Bioorganic & Medicinal Chemistry Letters 21 (2011) 892–898

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Structure–activity studies of a novel series of isoxazole-3-carboxamide derivatives as TRPV1 antagonists

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ARTICLE INFO

Article history:
Received 29 October 2010
Revised 16 December 2010
Accepted 18 December 2010
Available online 23 December 2010

Keywords:
TRPV1 antagonist
Antihyperalgesia
Isoxazoles
Hyperthermia

ABSTRACT

Optimisation of a screening hit incorporating both TRPV1 activity and solubility was conducted. Substitution of the isoxazole-3-carboxamide with the bespoke 1*S*, 3*R*-3-aminocyclohexanol motif afforded the requisite balance of potency and solubility. Compounds **32** and **40** were found to have antihyperalgesic effects in the rat CFA Hg assay and induce a mechanism based hyperthermia.

Transient receptor potential vanilloid 1 (TRPV1) is a Ca^{2+} permeant non-selective cation channel expressed in a subpopulation of primary afferent neurons.[1] TRPV1 is located both in the periphery and CNS on C and Aδ fibres, the afferents commonly associated with nociception. In addition to mediating the effects of exogenous capsaicin (the pungent component of chilli peppers) primary afferent TRPV1 receptors are thought to trigger the actions of heat (>43 °C), and protons (pH <6.8) and are modulated by a variety of endogenous lipid mediators including anandamide and bradykinin.[2] Consequently, TRPV1 is believed to act as an integrator of nociceptive responses to both chemical and thermal noxious stimuli.[3]

Since the receptor was cloned and characterised by Julius and co-workers in 1997[1] there has been a considerable amount of research interest into this ion channel. TRPV1 knockout mice provided evidence that TRPV1 played a key role in pain, with a clear attenuation to thermal hyperalgesia in response to proinflammatory agents.[4] TRPV1 also shows increased expression in pain states in both rat and human.[5] A number of small molecule ligands have also been published that show a clear reversal of both thermal and mechanical hyperalgesia in preclinical pain models.[6] This data clearly supports the role of TRPV1 antagonists in the management of acute and chronic pain.[7]

A staggering recent investment in R&D has led to a plethora of small molecule antagonists in the area.[8] Among the earliest TRPV1 antagonists to appear in the literature were the aryl ureas, for example, BCTC[9] (**1**) (Fig. 1). These served only as tools to explore pre-clinical pharmacology because of the poor aqueous solubility, poor oral bioavailability and metabolic instability. Other researchers have reported efforts to improve upon the poor solubility and metabolic instability of BCTC replacing the central piperazine ring with a phenyl ring, maintaining TRPV1 antagonism but failing to improve solubility.[10]

Over the past few years a number of small molecule TRPV1 antagonists such as **2**, **3** and **4** (Fig. 1) have entered clinical trials.[11] However, the sensitivity of TRPV1 to heat has suggested a role in maintenance of body temperature, and clinical trials of at least one TRPV1 antagonist have been stopped because of unacceptable levels of hyperthermia.[8d]

These compounds have in common a flat, relatively linear shape, with moderate to high lipophilicity and consequently low solubility.

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doi:10.1016/j.bmcl.2010.12.092

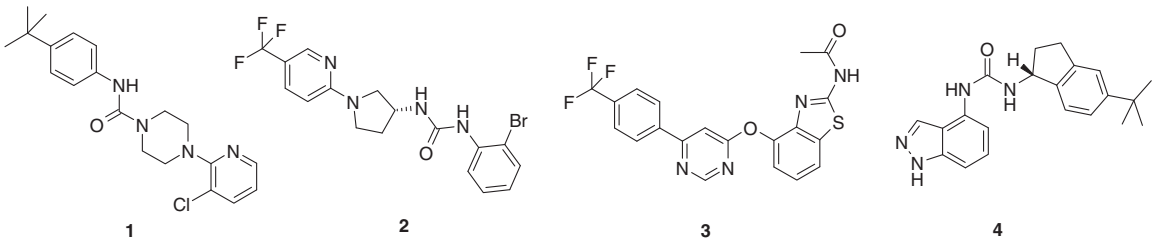


Figure 1.

