European Journal of Medicinal Chemistry 100 (2015) 129–138

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

TRPV1 modulators: Synthesis and in vitro evaluation of 1-heteroaryl piperidinecarboxamide and piperazinylurea derivatives

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ARTICLE INFO

Article history:
Received 4 February 2015
Received in revised form
21 April 2015
Accepted 26 May 2015
Available online 6 June 2015

Keywords:
TRPV1 receptor
Calcium channel assay
Piperidinecarboxamides
Piperazinylureas

ABSTRACT

A series of new 1-heteroaryl piperidinecarboxamide and piperazinylurea derivatives was synthesized and evaluated as TRPV1 modulators in a Ca^{2+} channel assay in HEK-293 cells overexpressing the human recombinant TRPV1 channel. Structural variations in the putative key portions of the molecules afforded several compounds endowed with agonist and/or antagonist/desensitizing activity at low micromolar concentration. As promising examples from this series, the piperidine-3-carboxamide derivative **31** exerts agonist/desensitizing activity at low micromolar concentration, while piperazinylurea derivatives **39** and **41** act as antagonists with sub-micromolar potency.

1. Introduction

The transient receptor potential vanilloid-1 (TRPV1) is a nonselective cation channel, with a preference for calcium, expressed predominantly on unmyelinated pain-sensing nerve fibers (C-fibers) and small Aδ fibers in the dorsal root, trigeminal, and nodose ganglia [1]. TRPV1, identified as the receptor for the vanilloid compound, capsaicin (Fig. 1, **A**), is activated by endogenous stimuli such as heat and low pH (<5.9) [2], and endogenous ligands including anandamide [3], and arachidonic acid metabolites [4].

The activation of the vanilloid receptor by agonists triggers cation influx resulting in excitation of primary sensory neurons, and ultimately the central perception of pain. The initial excitation is followed by a refractory state of desensitization, where the C-fiber sensory neurons become unresponsive to TRPV1 agonists and other inflammatory mediators [1,5]. This desensitization represents a basis for therapeutic use of vanilloid receptor agonists in the management of acute and chronic nociceptive pain, produced by osteoarthritis, rheumatoid arthritis [6], and diabetic peripheral neuropathy [7]. Moreover, desensitization of the afferent nerves using TRPV1 agonists such as capsaicin has been shown to give encouraging results in the treatment of bladder dysfunction associated with spinal cord injury and multiple sclerosis [8]. On the other hand, the identification of capsazepine (Fig. 1, **B**) [9], a competitive antagonist of capsaicin binding, provided a proof of principle for the discovery of novel analgesics based on the blocking of activation of TRPV1 by endogenous stimuli [10]. Given the therapeutic potential expected for both TRPV1 agonists [11], and antagonists [12] in the last years there has been a growing interest in developing TRPV1 ligands that could be used for prophylaxis and treatment of conditions and diseases including acute and chronic nociceptive pain, ischaemia, inflammatory disorders, urinary incontinence, and/or overactive bladder (for a recent review see Ref. [13]).

As a continuation of our efforts to identify anti-inflammatory/analgesic agents [14], we designed new compounds as TRPV1 modulators, based on the structural motif developed for several series of TRPV1 agonists and antagonists. In this context, Purdue Pharma, following an initial high throughput screening (HTS) approach, reported the design and synthesis of a series of 4-(2-pyridyl)piperazine-1-carboxamide analogs including N-(4-*tert*-butylphenyl)-4-(3-chloropyridin-2-yl)piperazine-1-carboxamide

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http://dx.doi.org/10.1016/j.ejmech.2015.05.041
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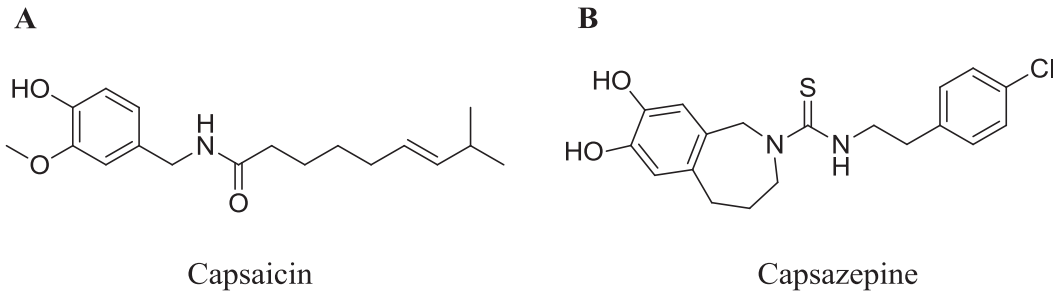


Fig. 1. Structure of prototypical TRPV1 ligands: Capsaicin (**A**, agonist), Capsazepine (**B**, antagonist).

(BCTC). BCTC showed potent TRPV1 antagonist activity in a HEK293 cell line against capsaicin and pH 5.5 activation in vitro, and effectively reversed the behavioral effects of inflammatory and neuropathic pain in rats [15]. In a similar manner, through internal high-throughput screening efforts, Swanson, et al. identified several series of agonists and antagonists, including a series of pyridinylpiperazine ureas [16]. In the present study, we planned to explore a series of 1-heteroaryl piperidinecarboxamides and piperazinylureas, and related structures bearing an aryl-, benzyl-, and phenethyl-amide moiety (Fig. 2).

In an effort to define the critical requirements for activity, and towards the understanding of molecular determinants of novel compounds as TRPV1 ligands, our approaches involved making structural modifications in the putative key pharmacophoric portions of the molecule. Our starting approach was focused to synthesize a series of new compounds bearing a 6-CF_3-3-CN-pyridine fragment in the **A** region, and to explore the structure–activity relationships (SARs) established around the heteroaryl group following structural changes. Then we evaluated the effect of some variation of the cycloaliphatic linker bearing the amide function (region **B**); and finally we studied the effect of the introduction of a differently polar substituent in the aromatic ring (region **C**). Herein we report the synthesis of a series of these ligands and preliminary results of a cell-based assay utilizing the Ca^{2+} permeability of the TRPV1 channel.

2. Results and discussion

2.1. Chemistry

The synthetic routes employed to obtain the target compounds are shown in Schemes 1–4. According to Scheme 1, a series of 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)piperidine-4-carboxamides **4**–**23** was synthesized. Heterocyclization of enaminonitrile **1** [17] with trifluoroacetylvinyl ether **2** in boiling MeCN, followed by alkaline cleavage afforded 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)piperidine-4-carboxylic acid (**3**). Coupling of the carboxylic acid **3** with an appropriately substituted aryl-, benzyl-, or phenethyl-amine mediated by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt) in DCM as the solvent gave the amides **4**–**23** in 46–92% yields.

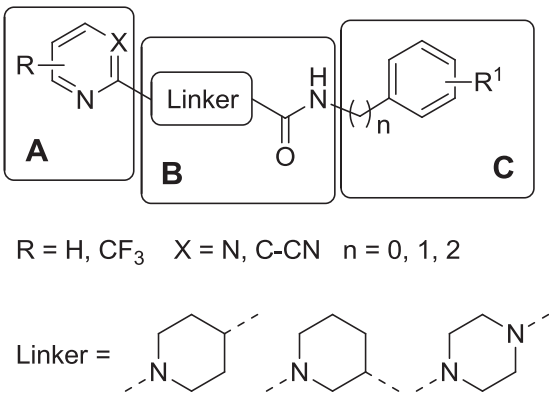


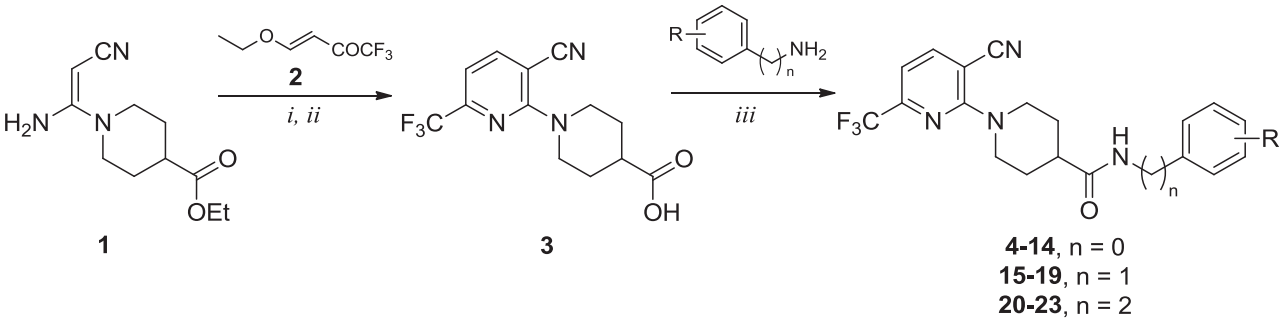
Fig. 2. Structural modifications of new modulators of the TRPV1 channel.

In a similar manner, amidation of acids 1-(3-cyanopyridin-2-yl) piperidine-4-carboxylic (**24**) [18], 1-(4-(trifluoromethyl)pyrimidin-2-yl)piperidine-4-carboxylic (**25**) [19], and 1-(4-(trifluoromethyl) pyrimidin-2-yl)piperidine-3-carboxylic (**26**) with 4-fluoroaniline and 4-hydroxyaniline led to analogs **27**–**32** in 52–89% yields (Scheme 2). Compound **26** has been prepared upon coupling 2-chloro-4-trifluoromethylpyrimidine (**45**) with ethyl piperidine-3-carboxylate (**46**) in DMF solution in presence of potassium carbonate, followed by alkaline hydrolysis (Scheme 2).

