with water, and extracted with AcOEt. The organic phase was washed twice with water, dried (Na_2SO_4), and evaporated under vacuum. The residue was purified by preparative layer chromatography (silica gel, 0.5 cm thick) and CH_2Cl_2/AcOEt = 7/3 as eluent to afford 18 mg (47%) of **6**: wax; IR (ATR) 3347, 2925, 1630, 1570, 1439, 1253 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.60 (3H, s), 1.65 (1H, s), 1.68 (3H, s), 1.70 (3H, s), 2.01–2.08 (4H, m), 2.77 (2H, t, J = 6.6 Hz), 3.52 (2H, q, J = 6.6 Hz), 3.73 (2H, t, J = 6.0 Hz),), 5.07 (2H, m), 5.21 (1H, t, J = 6.9 Hz), 5.59 (1H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.68, 23.37, 25.71, 26.64, 32.06, 38.13, 38.89, 121.96, 123.82, 132.07, 139.19, 158.50. By comparison, no reaction occurred under the same conditions with the most potent ester **2f**.
21. Ruparel, N. B.; Patwardhan, A. M.; Akopian, A. N.; Hargreaves, K. M. *Pain* **2008**, *135*, 271.
22. Copeland, K. W.; Boezio, A. A.; Cheung, E.; Lee, J.; Olivieri, P.; Schenkel, L. B.; Wan, Q.; Wang, W.; Wells, M. C.; Youngblood, B.; Gavva, N. R.; Lehto, S. G.; Geuns-Meyer, S. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3464. and references therein.
23. Horne, D. B.; Tamayo, N. A.; Bartberger, M. D.; Bo, Y.; Clarine, J.; Davis, C. D.; Gore, V. K.; Kaller, M. R.; Lehto, S. G.; Ma, V. V.; Nishimura, N.; Nguyen, T. T.; Tang, P.; Wang, W.; Youngblood, B. D.; Zhang, M.; Gavva, N. R.; Monenschein, H.; Norman, M. H. *J. Med. Chem.* **2014**, *57*, 2989. and references therein.
24. Zhang, L.; Barritt, G. J. *Cancer Res.* **2004**, *64*, 8365.
25. (a) Rooney, L.; Vidal, A.; D'Souza, A.-M.; Devereux, N.; Masick, B.; Boissel, V.; West, R.; Head, V.; Stringer, R.; Lao, J.; Petrus, M. J.; Patapoutian, A.; Nash, M.; Stoakley, N.; Panesar, M.; Verkuyl, J. M.; Schumacher, A. M.; Petrassi, H. M.; Tully, D. C. *J. Med. Chem.* **2014**, *57*, 5129; (b) Laliberté, S.; Vallée, F.; Fournier, P.-A.; Bedard, L.; Labrecque, J.; Albert, J. S. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3204; (c) Hu, Y.-J.; St.-Onge, M.; Laliberté, S.; Vallée, F.; Jin, S.; Bedard, L.; Labrecque, J.; Albert, J. S. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3199; (d) Bassoli, A.; Borgonovo, G.; Morini, G.; De Petrocelllis, L.; Schiano Moriello, A.; Di Marzo, V. *Food Chem.* **2013**, *141*, 2044; (e) Vallin, K. S. A.; Sterky, K. J.; Nyman, E.; Bernström, J.; From, R.; Linde, C.; Minidis, A. B. E.; Nolting, A.; Närhi, K.; Santangelo, E. M.; Sehgelmeble, F. W.; Sohn, D.; Strindlund, J.; Weigelt, D. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5485; (f) Gijsen, H. J. M.; Berthelot, D.; De Cleyn, M. A. J.; Geuens, I.; Brône, B.; Mercken, M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 797; (g) Baraldi, P. G.; Romagnoli, R.; Saponaro, G.; Tabrizi, M. A.; Baraldi, S.; Pedretti, P.; Fusi, C.; Nassini, R.; Materazzi, S.; Geppetti, P.; Preti, D. *Bioorg. Med. Chem.* **2012**, *20*, 1690; (h) Ryckmans, T.; Aubdool, A. A.; Bodkin, J. V.; Cox, P.; Brain, S. D.; Dupont, T.; Fairman, E.; Hashizume, Y.; Ishii, N.; Kato, T.; Kitching, L.; Newman, J.; Omoto, K.; Rawson, D.; Strover, J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4857.