After an HTS campaign we identified isoxazole-3-carboxamide **5** (Fig. 2), with moderate potency at TRPV1 (Table 1). In this report we describe the synthesis and biological evaluation of a series of isoxazole-3-carboxamides that modulate TRPV1. Initial optimisation goals for this series were improvements in potency and solubility sufficient to demonstrate in vivo efficacy and to provide confidence for full LO resourcing. This study culminated in the identification of potent new TRPV1 antagonists with good oral bioavailability and efficacy demonstrated in models of inflammatory pain suitable for further optimisation.

The general synthesis towards isoxazole-3-carboxamide derivatives is outlined in Scheme 1. The strategy involved the reaction of various substituted acetophenones (**6a**–**f**) with diethyl oxalate in the presence of sodium ethoxide. The resulting 2,4-diketo esters (**7a**–**f**) on treatment with hydroxylamine hydrochloride furnished substituted 3-isoxazole esters (**8a**–**f**) in excellent yields. The 4-position was further elaborated by bromination in the presence of NBS in acetic acid at elevated temperatures to yield the tri-substituted isoxazoles (**9a**–**f**). The esters were then heated to high temperature in the presence of cyclopentylamine and base to deliver amides (**10a**–**f**).

In order to exploit the 4-position the strategy outlined in Scheme 2 was adopted. The previously prepared 3-isoxazole ester (**8f**) was exposed to either NCS to deliver the chloro substituted derivative (**11fa**) or selectfluor to install the fluoro moiety (**11fb**). The latter method often required elevated temperatures and delivered moderate yields. The halogenated derivatives were then saponified to reveal the acids (**12fa** and **12fb**) and amides formed via the acid chlorides (**13fa** and **13fb**) or directly using standard coupling conditions to give **14**–**40**.

The preparation of the bespoke amine **41** is outlined in Scheme 3 following modified methods of Sammes and Thetford.[12] Reaction of potassium phthalimide with 3-bromocyclohexene (**42**) underwent the Gabriel reaction to give compound **43**. Treatment of **43** with NBS in ethanolic chloroform gave only the 1,3-adduct because the phthalimide group prefers to react by a six-membered transition state.[12] Release of the alcohol was realised by treating with dilute acid. This was readily reduced to furnish the desbromo compound **44** as a racemic *cis* mixture. Resolution to the enantiomerically pure amino alcohol **41** was achieved using the lipase-catalysed methods of Gotor and co-workers.[13] Treating the N-protected derivative **44** with lipase B from *Candida antarctica* (CAL-B) in THF for 3 h in the presence of vinyl acetate gave **45** and **46** which was easily separated using column chromatography. Hydrazinolysis of the N-protected aminocyclohexanol **45** produced the dihydrophthalazine-1,4-dione side-product from which the chemically pure 3-aminocyclohexanol **41** was not easily isolated. The crude was treated with benzyl chloroformate in the presence of base to give the N-protected carbamate which allowed for easier purification on silica gel. Hydrogenolysis of the protecting group then afforded the desired enantiomerically pure aminocyclohexanol **41** in >99% ee. The absolute configuration was tentatively assigned as 1*S*, 3*R* for **41** based on the literature data.[12]

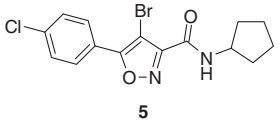
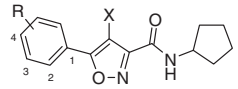
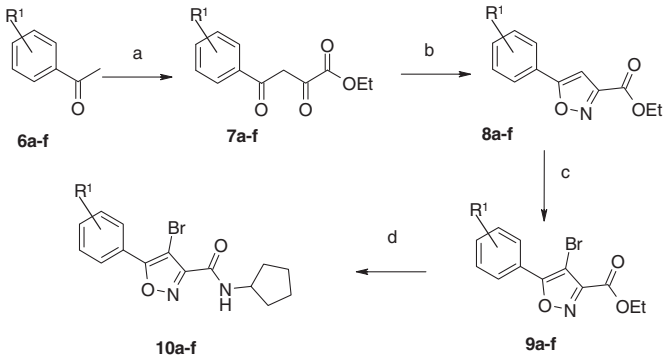


Figure 2.