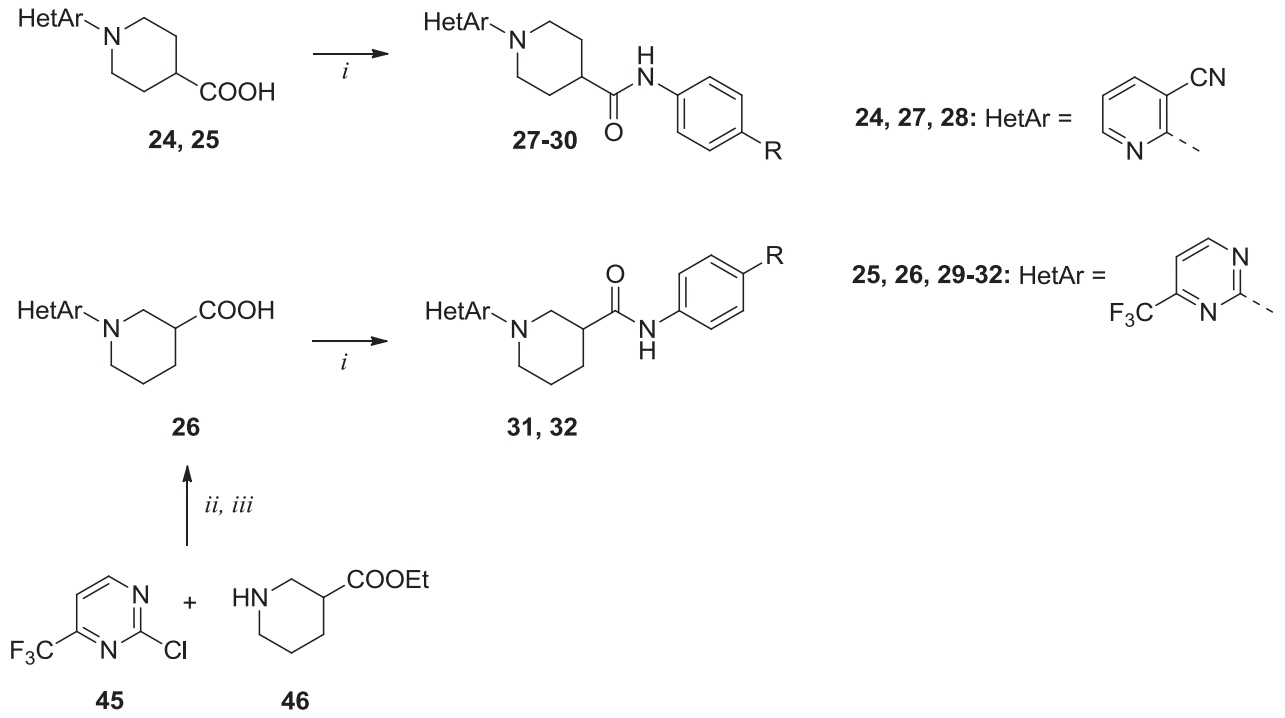
Piperazinylurea derivatives have been prepared as shown in Scheme 3. Coupling of 1-heteroarylpiperazine **33** [20] and **34** [21] with a variety of phenyl N-aryl-carbamates **35a-f** in DMSO solution provided piperazinylureas **36**–**44** in 64–93% yields. With some modification of reported procedures [22], phenyl N-aryl-carbamates **35a-f** were synthesized upon reacting commercially available substituted anilines with phenyl chloroformate in THF solution in presence of diisopropyl ethylamine (DIPEA) as a base (Scheme 4).

2.2. Biological activity

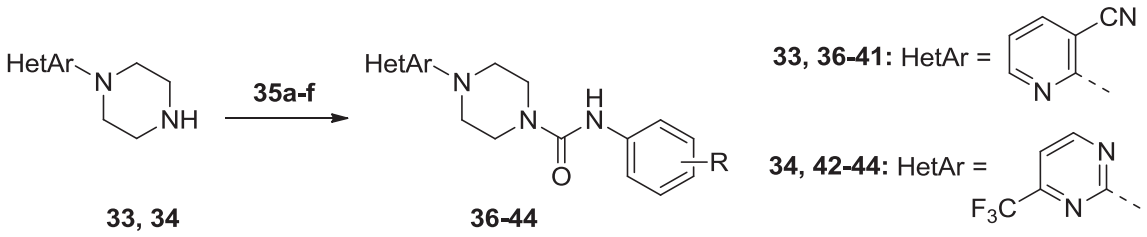
The activity of the new compounds on TRPV1 channel was determined by measuring the effect on intracellular Ca^{2+} ($[Ca^{2+}]_i$) elevation in HEK-293 cells overexpressing the human recombinant TRPV1 receptor (hTRPV1), with or without the subsequent (after 5 min) stimulation of cells with capsaicin (0.1 μM), as previously reported [23]. The efficacy of TRPV1 agonism was determined by normalizing the effect of the compounds to the maximum effect on $[Ca^{2+}]_i$ increase observed with application of 4 μM ionomycin, i.e. the efficacy was expressed as percent of the effect obtained with 4 μM ionomycin. Potency was instead expressed as the concentration of test substance exerting a half-maximal agonist effect (EC_{50}). Antagonist/desensitizing behavior was evaluated against 0.1 μM capsaicin. Data were expressed as the concentration exerting a half-maximal inhibition of capsaicin-induced $[Ca^{2+}]_i$ elevation (IC_{50}). The effect on intracellular Ca^{2+} exerted by capsaicin alone was taken as 100%. All determinations were performed at least in triplicate. The effects on functional activity on $[Ca^{2+}]_i$ elevation in HEK-293 cells mediated by the TRPV1 channel of the compounds herein studied are summarized in Table 1. Data for capsazepine (CPZ) [24] and BCTC [15a] as standard compounds are also included



Scheme 1. Reagents and conditions: (*i*) MeCN, reflux, 6 h; (*ii*) MeOH, 2 N aq NaOH, rt, 16 h, then HCl; (*iii*) EDC, HOBt, DCM, rt, overnight.



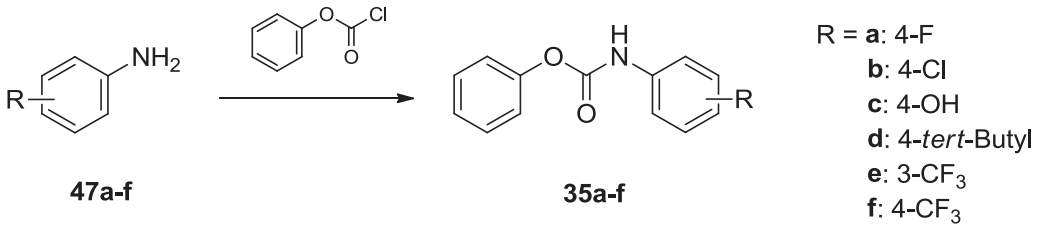
Scheme 2. Reagents and conditions: (*i*) 4-Fluoro- or 4-hydroxyaniline, EDC, HOBt, DCM, rt, overnight; (*ii*) DMF, K_2CO_3, 100 °C, overnight; (*iii*) MeOH, 2 N aq NaOH, rt, overnight, then HCl.



Scheme 3. Reagents and conditions: DMSO, rt, 18 h.

in the Table. This assay led to the identification of various hTRPV1 modulators that exhibit agonist and/or antagonist/desensitizing activity.

When exploring the SAR for the series of piperidine-4-carboxamides bearing a 3-cyano-6-(trifluoromethyl)pyridine moiety **4**–**23**, the 4-fluorophenylamide **4** exhibits agonist/desensitizing activity at low micromolar concentration. The substitution of the 4-fluorine atom of **4** with either a 4-chlorine (**5**), 4-*tert*-butyl (**8**), 3-CF_3 (**9**), or 4-CF_3 (**10**) group affords analogs showing antagonist behavior with moderate inhibitory activity. In contrast, the substitution with oxygen-bearing groups to produce compounds **7**, **11**–**14**, results in loss of TRPV1 ligand property. However, the phenol derivative **6** maintains a low activity as TRPV1 modulator. Introduction of a benzyl or phenethyl substituent on the amide function results in general loss of activity, probably due to a steric hindrance, even though compounds **15** and **19**, bearing a 4-fluoro-



Scheme 4. Reagents and conditions: THF, DIPEA, 0 °C to rt, 4 h.

and 4-trifluoromethylbenzylamide group respectively, retain micromolar agonist/desensitizing activity in the TRPV1 Ca^{2+} assay. Introduction of a 4-trifluoromethylpyrimidine group for the heteroaryl fragment (region **A**) of **4** leads to the analog **29** that exhibits mixed functional activity with mild efficacy in the calcium channel influx assay. However, the substitution of the piperidine-4-carboxamide linker (region **B**) of **29** with a piperidine-3-carboxamide fragment affords compound **31**, which acts as agonist/desensitizer at low micromolar concentration, with increased efficacy on $[Ca^{2+}]_i$ elevation when compared to both analogs **4** and **29**. In contrast, removal of the CF_3 on the heteroaryl fragment of **4** to give compound **27** results in loss of activity at the TRPV1 receptor. Among piperazinylureas, compounds **39** and **41**, bearing a 3-cyanopyridine group in the **A** region, exhibit antagonist activity with sub-micromolar IC_{50} inhibitory values comparable to that of the prototypical competitive antagonist capsazepine [24]. Noteworthy, compound **39**, bearing a 4-*tert*-butylphenyl substituent in the aromatic region, shows an IC_{50} inhibitory value of 0.16 μM, lower than that reported for the analog bearing a 4-*iso*-propylphenyl substituent in the aromatic region (IC_{50} = 0.287 μM) [15a]. On the other hand, the 4-trifluoromethylpyrimidine derivatives **42**, **43** retain mixed functional activity at micromolar concentrations, whereas **44** acts as agonist/desensitizing with residual functional efficacy at the channel.

