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Identification of potent, soluble, and orally active TRPV1 antagonists

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ABSTRACT

Optimization of a water soluble, moderately potent lead series of isoxazole-3-carboxamides was conducted, affording a compound with the requisite balance of potency, solubility and physicochemical properties for in vivo use. Compound **8e** was demonstrated to be efficacious in a rat model of inflammatory pain, following oral administration.

The TRPV1 (transient receptor potential vanilloid 1) receptor is a well-characterized member of the transient receptor potential (TRP) superfamily of ion channels.[1–3] TRPV1 is located in both the periphery and CNS on C and Aδ fibres, the afferents commonly associated with nociception, and its role in the transmission of pain signaling has been extensively discussed.[4,5] 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.[6] Consequently, TRPV1 is believed to act as an integrator of nociceptive responses to both chemical and thermal noxious stimuli.[7]

TRPV1 knockout mice provided evidence that the channel played a key role in pain, with a clear attenuation of thermal hyperalgesia in response to proinflammatory agents.[8] TRPV1 also shows increased expression in pain states in both rat and human.[9] In addition to the treatment of inflammatory pain, evidence suggests that a number of other disorders, including urinary urge incontinence, cough, and irritable bowel syndrome, may be treatable through modulation of TRPV1 signaling.[10]

Over recent years, a substantial effort has been made across all sectors of the pharmaceutical industry to target TRPV1 antagonists for the treatment of pain.[11] This has lead to several novel compounds entering clinical trials as analgesic agents.[12] The outcomes of these trials have been discussed in detail on a number of occasions,[11,12] however to date no clear evidence of analgesic efficacy has been demonstrated in man. Furthermore, despite being viewed as potentially the next generation of pain therapeutics, the observations that TRPV1 plays a role in maintenance of body temperature, and that blockade of the receptor can cause hyperthermia both pre-clinically and in humans, has resulted in the termination of at least one clinical trial because of dose-limiting hyperthermia that occurred at sub-therapeutic doses (Fig. 1).[11]

We have previously described the discovery of a novel isoxazole-3-carboxamide series of TRPV1 antagonists, which was optimized to a potent, selective and efficacious compound **1**.[13] Unfortunately, **1** suffered from poor solubility and high plasma protein binding. This was further optimized to compound **2** which had greatly improved solubility and lower plasma protein binding, albeit with reduced in vitro potency.[14]

In this Letter, we report on the optimization of compound **2**, focusing in particular on our approaches to target TRPV1 antagonists with increased potency, while maintaining the improved level of solubility, as well as favorable analgesic and pharmacokinetic properties.

In an effort to increase potency while retaining solubility, we set about expanding the structure–activity relationship around the amine region of the isoxazole (Fig. 2). A general synthesis of the isoxazole-3-carboxamides has been reported previously,[13,14]

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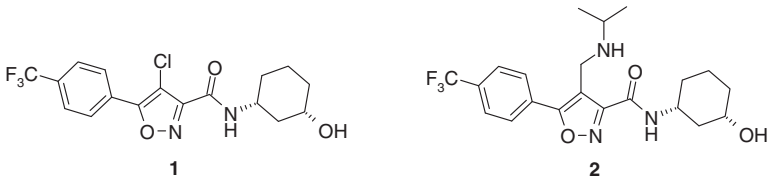


Figure 1. Structures of progenitor examples **1** and **2**.