Table 1
SAR of the 4- and 5-position of the central isoxazole ring



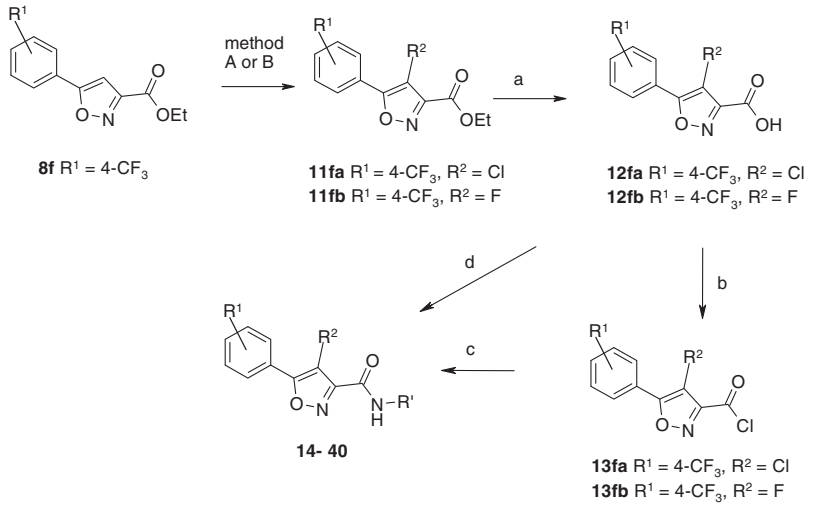
No.	R	X	TRPV1 IC_{50} (nM)
10a	2-Cl	Br	Inactive
10b	3-Cl	Br	4222
5	4-Cl	Br	370
10c	4-F	Br	377
10d	4-Br	Br	410
10e	4-OCF_3	Br	1252
10f	4-CF_3	Br	46
14	4-CF_3	H	201
15	4-CF_3	F	112
16	4-CF_3	Cl	15



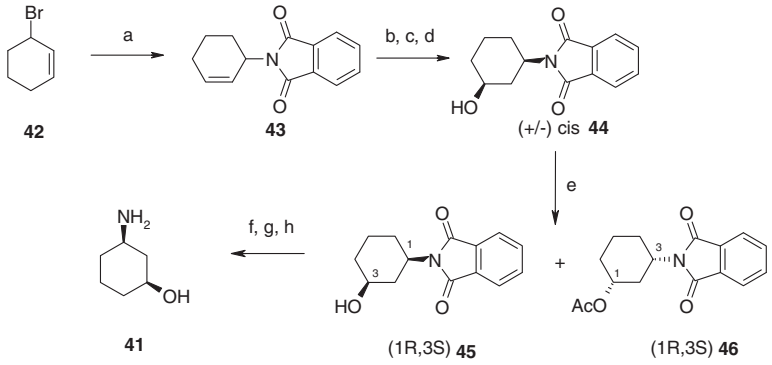
a R^1 = 2-Cl, b R^1 = 3-Cl, c R^1 = 4-F, d R^1 = 4-Br, e R^1 = 3-OCF_3, f R^1 = 4-CF_3

Scheme 1. Reagents and conditions: (a) diethyl oxalate, 21% wt sodium ethoxide in EtOH, THF; (b) hydroxylamine hydrochloride, EtOH, 85 °C; (c) *N*-bromosuccinimide, AcOH, 100 °C; (d) amine, *N*,*N*-diisopropylethylamine, DCM.

Compounds were tested in a TRPV1 assay.[14] Initial investigations centred on scanning at the 5-position of the central isoxazole core (Table 1). 2- or 3-chloro substituted aryl groups (**10a** and **10b**) leading to inactive or weakly active compounds respectively. In extreme cases agonism could be obtained by substituting in the 2-position (data not shown). Replacing the 4-chloro substituent with either 4-fluoro (**10c**) or 4-bromo (**10d**) led to equally potent



Scheme 2. Reagents and conditions: Method A: *N*-chlorosuccinimide, AcOH, 100 °C; Method B: selectfluor, tetramethylene sulfone, 120 °C: (a) Lithium hydroxide, H_2O, THF; (b) oxalyl chloride, DCM, cat. DMF; (c) amine, *N*,*N*-diisopropylethylamine, DCM; (d) amine, 1-hydroxybenzotriazole, *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide, triethylamine, THF.