Thus, in this series of compounds, the presence of a 4-CF_3-pyrimidine moiety in the **A** region leads to partial agonists with moderate efficacy at the TRPV1 channel; whereas, a 3-CN-pyridine moiety, preferably combined with a bulky lipophilic substituent such as a *tert*-butyl or CF_3 group in the *para* position of the phenyl ring (region **C**), seems to favor antagonist activity. Moreover, comparing compounds **8** and **10** with analogs **39** and **41**, respectively, removal of the CF_3 from heteroaryl fragment along with introduction of a piperazine for the piperidine linker in the **B** region leads to increased inhibitory effects (Table 1). Such effects seem to be correlated at least in part with enhanced hydrophilicity, as assessed by the calculated logP values [25]. In fact, with respect to compound **8** (logP = 5.10), the above described structural changes to give the analog **39** (logP = 3.79) result in about 100-fold increase in antagonistic effect; in a similar manner, passing from **10** (logP = 4.29) to the analog **41** (logP = 2.98) produces a ~10-fold enhancement of activity. Probably, differences in hydrophilicity combined with electronic and/or steric effects across this series of compounds affect significantly their ability to interact with the TRPV1 binding site, and influence both the kinetic and inhibitory activity of antagonists at the channel [1,12c,26].

3. Conclusions

A series of new 1-heteroaryl piperidinecarboxamide and piperazinylurea derivatives was synthesized and evaluated as TRPV1 modulators in a Ca^{2+} channel assay in HEK-293 cells over-expressing the human recombinant TRPV1 receptor. Structural variations in the putative key portions of the molecules allowed to identify a series of compounds endowed with agonist and/or antagonist/desensitizing activity with moderate to good potency. As promising examples from this series, the piperidine-3-carboxamide derivative **31** exerts agonist/desensitizing activity at low micromolar concentration, while piperazinylurea derivatives **39** and **41** act as antagonists with sub-micromolar potency. Across the series of piperazinylurea derivatives, the presence of a 4-CF_3-pyrimidine moiety in the **A** region results in partial agonists of the TRPV1 receptor; whereas, a 3-CN-pyridine moiety, preferably combined with a bulky lipophilic substituent such as a *tert*-butyl or CF_3 group in the *para* position of the phenyl ring (region **C**), seems to favor antagonistic activity. Prompted by herein reported preliminary results, further developments across the putative key portions of the molecules are now in progress, and the results will be presented in due course.

4. Experimental section

4.1. Chemistry

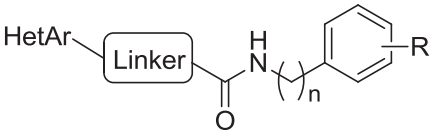
4.1.1. General methods

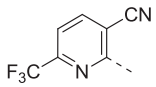
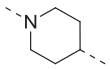
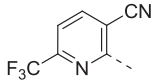
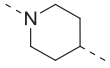
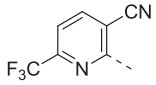
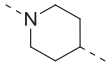
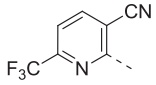
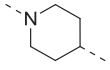
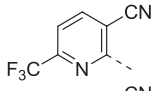
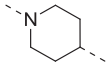
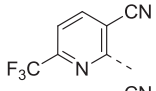
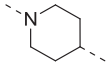
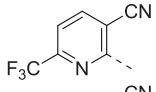
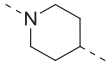
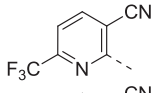
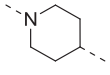
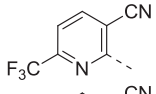
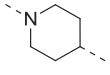
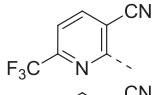
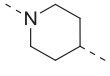
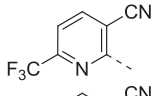
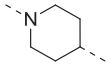
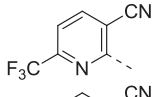
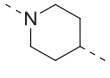
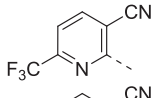
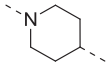
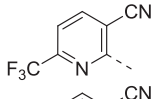
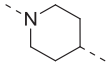
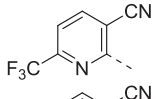
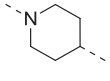
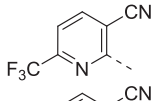
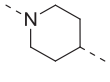
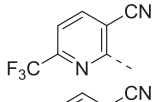
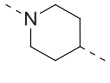
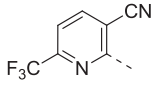
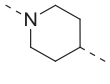
Unless otherwise noted, all solvents, including anhydrous solvents and chemicals, were purchased from Aldrich Co. and/or Alfa Aesar, and used without further purification. Melting points were recorded on a Stuart Scientific melting point SMP1 apparatus and are uncorrected. Positive-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing Finnigan MAT 95 instrument with BE geometry. Proton nuclear magnetic resonance spectra were recorded on a Varian Inova 500 spectrometer at 500 MHz, in DMSO-d_6 as the solvent, and TMS as the internal standard. Chemical shifts are expressed in ppm relative to tetramethylsilane. Splitting patterns are designated as singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). Infrared spectra were run on Bruker Vector 22 spectrophotometer. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F_{254} Merck plates). Developed plates were visualized by a Spectroline ENF 260C/F UV apparatus. Concentration and evaporation of the solvent after reaction or extraction were carried out on a rotary evaporator (Büchi Rotavapor) operating at reduced pressure. Elemental analyses were carried out with a Carlo Erba model 1106 elemental analyzer, and all values were within 0.4% of the calculated values, which indicates >95% purity of the tested compounds.

4.1.2. 1-(3-Cyano-6-(trifluoromethyl)pyridin-2-yl)piperidine-4-carboxylic acid (**3**)

A solution of 3-amino-3-(4-(ethoxycarbonyl)piperidin-1-yl) propenenitrile (**1**, 2.23 g, 10 mmol) and 4-ethoxy-1,1,1-trifluoro-3-buten-2-one (**2**, 1.7 g, 10 mmol) in MeCN (50 ml) was refluxed under stirring for 6 h. After evaporation of the solvent in vacuo, the residue was dissolved in methanol (100 ml), and 2 N NaOH (25 ml) was added. The mixture was stirred overnight at room temperature, and after evaporation of the solvent, the residue was dissolved

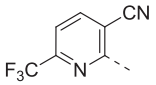
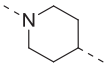
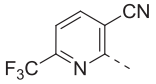
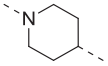
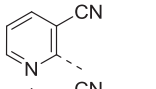
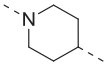
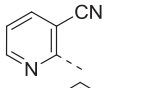
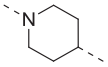
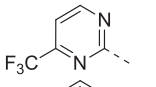
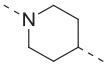
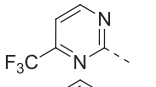
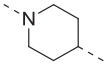
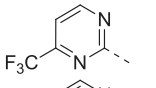
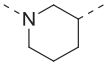
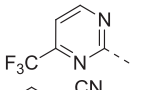
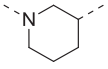
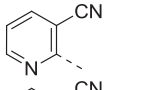
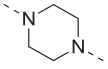
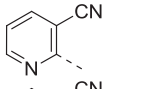
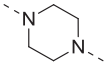
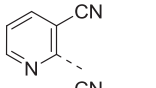
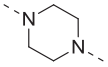
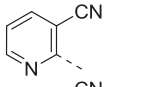
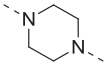
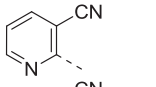
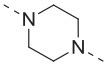
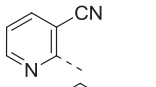
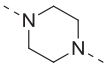
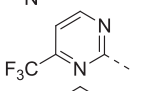
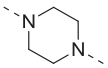
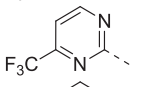
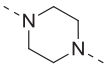
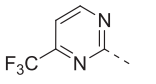
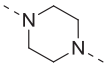
Table 1
Results of TRPV1 Ca^{2+} channel assays of piperidinecarboxamides **4**–**23**, **27**–**32**, and piperazinylureas **36**–**44**.[a]



Compd	HetAr	Linker	n	R	Antagonism/desensitization IC_{50} (μM) [b]	Efficacy at 10 μM [c]	Potency EC_{50} (μM)
4			0	4-F	1.6 ± 0.1	44.3 ± 2.2	1.1 ± 0.1
5			0	4-Cl	8.8 ± 0.7	<10	Nd [d]
6			0	4-OH	5.4 ± 0.1	48.0 ± 1.3	3.8 ± 0.5
7			0	4-OMe	>10	<10	Nd
8			0	4-*t*-Butyl	15.7 ± 1.8	<10	Nd
9			0	3-CF_3	6.9 ± 0.1	<10	Nd
10			0	4-CF_3	7.4 ± 0.3	<10	Nd
11			0	4-CH_2OH	>10	<10	Nd
12			0	4-$CH(CH_3)OH$	>10	<10	Nd
13			0	3,4-(OCH_2O)	>10	<10	Nd
14			0	3,4-($O(CH_2)_2O$)	>10	<10	Nd
15			1	4-F	7.0 ± 0.3	42.2 ± 2.9	4.4 ± 1.2
16			1	4-Cl	9.2 ± 0.1	26.6 ± 0.2	2.5 ± 0.1
17			1	4-OMe	>10	<10	Nd
18			1	4-*t*-Butyl	>10	<10	Nd
19			1	4-CF_3	8.1 ± 0.4	48.0 ± 0.7	5.5 ± 2.6
20			2	4-F	>10	18.0 ± 0.1	2.1 ± 0.1
21			2	4-Cl	>10	24.0 ± 0.5	5.1 ± 0.2

(*continued on next page*)

Table 1 (*continued*)