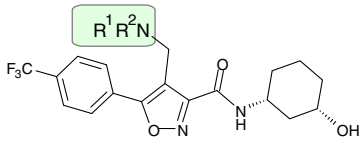


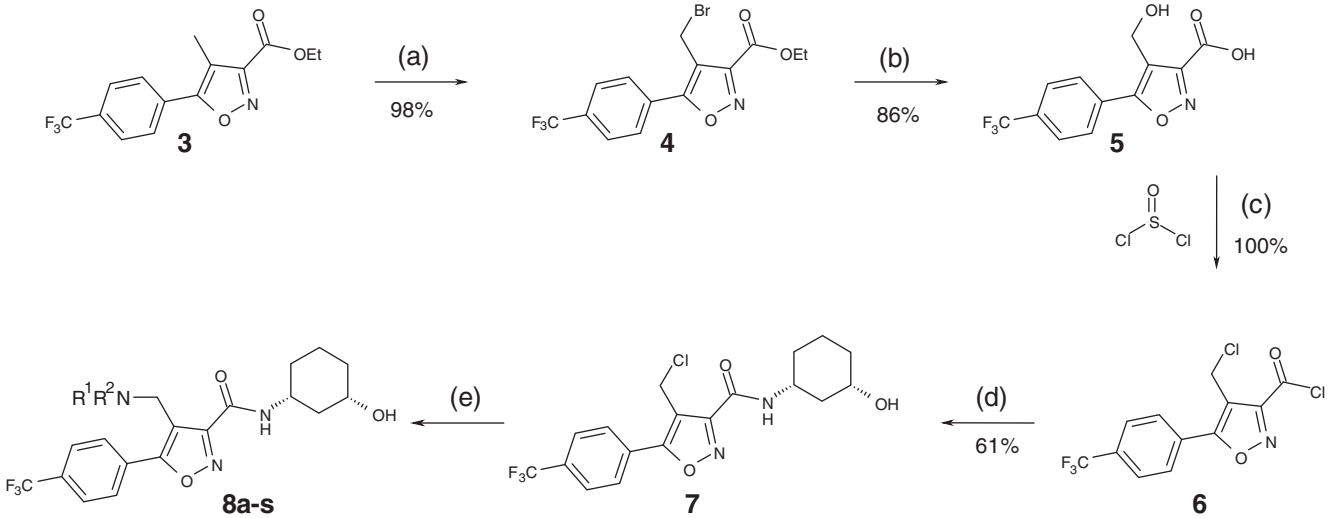
Figure 2. Generic scaffold of examples **8a**–**s**.

however for the purposes of probing this polar region more extensively an alternative route was established (Scheme 1). Starting from compound **3**,[14] simple bromination of the pendant methyl substituent was achieved in near quantitative yield using *N*-bromosuccinimide in the presence of benzoyl peroxide to give compound **4**. Treatment with trifluoroacetic acid in the presence of water furnished the hydroxy acid, compound **5**, which could be reacted with thionyl chloride in toluene and DMF to simultaneously furnish the acid chloride and transform the hydroxyl moiety into the desired alkyl halide **6**. Amide formation was achieved using the appropriate amine in the presence of triethylamine and DCM which furnished the important precursor, compound **7**, for probing tolerance to amine containing compounds **8a–s**.

Compounds were evaluated for their ability to modulate influx of Ca^{2+} in cells (CHO-K1) stably expressing human TRPV1 (VR1) using a Molecular Devices FLIPR. TRPV1 agonists stimulated intracellular fluorescence when applied to the channel, while antagonists inhibited the fluorescent response when co-applied with an agonist. Thus the same TRPV1 transfected cell line was used in both agonist and antagonist assays. Results are reported as pIC_{50} and are an average of at least two independent experiments in duplicate.[15]

Initial investigations centered on subtle modifications to compound **2**, probing tolerance to the presence of a tertiary amine (Table 1, Fig. 3). Introduction of methyl **8a** or ethyl **8b** units moderately improved TRPV1 potency (pIC_{50}'s of 7.6) without effecting solubility, albeit at the expense of a log unit increase in lipophilicity (*e* Log *D*).[16] Retaining a secondary amine, we began to probe the steric constraints around the amine nitrogen, the effect of variations in lipophilicity/polarity and of course changes which would influence amine basicity. As is illustrated by **8c**, **8d**, **8i** and **8j**, constraining the isopropyl group in the form of a cycloalkyl or heteroalkyl group was tolerated, and indeed for **8c** lead to improvement in TRPV1 potency, showing a pIC_{50} of 8.5. Significantly, the *e* Log *D* of these compounds was increasing with potency. An extreme example was provided by the introduction of the 1,1,1-trifluoropropan-2-amine moiety **8h**, which increased potency dramatically to furnish our most active compound to date (pIC_{50} of 9.8) but lost solubility, probably as a consequence of both reducing the amine basicity and increasing lipophilicity. Noting that polarity in the form of ether functionality was tolerated (**8i**–**j** with pIC_{50}'s of 7.9 and 7.1, respectively), the potential to balance potency and lipophilicity was further probed by synthesizing compounds **8k**–**s**. While the sulfone moiety present in **8q** and the amide moiety in **8s** furnished compounds with reduced TRPV1 antagonist activity, there appeared to be tolerance for a wide range of functionalities, especially for hydroxyl groups, as well as cyclic and acyclic ethers, leading to compounds that combined good levels of potency and solubility, for example, **8l** and **8n** with pIC_{50}'s of 8.6 and 8.5 and solubilities of 79 and 89 mg/L, respectively.[17] Finally, as can be seen from **8e** to **8g**, a happy compromise of our desired properties could also be achieved through subtle modification of the alkyl group.