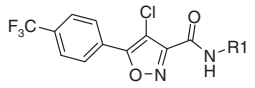
Scheme 3. Reagents and conditions: (a) potassium phthalimide, DMF, 100 °C; (b) *N*-bromosuccinimide, EtOH, $CHCl_3$; (c) 2 N HCl, MeOH; (d) tributyltin hydride, AIBN, MeOH; (e) vinyl acetate, *C. antarctica*; (f) hydrazine hydrate, MeOH; (g) benzyl chloroformate, Na_2CO_3, H_2O; (h) 10% palladium on carbon, H_2 (1 atm), MeOH.

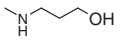
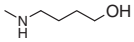
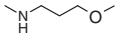
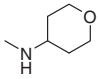
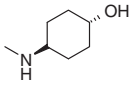
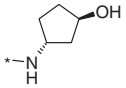
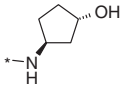
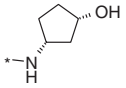
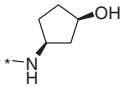
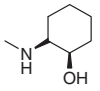
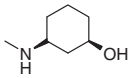
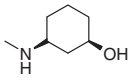
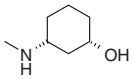
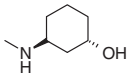
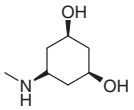
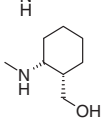
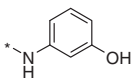
compounds, whilst the 4-trifluoromethoxy group (**10e**) appeared detrimental to activity when compared to the progenitor compound (**5**). The 4-trifluoromethyl (**10f**) group led to a dramatic improvement in activity and was the optimal group for potency in this region. Exploration of the 4-position of the isoxazole core showed halogens to be optimal. The unsubstituted compound (**14**) showed a fourfold loss in potency whilst the fluoro (**15**) and chloro (**16**) substituents in this position showed a twofold and >10-fold increase in potency respectively. Therefore it appears that 4-trifluoromethyl on the aromatic and chloro on the 4-position of the isoxazole are the optimal groups when the cyclopentylamine is in place.

A variety of 3-carboxamide substituents were investigated (Table 2) whilst retaining the 4-trifluoromethyl on the aromatic and the chloro on the 4-position of the isoxazole. The majority of the functionality probed in this region reflected the fact that the physicochemical properties required to increase the solubility. Aliphatics show a degree of activity (**21** and **22**) however the solubility was generally poor as expected. Pendant groups containing a strongly basic nitrogen or acidic group were poorly tolerated (data not shown). Interestingly the tertiary amide (**20**) switched the chemotype from antagonism to agonism. This was also true for a number of other examples (data not shown) suggesting the importance of the NH functionality for antagonistic effects. This has also been shown by a number of other groups in the area.[15]

Aminoalcohols were generally more potent than the aliphatic counterparts with small cyclic systems more potent than the acyclic progenitors. Interestingly the free hydroxyl of **17** appeared almost fourfold more potent than when the hydroxyl was masked as a methoxy group (**19**). The scope of the contribution of the aminocycloalkanols to potency and solubility was examined further through incorporation of a series of commercially available 3-aminocyclopentanols. It was discovered that potency at TRPV1 was strongly influenced by the stereochemistry of the amino and hydroxyl substituents on the cyclopentyl core. The 1*S*, 3*R*-3-aminocyclopentanol (**27**) was established with the greatest potency, twofold more potent that the corresponding *cis* enantiomer (**28**), and at least 10-fold more potent than the *trans* variants (**25** and **26**). However it was equipotent with the cyclopentylamine progenitor (**16**) although the hydroxyl moiety was found to impart solubility in all of the aminocyclopentanols. Based on these encouraging findings a series of compounds based on cyclohexyl containing pendant groups was investigated. At the time only mixtures of isomers were available commercially and served as the de facto scanning mode for the aminocyclohexanols. The mixture of four compounds was found to be 20-fold more potent than the progenitor cyclohexylamine (**22**). The racemates of the *cis* (**30**) and *trans* (**33**) isomers of the 3-aminocyclohexanol showed that the *cis* isomer was preferred being 20-fold more potent. This was also true for the 2-aminocyclohexanol (**29**) although this was ninefold less