Compd	HetAr	Linker	n	R	Antagonism/desensitization IC_{50} (μM) [b]	Efficacy at 10 μM [c]	Potency EC_{50} (μM)
22			2	4-OH	>10	<10	Nd
23			2	4-OMe	>10	18.5 ± 0.1	2.8 ± 0.1
27			0	4-F	>10	<10	Nd
28			0	4-OH	>10	<10	Nd
29			0	4-F	7.5 ± 1.5	23.5 ± 1.8	2.1 ± 0.9
30			0	4-OH	>10	19.7 ± 2.1	3.3 ± 1.6
31			0	4-F	1.1 ± 0.05	61.8 ± 1.1	1.1 ± 0.1
32			0	4-OH	5.1 ± 0.3	42.7 ± 0.6	2.8 ± 0.2
36			0	4-F	>10	<10	Nd
37			0	4-Cl	10.2 ± 0.1	<10	Nd
38			0	4-OH	>10	<10	Nd
39			0	4-*t*-Butyl	0.16 ± 0.01	<10	Nd
40			0	3-CF_3	8.7 ± 0.2	<10	Nd
41			0	4-CF_3	0.72 ± 0.01	<10	Nd
42			0	4-F	2.3 ± 0.1	40.2 ± 1.0	2.5 ± 0.3
43			0	4-OH	4.2 ± 0.1	37.7 ± 0.3	2.0 ± 0.1
44			0	4-*t*-Butyl	0.88 ± 0.04	10.6 ± 0.1	5.0 ± 0.1
CPZ					0.105 ± 0.01		
BCTC					0.0349 ± 0.019		

[a] Data are means ± SEM of N = 3 determinations.
[b] Determined against the effect of 0.1 μM capsaicin.
[c] As percent of 4 μM ionomycin.
[d] Not determined, when efficacy is lower than 10.

in water. 2 N hydrochloric acid was added to pH 3, the solid formed was filtered off and dried in vacuo to yield 1-[3-cyano-6-(trifluoromethyl)pyridin-2-yl]piperidine-3-carboxylic acid (**3**) (2.18 g, 73% yield), which was used without further purification in the next step. ^{1}HNMR (DMSO-d_6) δ 1.61 (m, 2H), 1.94 (m, 2H), 2.57 (m, 1H), 3.18 (m, 2H), 4.21 (m, 2H), 7.24 (d, J = 7.8 Hz, 1H), 8.66 (d, J = 7.8 Hz, 1H), 11.94 (s, 1H). IR (Nujol) 2880, 2208, 1708, 1595 cm^{-1}. ESI-MS (*m/z*): 300 $(M + H)^+$.

4.1.3. 1-[4-(Trifluoromethyl)pyrimidin-2-yl]piperidine-3-carboxylic acid (**26**)

To a solution of 2-chloro-4-trifluoromethylpyrimidine (**45**, 1.83 g, 10 mmol) and ethyl piperidine-3-carboxylate (**46**, 1.90 g, 12 mmol) in DMF (100 ml) potassium carbonate (2.8 g, 20 mmol)

was added, and the mixture was stirred overnight at 100 °C. The reaction mixture was taken up with brine and extracted with AcOEt. The organic layer was washed with 2 N hydrochloric acid, saturated $NaHCO_3$, and evaporated in vacuo. The residue was dissolved in methanol (100 ml) and 2 N NaOH (25 ml) was added. The mixture was stirred overnight at room temperature, and after evaporation of the solvent, the residue was dissolved in water. 2 N hydrochloric acid was added to pH 3, the solid formed was filtered off and dried in vacuo to yield 1-[4-(trifluoromethyl)pyrimidin-2-yl]piperidine-3-carboxylic acid (**26**) (1.71 g, 62% yield), which was used in the next step without further purification. ^{1}HNMR (DMSO-d_6) δ 1.43 (m, 1H), 1.79 (m, 2H), 1.99 (m, 1H), 3.08 (m, 1H), 3.21 (m, 2H), 4.50 (m, 2H), 6.98 (d, J = 3.0 Hz, 1H), 8.66 (d, J = 3.0 Hz, 1H), 12.36 (s, 1H). IR (Nujol) 2856, 1713, 1597 cm^{-1}. ESI-MS (*m/z*): 276 $(M + H)^+$.

4.1.4. General procedure for the preparation of 1-heteroarylpiperidinecarboxamides ***4*–*23*, *27*–*32***

To a stirred solution of **3**, or **24**–**26** (0.50 mmol) in DCM (5 mL) were added HOBt (102 mg, 0.60 mmol) and EDC (116 mg, 0.60 mmol). The mixture was stirred for 30 min at room temperature. Then a substituted aryl, benzyl, or phenethylamine (0.60 mmol) was added, and the mixture was stirred 24 h at room temperature. After evaporation of the solvent, the residue was taken up with brine and extracted with AcOEt. The organic phase was washed with 0.5 N hydrochloric acid, saturated $NaHCO_3$, and brine, dried (Na_2SO_4), and evaporated under vacuum. The residue was crystallized with *i*-Pr_2O/MeOH to give piperidinecarboxamides **4**–**23**, **27**–**32** in 46–92% yields.

*4.1.4.1. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-fluorophenyl)piperidine-4-carboxamide (**4**).* Following the general procedure, the title compound was obtained from **3** and 4-fluoroaniline in 68% yield; mp 172–174 °C. ESI-MS (*m/z*): 393 $(M + H)^+$. ^{1}HNMR: δ 1.74 (m, 2H), 1.92 (m, 2H), 2.65 (m, 1H), 3.15 (m, 2H), 4.37 (m, 2H), 7.10 (m, 2H), 7.27 (d, J = 7.5 Hz, 1H), 7.60 (m, 2H), 8.32 (d, J = 7.5 Hz, 1H), 10.03 (s, 1H); IR (Nujol) 3272, 2220,1661, 1590 cm^{-1}. Anal. Calcd for $C_{19}H_{16}F_4N_4O$: C, 58.16; H, 4.11; N, 14.28. Found: C, 58.08; H, 4.06; N, 14.21.

*4.1.4.2. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-chlorophenyl)piperidine-4-carboxamide (**5**).* Following the general procedure, the title compound was obtained from **3** and 4-chloroaniline in 92% yield; mp 197–199 °C. ESI-MS (*m/z*): 410 $(M + H)^+$. ^{1}HNMR: δ 1.74 (m, 2H), 1.93 (m, 2H), 2.67 (m, 1H), 3.19 (m, 2H), 4.38 (m, 2H), 7.27 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 8.4 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H), 8.33 (d, J = 7.8 Hz, 1H), 10.04 (s, 1H); IR (Nujol) 3279, 2218,1660, 1592 cm^{-1}. Anal. Calcd for $C_{19}H_{16}ClF_3N_4O$: C, 55.82; H, 3.94; N, 13.71. Found: C, 55.73; H, 3.90; N, 13.68.

*4.1.4.3. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-hydroxyphenyl)piperidine-4-carboxamide (**6**).* Following the general procedure, the title compound was obtained from **3** and 4-hydroxyaniline in 50% yield; mp 218–220 °C. ESI-MS (*m/z*): 391 $(M + H)^+$. ^{1}HNMR: δ 1.72 (m, 2H), 1.88 (m, 2H), 2.58 (m, 1H), 3.14 (m, 2H), 4.37 (m, 2H), 6.66 (d, J = 8.0 Hz, 2H), 7.25 (d, J = 7.6 Hz, 1H), 7.34 (d, J = 8.0 Hz, 2H), 8.29 (d, J = 7.6 Hz, 1H), 9.24 (s, 1H), 9.69 (s, 1H); IR (Nujol) 3311, 2225, 1660, 1592 cm^{-1}. Anal. Calcd for $C_{19}H_{17}F_3N_4O_2$: C, 58.46; H, 4.39; N, 14.35. Found: C, 58.40; H, 4.35; N, 14.28.

*4.1.4.4. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-methoxyphenyl)piperidine-4-carboxamide (**7**).* Following the general procedure, the title compound was obtained from **3** and 4-methoxyaniline in 74% yield; mp 193–195 °C. ESI-MS (*m/z*): 405 $(M + H)^+$. ^{1}HNMR: δ 1.75 (m, 2H), 1.93 (m, 2H), 2.65 (m, 1H), 3.18 (m, 2H), 3.71 (s, 3H), 4.39 (m, 2H), 6.87 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 7.5 Hz, 1H), 7.51 (d, J = 8.4 Hz, 2H), 8.34 (d, J = 7.5 Hz, 1H), 9.80 (s, 1H); IR (Nujol) 3262, 2220, 1640, 1589 cm^{-1}. Anal. Calcd for $C_{20}H_{19}F_3N_4O_2$: C, 59.40; H, 4.74; N, 13.85. Found: C, 59.33; H, 4.70; N, 13.78.

*4.1.4.5. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-tert-butylphenyl)piperidine-4-carboxamide (**8**).* Following the general procedure, the title compound was obtained from **3** and 4-*tert*-butylaniline in 85% yield; mp 202–204 °C. ESI-MS (*m/z*): 431 $(M + H)^+$. ^{1}HNMR: δ 1.23 (s, 9H), 1.78 (m, 2H), 1.91 (m, 2H), 2.66 (m, 1H), 3.16 (m, 2H), 4.38 (m, 2H), 7.25 (m, 3H), 7.49 (d, J = 8.5 Hz, 2H), 8.31 (d, J = 7.5 Hz, 1H), 9.87 (s, 1H); IR (Nujol) 3349, 2220, 1652, 1590 cm^{-1}. Anal. Calcd for $C_{23}H_{25}F_3N_4O$: C, 64.17; H, 5.85; N, 13.02. Found: C, 64.09; H, 5.80; N, 12.90.

*4.1.4.6. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(3-trifluoromethtylphenyl)piperidine-4-carboxamide (**9**).* Following the general procedure, the title compound was obtained from **3** and 3-(trifluoromethyl)aniline in 76% yield; mp 178–180 °C. ESI-MS (*m/z*): 443 $(M + H)^+$. ^{1}HNMR: δ 1.74 (m, 2H), 1.96 (m, 2H), 2.70 (m, 1H), 3.16 (m, 2H), 4.38 (m, 2H), 7.26 (d, J = 7.8 Hz, 1H), 7.37 (m, 1H), 7.53 (m, 1H), 7.78 (m, 1H), 8.10 (s, 1H), 8.32 (d, J = 7.8 Hz, 1H), 10.30 (s, 1H); IR (Nujol) 3282, 2224, 1662, 1589 cm^{-1}. Anal. Calcd for $C_{20}H_{16}F_6N_4O$: C, 54.30; H, 3.65; N, 12.67. Found: C, 54.22; H, 3.60; N, 12.58.