As illustrated in Table 2, a number of compounds were selected for in vitro ADME and hERG profiling. Apart from **8f** and **8h**, they demonstrated good stability in human hepatic microsomes in vitro. In some cases there did appear to be a disconnect between



Scheme 1. Reagents and conditions: (a) *N*-bromosuccinimide, benzoyl peroxide, CCl_4, 88 °C, 4 h, (98%); (b) TFA, water, microwave, 15 min, 150 °C, (86%); (c) thionyl chloride, toluene, DMF, 90 °C, 4 h, (100%); (d) (1*R*, 3*S*)-3-aminocyclohexanol, Et_3N, DCM; rt, 2 h, (61%); (e) NHR1, DIPEA, acetonitrile, microwave, 15 min, 165 °C, (7–68%).

Table 1
SAR of amine modifications; effect on potency and solubility

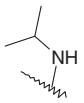
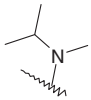
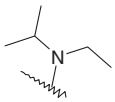
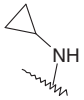
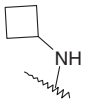
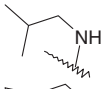
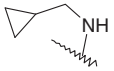
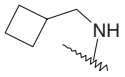
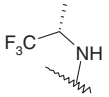
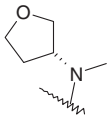
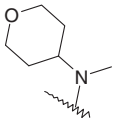
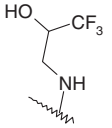
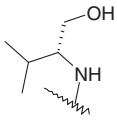
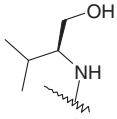
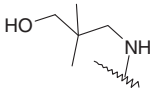
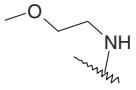
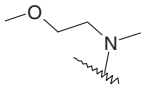
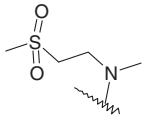
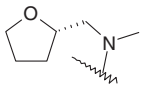
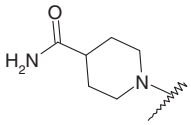
Compd	Structure	TRPV1 pIC_{50}	Solkin (mg L^{-1})	*e* Log *D*
2		7.2	82	3.0
8a		7.6	80	4.0
8b		7.6	85	4.1
8c		8.5	21	4.5
8d		7.8	26	4.3
8e		9.0	78	4.3
8f		8.1	77	3.5
8g		8.9	ND*	4.4
8h		9.8	3.5	5.6
8i		7.9	6.6	4.5
8j		7.1	82	3.7
8k		8.0	87	4.7
8l		8.6	79	4.2
8m		8.0	81	4.1
8n		8.5	89	3.5
8o		6.9	80	3.7
8p		8.1	83	4.0

Table 1 (*continued*)

Compd	Structure	TRPV1 pIC_{50}	Solkin (mg L^{-1})	*e* Log *D*
8q		5.9	26	3.6
8r		8.3	31	3.6
8s		5.5	30	3.6

* ND—not determined.

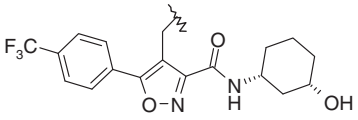


Figure 3.

the in vitro stabilities in human and rat microsomes with compounds **8e**, **8g** and **8n** having significantly reduced stability in rat compared to human, but compounds **8c**, **8f** and **8h** having comparable stability across species. Apart from compound **8g**, the remaining compounds in Table 2 displayed similar or lower affinity for the hERG receptor, and all demonstrated moderate to good levels of unbound compound as illustrated by the rat and human plasma protein binding assays.

Based on the combination of TRPV1 antagonist potency, inherent solubility and favourable overall physicochemical characteristics, compounds **8e** and **8l** were selected for further ADME profiling. As demonstrated in Table 3, compound **8l** has low to moderate in vivo clearance in rat, achieves a moderate plasma half-life of 4.3 h, but suffers from poor oral bioavailability (4%). Pleasingly, compound **8e** demonstrated improved oral bioavailability (33%) as compared to compound **2**. Furthermore, the rat in vitro and in vivo clearance (Tables 2 and 3) appear to correlate well, with compound **8e** having a relatively high rate of metabolic clearance, as anticipated due to its low in vitro microsomal stability and because its lower plasma protein binding, as compared to **8l**, leads to a larger free fraction in rat. Perhaps as a consequence of the extensive volume of distribution a moderate half-life of 4 h was also observed.