Table 2
SAR of the 3-carboxamide position of the central isoxazole ring



No.	R1	TRPV1 IC_{50} (nM)	Stereochemistry	Solkin[16] (mg/L)
17		595	—	7
18		238	—	5
19		2212	—	NT
20		263[a]	—	5
21		87	—	1
22		92	—	1
23		43	—	7
24		392	Trans	NT
25		197	1*R*, 3*R*	4
26		762	1*S*, 3*S*	NT
27		23	1*S*, 3*R*	5
28		55	1*R*, 3*S*	6
29		65	*cis* racemate	NT
30		7.4	*cis* racemate	NT
31		112	1*R*, 3*S*	3
32		3	1*S*, 3*R*	2
33		149	*trans* racemate	1
34		120	*cis* racemate	2
35		48	*cis* racemate	NT
36		118	—	1

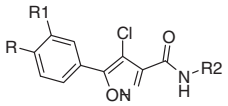
NT = not tested.
[a] Compound shown to be an agonist.

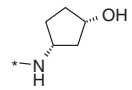
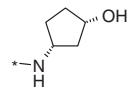
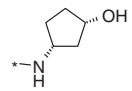
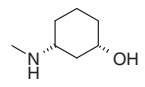
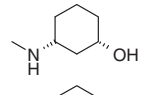
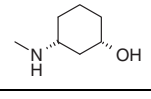
potent than the 3-aminocyclohexanol counterpart (**30**). It was clear that the *cis* orientation was linked to the strongest TRPV1 potency, and one particular isomer assigned as 1*S*, 3*R*-aminocyclohexanol (**32**) conferred TRPV1 potency of 3 nM, 37-fold more potent than its enantiomer.

Whilst the increase from five to six-membered ring size imparted an increase in TRPV1 potency the physiochemical properties of the cyclohexyl based system resulted in lower solubility. This proved to be the general trend whereby cyclohexyl based compounds were more potent but less soluble, requiring a return to intervention elsewhere in the molecule. A survey was envisaged in which the isoxazole 4-substituent was fixed as chloro, whilst varying the pendant 5-isoxazole aryl and 3-carboxamide groups. This search for moieties associated with improved solubility led to a number of interesting patterns and identified combinations of groups important for both solubility and potency (Table 3). When the 4-trifluoromethyl substituent on the aryl was replaced with 4-fluoro (**37**) a 19-fold weaker but much more soluble compound emerged. This marked the identification of the 4-fluoro aryl substituent as a moiety associated with improved solubility. This was then combined with the 1*S*, 3*R*-3-aminocyclohexanol carboxamide substituent of the highly potent TRPV1 antagonist **32** resulting in compound **39**. This displayed a threefold improvement in potency over **37** but resulted in a higher solubility of 39 mg/L. The aryl group was probed further for different substitution patterns that might bring similar solubility and improved TRPV1 potency. Compound **40** was identified containing a 3,4-difluoroaryl substituent and 1*S*, 3*R*-3-aminocyclohexanol with the crucial combination of both TRPV1 potency (79 nM) and solubility (17 mg/L).

Given the data it is obvious the aminoalcohol moiety in **40** plays a pivotal role in both activity and solubility. In an attempt to rationalise the interactions we investigated the conformation of this part of the molecule. Conformational analysis of **40** was carried out by NMR. ^{1}H NMR spectra show that there is a significant difference in the H-1 and H-3 signals when going from the hydrophobic ($CDCl_3$) to the polar (CD_3OD) solvent. The large coupling constants

Table 3
SAR of the 3-carboxamide and 5-aryl positions of the central isoxazole ring



No.	R2	R1	R	TRPV1 IC_{50} (nM)	Solkin (mg/L)
37		H	F	434	19
27		H	CF_3	23	5
38		F	F	404	33
39		H	F	141	39
32		H	CF_3	3	2
40		F	F	79	17

All compounds shown to be single enantiomers.

Table 4
The observed coupling constants (*J*) for H-1 and H-3 to H-2 determined from the PERCH simulation[17]

Proton	*J* (Hz) [CD_3OD]	*J* (Hz) [$CDCl_3$]
1→2	11.24 (ax-ax)	8.17
	3.95 (ax-eq)	4.08
3→2	10.43 (ax-ax)	7.33
	3.99 (ax-eq)	3.56

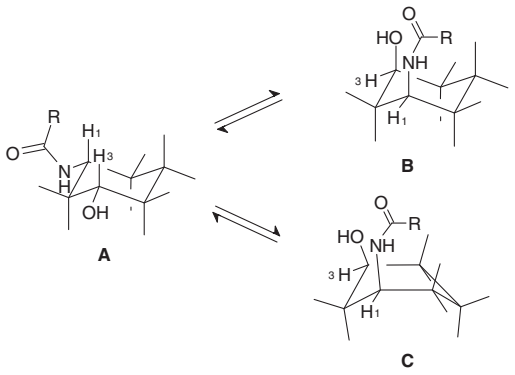


Figure 3. The possible conformations of 3-aminocyclohexanol moiety.