*4.1.4.7. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-trifluoromethtylphenyl)piperidine-4-carboxamide (**10**).* Following the general procedure, the title compound was obtained from **3** and 4-(trifluoromethyl)aniline in 88% yield; mp 226–228 °C. ESI-MS (*m/z*): 443 $(M + H)^+$. ^{1}HNMR: δ 1.75 (m, 2H), 1.95 (m, 2H), 2.72 (m, 1H), 3.20 (m, 2H), 4.38 (m, 2H), 7.27 (d, J = 7.6 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.80 (d, J = 8.4 Hz, 2H), 8.32 (d, J = 7.6 Hz, 1H), 10.33 (s, 1H); IR (Nujol) 3281, 2221, 1664, 1592 cm^{-1}. Anal. Calcd for $C_{20}H_{16}F_6N_4O$: C, 54.30; H, 3.65; N, 12.67. Found: C, 54.24; H, 3.59; N, 12.60.

*4.1.4.8. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-(hydroxymethyl)phenyl)piperidine-4-carboxamide (**11**).* Following the general procedure, the title compound was obtained from **3** and 4-(hydroxymethyl)aniline in 54% yield; mp 155–157 °C. ESI-MS (*m/z*): 405 $(M + H)^+$. ^{1}HNMR: δ 1.74 (m, 2H), 1.93 (m, 2H), 2.67 (m, 1H), 3.14 (m, 2H), 4.40 (m, 4H), 5.06 (s, 1H), 7.22 (d, J = 6.0 Hz, 2H), 7.27 (d, J = 7.2 Hz, 1H), 7.54 (d, J = 6.0 Hz, 2H), 8.35 (d, J = 7.2 Hz, 1H), 9.90 (s, 1H); IR (Nujol) 3340, 3296, 2221, 1659, 1591 cm^{-1}. Anal. Calcd for $C_{20}H_{19}F_3N_4O_2$: C, 59.40; H, 4.74; N, 13.85. Found: C, 59.32; H, 4.71; N, 13.76.

*4.1.4.9. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-(1-hydroxyethyl)phenyl)piperidine-4-carboxamide (**12**).* Following the general procedure, the title compound was obtained from **3** and 4-(1-hydroxyethyl)aniline in 46% yield; mp 112–114 °C. ESI-MS (*m/z*): 419 $(M + H)^+$. ^{1}HNMR: δ 1.25 (d, J = 6.0 Hz, 3H), 1.69 (m, 2H), 1.90 (m, 2H), 2.65 (m, 1H), 3.15 (m, 2H), 4.37 (m, 2H), 4.64 (m, 1H), 5.12 (m, 1H), 7.24 (m, 3H), 7.50 (d, J = 8.0 Hz, 2H), 8.29 (d, J = 7.8 Hz, 1H), 9.96 (s, 1H); IR (Nujol) 3338, 3292, 2220, 1660, 1592 cm^{-1}. Anal. Calcd for $C_{20}H_{19}F_3N_4O_2$: C, 60.28; H, 5.06; N, 13.39. Found: C, 60.20; H, 5.00; N, 13.31.

*4.1.4.10. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(3,4-methylenedioxyphenyl)piperidine-4-carboxamide (**13**).* Following the general procedure, the title compound was obtained from **3** and 3,4-methylendioxyaniline in 74% yield; mp 196–198 °C.

ESI-MS (*m/z*): 419 $(M + H)^+$. ^{1}HNMR: δ 1.73 (m, 2H), 1.92 (m, 2H), 2.63 (m, 1H), 3.16 (m, 2H), 4.38 (m, 2H), 5.96 (s, 2H), 6.83 (d, $J = 7.8$ Hz, 1H), 6.97 (d, $J = 7.8$ Hz, 1H), 7.27 (d, $J = 7.2$ Hz, 1H), 7.30 (s, 1H), 8.33 (d, $J = 7.2$ Hz, 1H), 9.85 (s, 1H); IR (Nujol) 3261, 2222, 1648, 1592 cm^{-1}. Anal. Calcd for $C_{20}H_{17}F_3N_4O_3$: C, 57.42; H, 4.10; N, 13.39. Found: C, 57.35; H, 4.06; N, 13.31.

*4.1.4.11. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(3,4-ethylenedioxyphenyl)piperidine-4-carboxamide (**14**).* Following the general procedure, the title compound was obtained from **3** and 3,4-ethylendioxyaniline in 86% yield; mp 203–205 °C. ESI-MS (*m/z*): 433 $(M + H)^+$. ^{1}HNMR: δ 1.76 (m, 2H), 1.91 (m, 2H), 2.61 (m, 1H), 3.16 (m, 2H), 4.19 (m, 4H), 4.38 (m, 2H), 6.75 (d, $J = 8.5$ Hz, 1H), 6.98 (d, $J = 8.5$ Hz, 1H), 7.23 (s, 1H), 7.27 (d, $J = 7.5$ Hz, 1H), 8.33 (d, $J = 7.5$ Hz, 1H), 9.76 (s, 1H); IR (Nujol) 3264, 2221, 1645, 1594 cm^{-1}. Anal. Calcd for $C_{21}H_{19}F_3N_4O_3$: C, 58.33; H, 4.43; N, 12.96. Found: C, 58.30; H, 4.38; N, 12.88.

*4.1.4.12. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-fluorobenzyl)piperidine-4-carboxamide (**15**).* Following the general procedure, the title compound was obtained from **3** and 4-fluorobenzylamine in 56% yield; mp 170–172 °C. ESI-MS (*m/z*): 407 $(M + H)^+$. ^{1}HNMR: δ 1.68 (m, 2H), 1.85 (m, 2H), 2.54 (m, 1H), 3.12 (m, 2H), 4.24 (d, $J = 6.0$ Hz, 2H), 4.34 (m, 2H), 7.21 (m, 2H), 7.25 (m, 3H), 8.29 (d, $J = 7.6$ Hz, 1H), 8.39 (br s, 1H); IR (Nujol) 3290, 2221, 1638, 1589 cm^{-1}. Anal. Calcd for $C_{20}H_{18}F_4N_4O$: C, 59.11; H, 4.46; N, 13.79. Found: C, 59.05; H, 4.41; N, 13.70.

*4.1.4.13. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-chlorobenzyl)piperidine-4-carboxamide (**16**).* Following the general procedure, the title compound was obtained from **3** and 4-chlorobenzylamine in 90% yield; mp 179–181 °C. ESI-MS (*m/z*): 424 $(M + H)^+$. ^{1}HNMR: δ 1.67 (m, 2H), 1.85 (m, 2H), 2.52 (m, 1H), 3.12 (m, 2H), 4.24 (d, $J = 6.0$ Hz, 2H), 4.33 (m, 2H), 7.24 (m, 3H), 7.35 (d, $J = 8.0$ Hz, 2H), 8.30 (d, $J = 7.8$ Hz, 1H), 8.41 (m, 1H); IR (Nujol) 3279, 2219, 1643, 1587 cm^{-1}. Anal. Calcd for $C_{20}H_{18}ClF_3N_4O$: C, 56.81; H, 4.29; N, 13.25. Found: C, 56.79; H, 4.25; N, 13.20.

*4.1.4.14. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-methoxybenzyl)piperidine-4-carboxamide (**17**).* Following the general procedure, the title compound was obtained from **3** and 4-methoxybenzylamine in 84% yield; mp 171–173 °C. ESI-MS (*m/z*): 419 $(M + H)^+$. ^{1}HNMR: δ 1.69 (m, 2H), 1.85 (m, 2H), 2.54 (m, 1H), 3.13 (m, 2H), 3.73 (s, 3H), 4.20 (d, $J = 5.5$ Hz, 2H), 4.35 (m, 2H), 6.88 (d, $J = 8.5$ Hz, 2H), 7.16 (d, $J = 8.5$ Hz, 2H), 7.26 (d, $J = 7.5$ Hz, 1H), 8.34 (m, 2H); IR (Nujol) 3286, 2220, 1636, 1588 cm^{-1}. Anal. Calcd for $C_{21}H_{21}F_3N_4O_2$: C, 60.28; H, 5.06; N, 13.39. Found: C, 60.20; H, 4.98; N, 13.32.

*4.1.4.15. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-tert-butylbenzyl)piperidine-4-carboxamide (**18**).* Following the general procedure, the title compound was obtained from **3** and 4-*tert*-butylbenzylamine in 78% yield; mp 138–140 °C. ESI-MS (*m/z*): 445 $(M + H)^+$. ^{1}HNMR: δ 1.25 (s, 9H), 1.70 (m, 2H), 1.85 (m, 2H), 2.52 (m, 1H), 3.12 (m, 2H), 4.22 (d, $J = 5.5$ Hz, 2H), 4.34 (m, 2H), 7.15 (d, $J = 8.5$ Hz, 2H), 7.25 (d, $J = 7.6$ Hz, 1H), 7.32 (d, $J = 8.5$ Hz, 2H), 8.32 (m, 2H); IR (Nujol) 3266, 2220, 1637, 1588 cm^{-1}. Anal. Calcd for $C_{24}H_{27}F_3N_4O$: C, 64.85; H, 6.12; N, 12.60. Found: C, 64.78; H, 6.09; N, 12.52.