Overall, compound **8e** demonstrated an acceptable pharmacokinetic profile sufficient to proceed to in vivo efficacy testing in a rat model of inflammatory pain. The efficacy of compound **8e** was evaluated in the capsaicin Hargreaves assay, a functional in vivo assay of TRPV1 induced thermal hyperalgesia in rats.[18] In the capsaicin Hargreaves model, **8e** (3, 10, 30 μmol/kg; po; 2 h pre-treatment) significantly reversed the thermal hyperalgesia induced by capsaicin, with an MED of 30 μmol/kg (see Fig. 4).

In summary, a series of isoxazole derivatives was optimized through a systematic SAR study of the tolerance to varying degrees of polarity at the isoxazole 4-position. This led to the identification of compounds with a better balance of solubility, physicochemical properties and in vitro potency. In particular compounds **8e** and **8l** were identified as the most promising examples from this series and both were progressed into animal studies. The improved oral bioavailability of compound **8e** lead to its advancement into efficacy testing where it showed statistically significant attenuation

Table 2
In vitro PK, hERG and plasma protein binding data for selected compounds

Compd	HLM CL_{int} ($\mu l\ min^{-1}\ mg^{-1}$)	RLM CL_{int} ($\mu l\ min^{-1}\ mg^{-1}$)	Rat Hep. CL_{int} ($\mu l\ min^{-1} 10^6\ cells^{-1}$)	hERG pK_i (% inhibition at 100 μM)	PPB%Bound (Human)	PPB% Bound (Rat Wistar)
2	<12	<12	<6	5.2	75	72.9
8c	17	<12	<6	(75)	88.2	93.6
8e	12	107	12	(86)	82.3	75.8
8f	48	55	ND*	5.3	ND*	ND*
8g	21	144	ND*	5.9	ND*	ND*
8h	66	49	ND*	(59)	95.2	96.3
8l	12	41	ND*	(83)	74.4	88.4
8n	12	93	10	(69)	63.9	83.2

* ND—not determined.

Table 3
In vivo PK, for selected compounds. All compounds were dosed at 10 mg/kg (po) and 2 mg/kg (iv)

Compd	CL ($ml\ min^{-1}\ kg^{-1}$)	V_{ss} ($L\ kg^{-1}$)	$T_{1/2}$ (h)	po C_{max} (μM)	po T_{max} (h)	%F
2	12.5	5.9	7.9	0.72	2	20
8e	69	7.6	4.0	0.42	2	33
8l	13.5	3.5	4.3	0.23	1.5	4

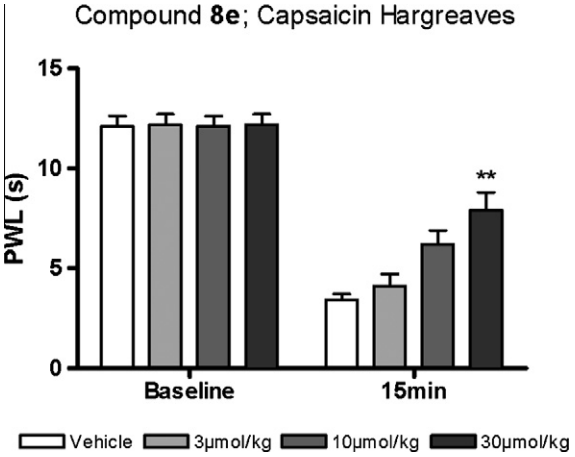


Figure 4. The effects of po administration of **8e** (3, 10, 30 μmol/kg) on paw withdrawal latency (PWL) in the capsaicin Hargreaves assay.

of the acute inflammatory thermal response in the rat capsaicin Hargreaves assay (p <0.01). We believe **8e** will be a valuable tool in exploring the potential role of TRPV1 antagonists in pain, and in potentially elucidating and interpreting the relationship between TRPV1 antagonism and hyperthermia.

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well and the subsequent change in the fluorescence of the cells measured to assess agonist activity. Ten minutes after test compound addition, 12.5 μl of 30 nM capsaicin, prepared in assay buffer, was added to each well and the change in the fluorescence of the cells measured again. In this way, the same assay was used to assess both the agonist activity and antagonist activity of test compounds.

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