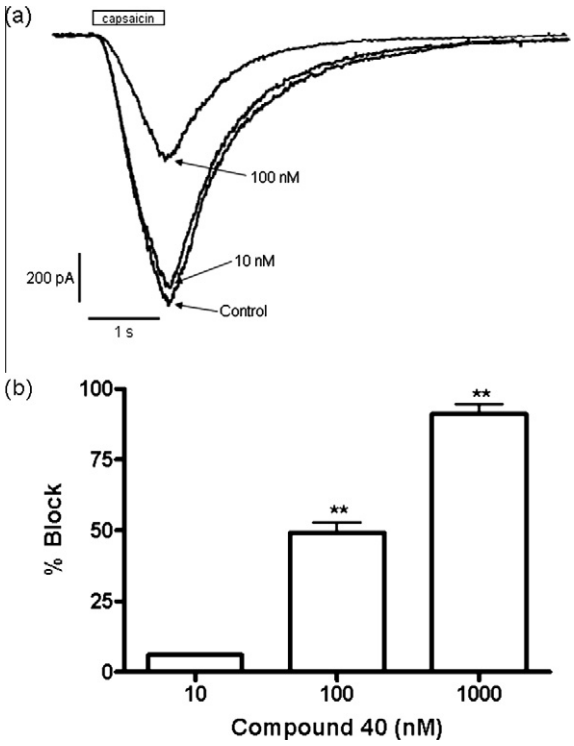


Figure 4. The effect **40** (10, 100, 1000 nM) on capsaicin-evoked currents recorded in adult rat DRGs. Reduction in the capsaicin-evoked currents is shown in a single neurone (a) and (b) mean data from three experiments. Currents are evoked by 1 s applications of capsaicin (500 nM) at the time illustrated by the rectangle in (A).

between H-1 and H-2 and H-3 and H-2 in CD_3OD (Table 4) indicate H-1 and H-3 are both in an axial position in this solvent and therefore the OH and amide groups are confirmed as being *cis*. The change in coupling constants on changing solvents is associated with the cyclohexyl ring flipping between different conformations. The lowest energy conformation of this system is expected to be the chair with the hydroxyl and amide groups equatorial (Fig. 3, **A**). Conformation **A** is seen to be dominant in CD_3OD, because the coupling constants of H-1 and H-3 to H-2 are large, (Table 4) and so these protons are axial. However, in $CDCl_3$ there is a significant contribution from either the inverted chair conformation (**B**) or the twist-boat conformations (**C**) and so the H-1 and H-3 coupling constants are of intermediate magnitude (Table 4).

The stabilization of **B** and **C** in $CDCl_3$ is likely to be due to hydrogen bonding from the OH to either the NH or the carbonyl oxygen. The fact that these conformations could be stabilized in a hydrophobic environment may be important if this group sits in a hydrophobic binding pocket. In fact this is speculated as the preferred conformation since literature examples suggest that a polar group cannot be tolerated in this region.

Together **32** and **40** were advanced into rat pharmacokinetic experiments (Table 5). The compounds were administered to Wistar BRL rats and showed good absorption; with T_{max} ~4 h (**32**) and 2 h (**40**) post dose. However **32** had considerably better oral bioavailability presumably due to the much lower clearance of **32** (5.5 mL/min/kg) compared with **40** (13.4 mL/min/kg).

Both **32** and **40** showed no significant inhibition of P450 enzymes (data not shown) and appeared clean across the five major isoforms and as such had no inhibition problems giving confidence that the risk of drug–drug interactions would be low. The P450 identification assay also showed that a range of enzymes were involved in the metabolism of **32** and **40** (data not shown). This is an excellent profile for compounds and further indicates that it is unlikely to suffer from severe drug–drug interactions.