*4.1.4.16. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-(trifluoromethyl)benzyl)piperidine-4-carboxamide (**19**).* Following the general procedure, the title compound was obtained from **3** and 4-(trifluoromethyl)benzylamine in 62% yield; mp 189–191 °C. ESI-MS (*m/z*): 457 $(M + H)^+$. ^{1}HNMR: δ 1.67 (m, 2H), 1.86 (m, 2H), 2.54 (m, 1H), 3.21 (m, 2H), 4.34 (m, 4H), 7.22 (d, $J = 7.5$ Hz, 1H), 7.43 (d, $J = 8.0$ Hz, 2H), 7.64 (d, $J = 8.0$ Hz, 2H), 8.27 (d, $J = 7.5$ Hz, 1H), 8.50 (m, 1H); IR (Nujol) 3286, 2218, 1646, 1588 cm^{-1}. Anal. Calcd for $C_{21}H_{18}F_6N_4O$: C, 55.27; H, 3.98; N, 12.28. Found: C, 55.20; H, 3.94; N, 12.21.

*4.1.4.17. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-fluorophenethyl)piperidine-4-carboxamide (**20**).* Following the general procedure, the title compound was obtained from **3** and 4-fluorophenethylamine in 68% yield; mp 163–165 °C. ESI-MS (*m/z*): 421 $(M + H)^+$. ^{1}HNMR: δ 1.63 (m, 2H), 1.76 (m, 2H), 2.41 (m, 1H), 2.70 (m, 2H), 3.11 (m, 2H), 3.26 (m, 2H), 4.31 (m, 2H), 7.08 (m, 2H), 7.24 (m, 3H), 7.89 (br s, 1H), 8.32 (d, $J = 7.0$ Hz, 1H); IR (Nujol) 3304, 2224, 1641, 1589 cm^{-1}. Anal. Calcd for $C_{21}H_{20}F_4N_4O$: C, 60.00; H, 4.80; N, 13.33. Found: C, 60.02; H, 4.74; N, 13.26.

*4.1.4.18. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-chlorophenethyl)piperidine-4-carboxamide (**21**).* Following the general procedure, the title compound was obtained from **3** and 4-chlorophenethylamine in 70% yield; mp 171–173 °C. ESI-MS (*m/z*): 438 $(M + H)^+$. ^{1}HNMR: δ 1.60 (m, 2H), 1.75 (m, 2H), 2.40 (m, 1H), 2.69 (m, 2H), 3.09 (m, 2H), 3.25 (m, 2H), 4.30 (m, 2H), 7.20 (d, $J = 8.0$ Hz, 2H), 7.24 (d, $J = 7.0$ Hz, 1H), 7.30 (d, $J = 8.0$ Hz, 2H), 7.90 (m, 1H), 8.29 (d, $J = 7.0$ Hz, 1H); IR (Nujol) 3299, 2223, 1640, 1589 cm^{-1}. Anal. Calcd for $C_{21}H_{20}ClF_3N_4O$: C, 57.74; H, 4.61; N, 12.82. Found: C, 57.69; H, 4.59; N, 12.74.

*4.1.4.19. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-hydroxyphenethyl)piperidine-4-carboxamide (**22**).* Following the general procedure, the title compound was obtained from **3** and 4-hydroxyphenethylamine in 48% yield; mp 134–136 °C. ESI-MS (*m/z*): 419 $(M + H)^+$. ^{1}HNMR: δ 1.61 (m, 2H), 1.76 (m, 2H), 2.40 (m, 1H), 2.56 (m, 2H), 3.08 (m, 2H), 3.18 (m, 2H), 4.30 (m, 2H), 6.65 (d, $J = 8.0$ Hz, 2H), 6.96 (d, $J = 8.0$ Hz, 2H), 7.23 (d, $J = 7.5$ Hz, 1H), 7.86 (m, 1H), 8.28 (d, $J = 7.5$ Hz, 1H), 9.19 (s, 1H); IR (Nujol) 3321, 2221, 1641, 1588 cm^{-1}. Anal. Calcd for $C_{21}H_{21}F_3N_4O_2$: C, 60.28; H, 5.06; N, 13.39. Found: C, 60.20; H, 5.01; N, 13.30.

*4.1.4.20. 1-(3-cyano-6-(trifluoromethyl)pyridin-2-yl)-N-(4-methoxyphenethyl)piperidine-4-carboxamide (**23**).* Following the general procedure, the title compound was obtained from **3** and 4-methoxyphenethylamine in 65% yield; mp 164–166 °C. ESI-MS (*m/z*): 433 $(M + H)^+$. ^{1}HNMR: δ 1.64 (m, 2H), 1.77 (m, 2H), 2.40 (m, 1H), 2.64 (m, 2H), 3.11 (m, 2H), 3.23 (m, 2H), 3.71 (s, 3H), 4.31 (m, 2H), 6.84 (d, $J = 8.8$ Hz, 2H), 7.10 (d, $J = 8.8$ Hz, 2H), 7.25 (d, $J = 7.6$ Hz, 1H), 7.87 (m, 1H), 8.32 (d, $J = 7.6$ Hz, 1H); IR (Nujol) 3296, 2224, 1639, 1591 cm^{-1}. Anal. Calcd for $C_{22}H_{23}F_3N_4O_2$: C, 61.10; H, 5.36; N, 12.96. Found: C, 61.04; H, 5.30; N, 12.87.

*4.1.4.21. 1-(3-cyanopyridin-2-yl)-N-(4-fluorophenyl)piperidine-4-carboxamide (**27**).* Following the general procedure, the title compound was obtained from **24** and 4-fluoroaniline in 84% yield; mp 204–206 °C. ESI-MS (*m/z*): 325 $(M + H)^+$. ^{1}HNMR: δ 1.74 (m, 2H), 1.87 (m, 2H), 2.58 (m, 1H), 3.06 (m, 2H), 4.28 (m, 2H), 6.67 (d, $J = 8.8$ Hz, 2H), 6.90 (m, 1H), 7.37 (d, $J = 8.8$ Hz, 2H), 8.05 (m, 1H), 8.40 (m, 1H), 9.65 (s, 1H); IR (Nujol) 3305, 2223,1660, 1585 cm^{-1}. Anal. Calcd for $C_{18}H_{17}FN_4O$: C, 66.65; H, 5.28; N, 17.27. Found: C, 66.59; H, 5.22; N, 17.20.

*4.1.4.22. 1-(3-cyanopyridin-2-yl)-N-(4-hydroxyphenyl)piperidine-4-carboxamide (**28**).* Following the general procedure, the title compound was obtained from **24** and 4-hydroxyaniline in 64% yield; mp 225–227 °C. ESI-MS (*m/z*): 323 $(M + H)^+$. ^{1}HNMR: δ 1.74 (m, 2H), 1.90 (m, 2H), 2.61 (m, 1H), 3.09 (m, 2H), 4.28 (m, 2H), 6.90 (m, 1H), 7.11 (d, $J = 8.5$ Hz, 2H), 7.62 (d, $J = 8.5$ Hz, 2H), 8.04 (m, 1H), 8.40

(m, 1H), 9.99 (s, 1H), 10.52 (s, 1H); IR (Nujol) 3427, 3299, 2220,1652, 1585 cm^{-1}. Anal. Calcd for $C_{18}H_{18}N_4O_2$: C, 66.65; H, 5.28; N, 17.27. Found: C, 66.59; H, 5.22; N, 17.20.

*4.1.4.23. N-(4-fluorophenyl)-1-(4-(trifluoromethyl)pyrimidin-2-yl) piperidine-4-carboxamide (**29**).* Following the general procedure, the title compound was obtained from **25** and 4-fluoroaniline in 89% yield; mp 191–193 °C. ESI-MS (*m/z*): 369 $(M + H)^+$. ^{1}HNMR: δ 1.60 (m, 2H), 1.90 (m, 2H), 2.66 (m, 1H), 3.04 (m, 2H), 4.68 (m, 2H), 6.99 (d, $J = 5.0$ Hz, 1H), 7.12 (m, 2H), 7.61 (m, 2H), 8.68 (d, $J = 5.0$ Hz, 1H), 9.98 (s, 1H); IR (Nujol) 3277, 1652, 1595 cm^{-1}. Anal. Calcd for $C_{17}H_{16}F_4N_4O$: C, 55.43; H, 4.38; N, 15.21. Found: C, 55.36; H, 4.35; N, 15.15.

*4.1.4.24. N-(4-hydroxyphenyl)-1-(4-(trifluoromethyl)pyrimidin-2-yl) piperidine-4-carboxamide (**30**).* Following the general procedure, the title compound was obtained from **25** and 4-hydroxyaniline in 52% yield; mp 232–234 °C. ESI-MS (*m/z*): 367 $(M + H)^+$. ^{1}HNMR: δ 1.57 (m, 2H), 1.87 (m, 2H), 2.61 (m, 1H), 3.04 (m, 2H), 4.68 (m, 2H), 6.67 (d, $J = 8.4$ Hz, 2H), 6.99 (d, $J = 4.8$ Hz, 1H), 7.36 (d, $J = 8.4$ Hz, 2H), 8.68 (d, $J = 4.8$ Hz, 1H), 9.13 (s, 1H), 9.66 (s, 1H); IR (Nujol) 3296, 1653, 1595 cm^{-1}. Anal. Calcd for $C_{19}H_{17}F_3N_4O_2$: C, 55.74; H, 4.68; N, 15.29. Found: C, 55.67; H, 4.64; N, 15.20.

*4.1.4.25. N-(4-fluorophenyl)-1-(4-(trifluoromethyl)pyrimidin-2-yl) piperidine-3-carboxamide (**31**).* Following the general procedure, the title compound was obtained from **26** and 4-fluoroaniline in 60% yield; mp 202–204 °C. ESI-MS (*m/z*): 369 $(M + H)^+$. ^{1}HNMR: δ 1.47 (m, 1H),1.75 (m, 2H), 2.03 (m, 1H), 3.02 (m, 1H), 3.17 (m, 2H), 4.64 (m, 2H), 6.99 (d, $J = 4.8$ Hz, 1H), 7.13 (m, 2H), 7.61 (m, 2H), 8.68 (d, $J = 4.8$ Hz, 1H), 10.04 (s, 1H); IR (Nujol) 3287, 1658, 1593 cm^{-1}. Anal. Calcd for $C_{17}H_{16}F_4N_4O$: C, 55.43; H, 4.38; N, 15.21. Found: C, 55.35; H, 4.32; N, 15.17.