The TRPV1 antagonist **40** was tested for effects at the native receptor using whole-cell patch clamp electrophysiology[18] to record capsaicin (500 nM)-evoked currents in adult rat dorsal root ganglion neurones in vitro (Fig. 4). The compound produced a concentration-dependent reduction in the current amplitude, with the top concentration tested, 1000 nM, reducing the capsaicin-evoked current by 91 ± 3% compared to control ($n = 3$; $P < 0.01$, ANOVA, followed by Dunnett's ad hoc test). This effect was reversible on washout of the compound.

Both compounds demonstrated a sufficient profile suitable to progress for in vivo evaluation in our analgesic behavioural models.

The TRPV1 receptor antagonists **40** and **32** were profiled in rat models of acute inflammatory thermal hyperalgesia (complete Freund's adjuvant (CFA)) and on body temperature in telemetered rats. In the CFA model, **40** (10, 30, 100 μmol/kg; po; 2 h pre-treatment) significantly reversed the thermal hyperalgesia induced by CFA, with an MED of 30 μmol/kg (see Fig. 5a). At this dose, the paw withdrawal latencies (PWLs) were significantly attenuated from baseline values of 4.7 ± 0.6 to 9.5 ± 1.2 s (~59%), with an ED_{50} value of 25.2 μmol/kg. **32** (10, 30, 100 μmol/kg; po; 3 h pre-treatment) also had an MED of 30 μmol/kg in this assay, with PWL's significantly attenuated from baseline values of 3.6 ± 0.5 to

Table 5
PK profile of compounds **32** and **40** in Wistar rats

No.	iv dose (mg/kg)	Cl (mL/min/kg)	V_{ss} (L/kg)	$T_{½}$ (h)	po dose (mg/kg)	AUC_{0-in} (ng h/mL)	T_{max} (h)	C_{max} (ng/mL)	*F* (%)
32	1.0	5.4	0.8	1.9	10	32368	4.0	3210	100
40	2.0	13.4	1.0	0.9	10	3313	2.2	408	27

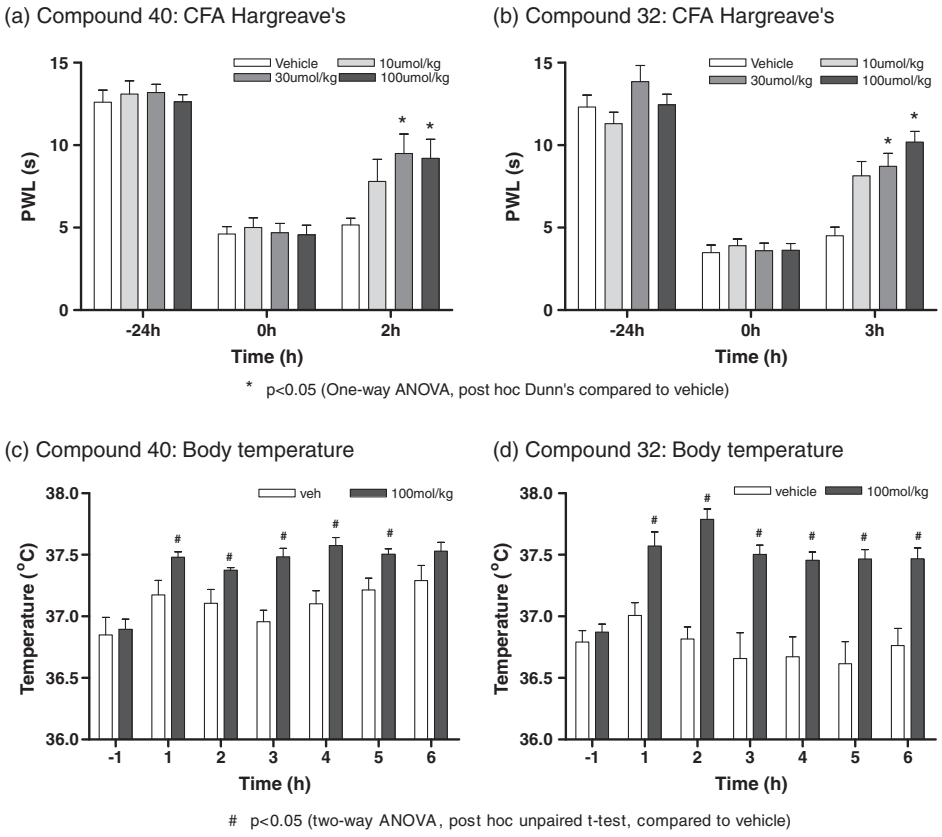


Figure 5. The effect of po administration of (a) **40** (10, 30, 100 μmol/kg) and (b) **32** (10, 30, 100 μmol/kg) on paw withdrawal latency (PWL) in the CFA Hg assay and (c) **40** (100 μmol/kg) and (d) **32** on body temperature in telemetered animals. Data are expressed as mean ± sem.