*4.1.4.26. N-(4-hydroxyphenyl)-1-(4-(trifluoromethyl)pyrimidin-2-yl) piperidine-3-carboxamide (**32**).* Following the general procedure, the title compound was obtained from **26** and 4-hydroxyaniline in 56% yield; mp 222–224 °C. ESI-MS (*m/z*): 367 $(M + H)^+$. ^{1}HNMR: δ 1.44 (m, 1H),1.78 (m, 2H), 1.99 (m, 1H), 2.99 (m, 1H), 3.13 (m, 2H), 4.64 (m, 2H),6.68 (d, $J = 8.0$ Hz, 2H), 6.98 (d, $J = 4.0$ Hz, 1H), 7.35 (d, $J = 8.0$ Hz, 2H), 8.67 (d, $J = 4.0$ Hz, 1H), 9.15 (s, 1H), 9.70 (s, 1H); IR (Nujol) 3297, 3208, 1644, 1597 cm^{-1}. Anal. Calcd for $C_{19}H_{17}F_3N_4O_2$: C, 55.74; H, 4.68; N, 15.29. Found: C, 55.69; H, 4.63; N, 15.22.

*4.1.5. General procedure for the preparation of phenyl N-arylcarbamates **35a–f***

To a stirred solution of a substituted aniline **47a–f** (1 mmol) and DIPEA (0.194 g, 1.5 mmol) in anhydrous THF (10 mL) at 0 °C phenyl chloroformate (0.189 g, 1.2 mmol) in THF (2.5 mL) was added dropwise. The mixture was stirred at room temperature for 4 h. After evaporation of the solvent, water (10 mL) was added; the formed solid was filtered off, washed with hexane and dried in vacuo to give **35a–f**, which were used without further purification in the next step.

*4.1.6. General procedure for the preparation of 4-heteroarylpiperazine-1-carboxamide derivatives **36–44***

A mixture of 1-substituted piperazine **33** or **34** (0.5 mmol) and phenyl arylcarbamate **35** (0.5 mmol) in anhydrous DMSO (5 mL) was stirred at room temperature for 18 h. The reaction mixture was then diluted with water (10 mL); the formed solid was collected with water, filtered off, air dried, and crystallized with MeOH to give **36–44** in 64–93% yields.

*4.1.6.1. 4-(3-cyanopyridin-2-yl)-N-(4-fluorophenyl)piperazine-1-carboxamide (**36**).* Following the general procedure, the title compound was obtained from **33** and **35a** in 72% yield; mp 189–191 °C. ESI-MS (*m/z*): 326 $(M + H)^+$. ^{1}HNMR: δ 3.61 (m, 4H), 3.68 (m, 4H), 6.95 (m, 1H), 7.08 (m, 2H), 7.48 (m, 2H), 8.09 (m, 1H), 8.44 (m, 1H), 8.61 (s, 1H); IR (Nujol) 3338, 2222, 1637, 1584 cm^{-1}. Anal. Calcd for $C_{17}H_{16}FN_5O$: C, 62.76; H, 4.96; N, 21.53. Found: C, 62.80; H, 4.90; N, 21.47.

*4.1.6.2. 4-(3-Cyanopyridin-2-yl)-N-(4-chlorophenyl)piperazine-1-carboxamide (**37**).* Following the general procedure, the title compound was obtained from **33** and **35b** in 81% yield; mp 178–180 °C. ESI-MS (*m/z*): 343 $(M + H)^+$. ^{1}HNMR: δ 3.62 (m, 4H), 3.67 (m, 4H), 6.95 (m, 1H), 7.28 (d, $J = 8.4$ Hz, 2H), 7.52 (d, $J = 8.4$ Hz, 2H), 8.10 (m, 1H), 8.44 (m, 1H), 8.70 (s, 1H); IR (Nujol) 3347, 2221, 1638, 1583 cm^{-1}. Anal. Calcd for $C_{17}H_{16}ClN_5O$: C, 59.74; H, 4.72; N, 20.49. Found: C, 59.69; H, 4.68; N, 20.42.

*4.1.6.3. 4-(3-cyanopyridin-2-yl)-N-(4-hydroxyphenyl)piperazine-1-carboxamide (**38**).* Following the general procedure, the title compound was obtained from **33** and **35c** in 66% yield; mp 203–205 °C. ESI-MS (*m/z*): 324 $(M + H)^+$. ^{1}HNMR: δ 3.58 (m, 4H), 3.65 (m, 4H), 6.65 (d, $J = 8.6$ Hz, 2H), 6.95 (m, 1H), 7.20 (d, $J = 8.6$ Hz, 2H), 8.09 (m, 1H), 8.30 (s, 1H), 8.44 (m, 1H), 9.00 (s, 1H); IR (Nujol) 3365, 3146, 2220, 1640, 1582 cm^{-1}. Anal. Calcd for $C_{17}H_{17}N_5O_2$: C, 63.15; H, 5.30; N, 21.66. Found: C, 63.08; H, 5.24; N, 21.60.

*4.1.6.4. 4-(3-cyanopyridin-2-yl)-N-(4-t-butylphenyl)piperazine-1-carboxamide (**39**).* Following the general procedure, the title compound was obtained from **33** and **35d** in 82% yield; mp 135–137 °C. ESI-MS (*m/z*): 364 $(M + H)^+$. ^{1}HNMR: δ 1.24 (s, 9H), 3.60 (m, 4H), 3.66 (m, 4H), 6.94 (m, 1H), 7.24 (d, $J = 8.4$ Hz, 2H), 7.36 (d, $J = 8.4$ Hz, 2H), 8.08 (m, 1H), 8.43 (m, 1H), 8.48 (s, 1H); IR (Nujol) 3357, 2214, 1633, 1584 cm^{-1}. Anal. Calcd for $C_{21}H_{25}N_5O$: C, 69.40; H, 6.93; N, 19.27. Found: C, 69.34; H, 6.88; N, 19.19.

*4.1.6.5. 4-(3-cyanopyridin-2-yl)-N-(3-(trifluoromethyl)phenyl)piperazine-1-carboxamide (**40**).* Following the general procedure, the title compound was obtained from **33** and **35e** in 70% yield; mp 116–118 °C. ESI-MS (*m/z*): 376 $(M + H)^+$. ^{1}HNMR: δ 3.66 (m, 8H), 6.95 (m, 1H), 7.27 (d, $J = 7.6$ Hz, 1H), 7.47 (m, 1H), 7.77 (m, 1H), 7.95 (s, 1H), 8.09 (d, $J = 7.6$ Hz, 1H), 8.43 (m, 1H), 8.92 (s, 1H); IR (Nujol) 3309, 2216, 1665, 1584 cm^{-1}. Anal. Calcd for $C_{20}H_{16}F_6N_4O$: C, 54.30; H, 3.65; N, 12.67. Found: C, 54.23; H, 3.58; N, 12.60.

*4.1.6.6. 4-(3-cyanopyridin-2-yl)-N-(4-(trifluoromethyl)phenyl)piperazine-1-carboxamide (**41**).* Following the general procedure, the title compound was obtained from **33** and **35f** in 93% yield; mp 174–176 °C. ESI-MS (*m/z*): 376 $(M + H)^+$. ^{1}HNMR: δ 3.66 (m, 8H), 6.96 (m, 1H), 7.59 (d, $J = 8.5$ Hz, 2H), 7.71 (d, $J = 8.5$ Hz, 2H), 8.10 (m, 1H), 8.44 (m, 1H), 8.97 (s, 1H); IR (Nujol) 3361, 2218, 1668, 1585 cm^{-1}. Anal. Calcd for $C_{18}H_{16}F_3N_5O$: C, 57.60; H, 4.30; N, 18.66. Found: C, 57.53; H, 4.28; N, 18.58.

*4.1.6.7. N-(4-fluorophenyl)-4-(4-(trifluoromethyl)pyrimidin-2-yl) piperazine-1-carboxamide (**42**).* Following the general procedure, the title compound was obtained from **34** and **35a** in 64% yield; mp 196–198 °C. ESI-MS (*m/z*): 370 $(M + H)^+$. ^{1}HNMR: δ 3.57 (m, 4H), 3.82 (m, 4H), 7.08 (m, 3H), 7.47 (m, 2H), 8.65 (s, 1H), 8.72 (d, $J = 4.5$ Hz, 1H); IR (Nujol) 3278, 1632, 1592 cm^{-1}. Anal. Calcd for $C_{16}H_{15}F_4N_5O$: C, 52.03; H, 4.09; N, 18.96. Found: C, 51.96; H, 4.06; N, 18.88.

*4.1.6.8. N-(4-hydroxyphenyl)-4-(4-(trifluoromethyl)pyrimidin-2-yl) piperazine-1-carboxamide (**43**).* Following the general procedure,

the title compound was obtained from **34** and **35c** in 83% yield; mp 218–220 °C. ESI-MS (*m/z*): 368 $(M + H)^+$. ^{1}HNMR: δ 3.54 (m, 4H), 3.81 (m, 4H), 6.65 (d, $J = 8.0$ Hz, 2H), 7.05 (d, $J = 5.0$ Hz, 1H), 7.20 (d, $J = 8.0$ Hz, 2H), 8.33 (s, 1H), 8.71 (d, $J = 5.0$ Hz, 1H), 9.00 (s, 1H); IR (Nujol) 3407, 3276, 1634, 1595 cm^{-1}. Anal. Calcd for $C_{16}H_{16}F_3N_5O_2$: C, 52.32; H, 4.39; N, 19.07. Found: C, 52.27; H, 4.34; N, 19.00.