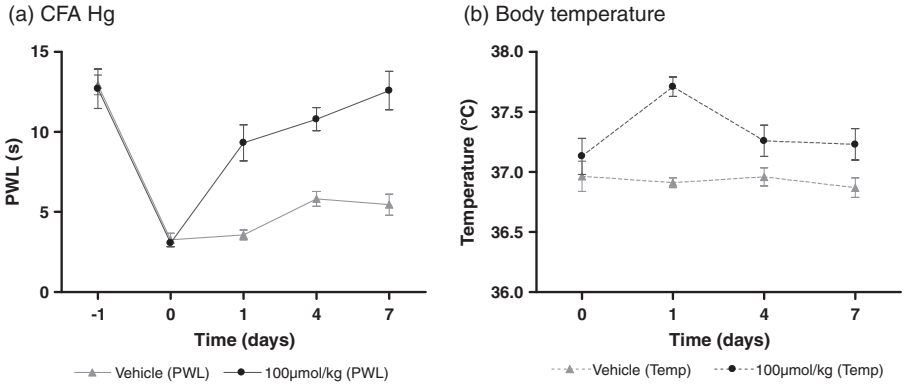


Figure 6. The effect of po administration **32** (100 μmol/kg) assessed 3 h after compound administration on (a) paw withdrawal latency (PWL) in the CFA assay and (b) on body temperature in telemetered rats. Data are expressed as mean ± sem.

8.7 ± 0.8 s (~51%). The ED_{50} value for **32** was calculated to be 7.4 μmol/kg (see Fig. 5b).

Compounds **40** (10 and 100 μmol/kg; po) and **32** (10, 30 and 100 μmol/kg) were also evaluated for effects on body temperature in rats. Compound **40** at 10 μmol/kg showed a small, but significant, increase in body temperature (data not shown); at 100 μmol/kg **40** showed a larger and more sustained (1–5 h) increase (~0.3–0.5 °C) in body temperature (see Fig. 5c). Similarly, **32** also showed a significant increase in body temperature at 100 μmol/kg (0.6–1.0 °C) from 1 to 10 h post-drug administration (see Fig. 5d). In an additional study with **32**, the effects of daily dosing of the compound, for 7 days on both the thermal hyperalgesia and body temperature were examined 3 h after drug administration to determine if tolerance would develop. In this study, analgesic efficacy was maintained throughout the period whilst tolerance to the effects on body temperature was apparent by day 4 (see Fig. 6).

In summary, we have identified a novel series of TRPV1 antagonists. We have shown key features of the isoxazole-3-carboxamide derivatives that confer both TRPV1 potency and solubility. The bespoke 1*S*, 3*R*-3-aminocyclohexanol unit is highlighted as a fundamental stereochemically defined group that aids both potency and solubility. The trifluoromethyl moiety was shown to impart strong potency to the molecules at TRPV1 whilst introduction of fluoro substituents were found to make important contributions to the compounds exhibiting the requisite balance of solubility and potency. In particular compounds **32** and **40** were identified as the most promising compounds from this series and progressed

into animal studies. Both compounds were able to attenuate the acute inflammatory thermal response in the rat CFA assay. However, dose related increases in body temperature in rats were observed although this did tolerate out over several days. Most TRPV1 antagonists described to date cause a modest increase in body temperature in preclinical studies.[19] However, the magnitude and duration of temperature elevation appears dependant on the PK profile and modality specific to blockade of TRPV1 activation.[20] Given AMG-517 elevates temperature in humans[8d] and subsequently has been withdrawn from clinical development, it will be interesting to see the clinical outcomes of future trials[21] with other TRPV1 antagonists to understand whether the hyperthermia liability can be managed either with anti-pyretics or through shortening the half-life of the compounds.

Acknowledgements

The work described here was conducted between November 2006 and December 2007. We would like to thank our colleagues in the Analytical section for structure and purity determination of all compounds.

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