*4.1.6.9. N-(4-t-butylphenyl)-4-(4-(trifluoromethyl)pyrimidin-2-yl) piperazine-1-carboxamide (**44**).* Following the general procedure, the title compound was obtained from **34** and **35d** in 69% yield; mp 163–165 °C. ESI-MS (*m/z*): 408 $(M + H)^+$. ^{1}HNMR: δ 1.26 (s, 9H), 3.56 (m, 4H), 3.82 (m, 4H), 7.06 (d, $J = 4.5$ Hz, 1H), 7.26 (d, $J = 8.5$ Hz, 2H), 7.37 (d, $J = 8.5$ Hz, 2H), 8.54 (s, 1H), 8.77 (d, $J = 4.5$ Hz, 1H); IR (Nujol) 3267, 1645, 1593 cm^{-1}. Anal. Calcd for $C_{20}H_{24}F_3N_5O_2$: C, 58.96; H, 5.94; N, 17.19. Found: C, 58.87; H, 5.89; N, 17.15.

Acknowledgments

This study was supported in part by the Italian Ministero dell'Istruzione, Università e della Ricerca (PRIN 2010–2011, Prot. no. 120105YY2HL_002), the Research Funds of the University of Cagliari (to C.C., V.O., G.B.), and Regione Autonoma Sardegna (project L.R. 7/2007, CRP-59473/2012, to C.C., V.O., G.B., V.D.M.).

References

[1] M.J. Caterina, D. Julius, Annu. Rev. Neurosci. 24 (2001) 487.
[2] M. Tominaga, M.J. Caterina, A.B. Malmberg, T.A. Rosen, H. Gilbert, K. Skinner, B.E. Raumann, A.I. Basbaum, D. Julius, Neuron 21 (1998) 531.
[3] (a) D. Smart, M.J. Gunthorpe, J.C. Jerman, S. Nasir, J. Gray, A.I. Muir, J.K. Chambers, A.D. Randall, J.B. Davis, Br. J. Pharmacol. 129 (2000) 227;
(b) L. De Petrocellis, T. Bisogno, M. Maccarrone, J.B. Davis, A. Finazzi-Agrò, V. Di Marzo, J. Biol. Chem. 276 (2001) 12856.
[4] (a) P.M. Zygmunt, J. Petersson, D.A. Andersson, H.-h. Chuang, M. Sørgård, V. Di Marzo, D. David Julius, E.D. Högestätt, Nature 400 (1999) 452;
(b) S.W. Hwang, H. Cho, J. Kwak, S.Y. Lee, C.-J. Kang, J. Jung, S. Cho, K.H. Min, Y.G. Suh, D. Kim, U. Oh, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 6155.
[5] P. Holzer, Pharmacol. Rev. 43 (1991) 143.
[6] M.S. Chong, J. Hester, Drugs 67 (2007) 569.
[7] P.G. Jensen, J.R. Larson, Drugs Aging 18 (2001) 737.
[8] (a) C.A. Maggi, Life Sci. 51 (1992) 1777;
(b) D. DeRidder, V. Chandiramani, P. Dasgupta, H. VanPoppel, L. Baert, C.J. Fowler, J. Urol. 158 (1997) 2087.
[9] (a) C.S.J. Walpole, S. Bevan, G. Bovermann, J.J. Boelsterli, R. Breckenridge, J.W. Davies, G.A. Hughes, I. James, L. Oberer, J. Med. Chem. 37 (1994) 1942;
(b) K.M. Walker, L. Urban, S.J. Medhurst, S. Patel, M. Panesar, A.J. Fox, P. McIntyre, J. Pharmacol. Exp. Ther. 304 (2003) 56.
[10] A. Szallasi, D.N. Cortright, C.A. Blum, S.R. Eid, Nat. Rev. Drug Discov. 6 (2007) 357.
[11] (a) K.R. Bley, Expert. Opin. Investig. Drugs 13 (2004) 1445;
(b) H. Knotkova, M. Pappagallo, A. Szallasi, Clin. J. Pain 24 (2008) 142;
(c) S.J. Conway, Chem. Soc. Rev. 37 (2008) 1530.
[12] (a) M. Pal, S. Angaru, A. Kodimuthali, N. Dhingra, Curr. Pharm. Des. 15 (2009) 1008;
(b) L.M. Broad, S.J. Keding, M.J. Blanco, Curr. Top. Med. Chem. 8 (2008) 1431;
(c) P.R. Kym, M.E. Kort, C.W. Hutchins, Biochem. Pharmacol. 78 (2009) 211;
(d) A. Szallasi, P.M. Blumberg, Pharmacol. Rev. 51 (1999) 159;
(e) A. Szallasi, G. Appendino, J. Med. Chem. 47 (2004) 2717;
(f) R.L. McLeod, C.C. Correll, Y. Jia, J.C. Anthes, Lung 186 (2008) S59;
(g) M.J. Gunthorpe, B.A. Chizh, Drug Discov. Today 14 (2009) 56;
(h) G.Y. Wong, N.R. Gavva, Brain Res. Rev. 60 (2009) 267.
[13] L. De Petrocellis, A. Schiano-Moriello, Recent Pat. CNS Drug Discov. 8 (2013) 180.
[14] (a) V. Onnis, C. Congiu, E. Björklund, F. Hempel, E. Söderström, C.J. Fowler, J. Med. Chem. 53 (2010) 2286;
(b) C.J. Fowler, E. Björklund, A.H. Lichtman, P.S. Naidu, C. Congiu, V. Onnis, J. Enzym. Inhib. Med. Chem. 28 (2013) 172;
(c) M. Cipriano, E. Björklund, A.A. Wilson, C. Congiu, V. Onnis, C.J. Fowler, Eur. J. Pharmacol. 720 (2013) 383.
[15] (a) Q. Sun, L. Tafesse, K. Islam, X. Zhou, S. Victory, C. Zhang, M. Hachicha, L. Schmid, A. Patel, Y. Rotshteyn, K.J. Valenzano, D.J. Kyle, Bioorg. Med. Chem. Lett. 13 (2003) 3611;
(b) K.J.Valenzano, E. R.Grant, G.Wu, M.Hachicha, L.Schmid, L.Tafesse, Q.Sun, Y.Rotshteyn, J.Francis, J.Limberis, S.Malik, E.R.Whittemore, D.J.Hodges, Pharmacol. Exp. Ther.. 306 (2003) 377. (c) J.D. Pomonis, J.E. Harrison, L. Mark, D.R. Bristol, K.J. Valenzano, K.J. Walker, Pharmacol. Exp. Ther. 306 (2003) 387.
[16] D.M. Swanson, A.E. Dubin, C. Shah, N. Nasser, L. Chang, S.L. Dax, M. Jetter, J.G. Breitenbucher, C. Liu, C. Mazur, B. Lord, L. Gonzales, K. Hoey, M. Rizzolio, M. Bogenstaetter, E.E. Codd, D.H. Lee, S.-P. Zhang, S.R. Chaplan, N.I. Carruthers, J. Med. Chem. 48 (2005) 1857.
[17] M.T. Cocco, C. Congiu, V. Onnis, A. Maccioni, Synthesis 7 (1991) 529.
[18] T. Nakagawa, T. Suzuki, K. Takenaka, S. Fujita, Y. Yamada, Y. Shima, T. Okuzumi, T. Yoshimura, M. Yoshida, M. Murata, Patent US2009/0258900 A1, 2009.
[19] C.M.S. Heeney, Patent WO2010/097576 A1, 2010.
[20] K. Ishizumi, A. Kojima, F. Antoku, Chem. Pharm. Bull. 39 (1991) 2288.
[21] C.-B. Xue, C. Zheng, H. Feng, M. Xia, J. Glenn, G. Cao, B.W. Metcalf, Patent WO2006/004741 A2, 2006.
[22] (a) C. Sheng, X. Che, W. Wang, S. Wang, Y. Cao, Z. Miao, J. Yao, W. Zhang, Eur. J. Med. Chem. 46 (2011) 5276;
(b) C.H. Mack, H.H. McGregor Jr., S.R. Hobart, J. Chem. Eng. Data 14 (1969) 258;
(c) P.F. Cirillo, S. Breitfelder, U. R. Patel, J. Proudfoot, A. Swinamer, Patent US2003/0225077 A1, 2003.
(d) L.A. Gharat, L. Narayana, N. Khairatkar-Joshi, V.G. Kattige, Patent WO2009/090548 A2, 2009.
[23] (a) L. De Petrocellis, T. Bisogno, J.B. Davis, R.G. Pertweed, V. Di Marzo, FEBS Lett. 483 (2000) 52;
(b) G. Ortar, M.G. Cascio, L. De Petrocellis, E. Morera, F. Rossi, A. Schiano Moriello, M. Nalli, V. de Novellis, D.F. Woodward, S. Maione, V. Di Marzo, J. Med. Chem. 50 (2007) 6554.
[24] S. Maione, L. De Petrocellis, V. de Novellis, A. Schiano Moriello, S. Petrosino, E. Palazzo, F.S. Rossi, D.F. Woodward, V. Di Marzo, Br. J. Pharmacol. 150 (2007) 766.
[25] http://www.molinspiration.com/cgi-bin/properties.
[26] (a) Y.G. Suh, U. Oh, Curr. Pharm. Des. 11 (2005) 474. J. Lazar, D.C. Braun, A. Tóth, Y. Wang, L.V. Pearce, V.A. Pavlyukovets, P.M. Blumberg, S.H. Garfield, S. Wincovitch, H.-K. Choi, J. Lee, Mol. Pharmacol. 69 (2006) 1166;
(b) J. Vriens, G. Appendino, B. Nilius, Mol. Pharmacol. 75 (2009) 1262;
(c) P. Ambrosino, M.V. Soldovieri, C. Russo, M. Taglialatela, Br. J. Pharmacol. 168 (2013) 1430;
(d) L. De Petrocellis, A. Schiano Moriello, G. Fontana, A. Sacchetti, D. Passarella, G. Appendino, V. Di Marzo, Br. J. Pharmacol. 171 (2014) 2608.