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α -Substituted 2-(3-fluoro-4-methylsulfonamidophenyl)acetamides as potent TRPV1 antagonists

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Abstract

A series of α -substituted acetamide derivatives of previously reported 2-(3-fluoro-4-methylsulfonamidophenyl)propanamide leads (**1**, **2**) were investigated for antagonism of *h*TRPV1 activation by capsaicin. Compound **34**, which possesses an α -*m*-tolyl substituent, showed highly potent and selective antagonism of capsaicin with $K_{i(\text{CAP})} = 0.1$ nM. It thus reflected a 3-fold improvement in potency over parent **1**. Docking analysis using our homology model indicated that the high potency of **34** might be attributed to a specific hydrophobic interaction of the *m*-tolyl group with the receptor.

Keywords

TRPV1 antagonist; Analgesic; Capsaicin; Molecular modeling

The transient receptor potential vanilloid 1 channel (TRPV1) has emerged as a very promising therapeutic target, reflecting its central role in nociception and its involvement in a range of diseases.^{1–3} Capsaicin⁴ and resiniferatoxin⁵ provided early structural leads for understanding the vanilloid pharmacophore, and it was soon recognized that appropriately modified derivatives were able to achieve antagonism.^{6,7} Intense medicinal chemical efforts have now afforded a broad base of structures for the further development of TRPV1 antagonists.⁸ Although a crystal structure for TRPV1 is not available, homology modeling of

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TRPV1 represents a complementary tool to further refine insights into ligand–TRPV1 interactions.⁹ As the field has matured, it has become apparent that compounds may have different potencies or efficacies as antagonists for different modes of TRPV1 activation and may have different effects at the whole animal level.^{10,11}

Of particular importance, hyperthermia represents a common side effect of TRPV1 antagonists and the pattern of antagonistic activities for different agonists has been suggested to be related to the ability of an antagonist to cause hyperthermia.¹¹ Further, different antagonists are differentially affected by the signaling pathways that regulate TRPV1.¹² The availability of novel antagonists will be critical for optimizing such characteristics.

Previously, we have described a series of *N*-(6-trifluoromethylpyridin-3-yl)methyl 2-(3-fluoro-4-methylsulfonamidophenyl)propanamides, originally designed based on a pharmacophoric combination approach, that showed potent *h*TRPV1 antagonism toward multiple activators.^{13–17} The structure of capsaicin (CAP) has been divided into three pharmacophoric regions.⁴ Correspondingly, the antagonistic template was subdivided into the same three pharmacophoric regions, namely the A-region (3-fluoro-4-methylsulfonamidophenyl), the B-region (propanamide), and the C-region ((6-trifluoromethylpyridin-3-yl)methyl) (Fig. 1). The structure activity relationship (SAR) studies of the template were initiated by incorporating a variety of functional groups including amino,¹³ oxy,¹⁴ thio,¹⁵ alkyl¹⁶ and aryl¹⁷ groups into the 2-position in the pyridine C-region. In these series, multiple compounds showed highly potent and (S)-stereospecific antagonism of *h*TRPV1 activators including capsaicin, pH, heat (45 °C) and *N*-arachidonoyl dopamine (NADA). Their *in vitro* mechanism of action as TRPV1 antagonists was confirmed *in vivo* by their ability to block capsaicin-induced hypothermia. Most importantly, the selected compounds showed promising antinociceptive activity in neuropathic and inflammatory pain models.

In an effort to optimize the properties of the antagonistic template, our focus was next directed to the propanamide (or α -methylacetamide) B-region. The modeling analysis of the B-region using our established *h*TRPV1 homology model^{9,13} indicated that the amide group made a hydrogen bond with Tyr511 and also contributed to the appropriate positioning of the C-region for hydrophobic interactions. In order to gain better positioning of the C-region with the receptor through α -substitution in the B-region, with consequent enhancement of activity, we have extensively investigated the structure activity relationships of α -substituted acetamide derivatives for *h*TRPV1 antagonism. Compounds **1** and **2**, previously described as highly potent and stereoselective antagonists, were selected as the parent compounds for this optimization of the B-region (Fig. 1).^{13,15} Here, we describe incorporation of various alkyl, dialkyl and aryl groups at the α -position in the B-region of **1** and **2**, with 4-methylpiperidinyl and cyclohexylthio groups at the 2-position in the pyridine C-region, respectively, and we have evaluated their antagonism of CAP stimulation of *h*TRPV1 expressed in CHO cells.

The syntheses of α -substituted acetamide derivatives were accomplished by one of four different synthetic routes (Schemes 1–4). For the synthesis of A/B-regions of the α -ethyl derivative **21**, the nucleophilic substitution of 2,4-difluoronitrobenzene **3** by diethyl

malonate afforded the adduct **4**,¹⁸ which was alkylated with ethyl iodide to provide **5**. Further elaboration by a known protocol provided the α -ethyl acetic acid **7** (Scheme 1). α -Substituted 2-phenylacetic acids as the A/B-regions of compounds **22–43** were prepared efficiently by vicarious nucleophilic substitution¹⁹ from the corresponding ethyl 2-substituted 2-chloroacetates **8**, respectively, using the method previously reported for the synthesis of parent compounds **1** and **2** (Scheme 2). For the synthesis of the A/B-regions of the α,α' -dialkyl derivatives **44–47**, the α -methyl ethyl ester **13**²⁰ was alkylated with the corresponding alkyl iodides to provide the dialkyl intermediates **14**, which were converted to the respective α,α' -dialkylated acetic acids **15** (Scheme 3). For the synthesis of the A/B-regions of the cyclopentyl derivatives **48–49**, the ethyl 2-(3-fluorophenyl)acetate **16** was nitrated and then alkylated with 1,4-dibromobutane. Further three-step elaboration provided the α -cyclopentyl acetic acid **18** (Scheme 4). The prepared α -substituted 2-phenylacetic acids (**7**, **12**, **15**, **18**) as A/B-regions were coupled with the two C-regions, **19**¹³ and **20**¹⁵, to provide the final compounds **21–49**, respectively (Scheme 5).

The synthesized TRPV1 ligands were evaluated in vitro for antagonism as measured by inhibition of activation by capsaicin (100 nM, CAP). The assays were conducted using a fluorometric imaging plate reader (FLIPR) with human TRPV1 heterologously expressed in Chinese hamster ovary (CHO) cells.¹³ The results are summarized in Table 1, together with the potencies of the parent antagonists **1** and **2** as racemates with $K_{i(\text{CAP})}$ = 0.3 and 0.9 nM, respectively.^{13,15}

First, we examined the SAR of the α -alkyl derivatives (**21–25**). The α -ethyl derivative **21**, the one-carbon elongated surrogate of **1**, showed 5-fold less antagonistic potency compared to **1**. However, a further increase in size, such as cyclopentyl (**22**, **23**) and cyclohexyl (**24**, **25**) groups, led to a dramatic loss in antagonism (>100-fold) compared to the parents **1** and **2**.

Next, the SAR of the α -phenyl derivatives (**26–37**) was examined. The α -phenyl derivatives **26** and **27** showed a moderate or a substantial reduction, respectively, in antagonism compared to the parents but still exhibited potent antagonism compared to the cyclohexyl derivatives (**24**, **25**) despite of a comparable size of the α -substituent. We therefore further investigated other phenyl derivatives substituted with small size groups such as fluoro and methyl. α -Fluorophenyl derivatives (**28–31**) displayed comparable potencies to the α -phenyl derivatives (**26–27**), respectively. However, there was a significant difference in potency with the α -tolyl derivatives (**32–37**) depending on the methyl position in the α -tolyl group as well as on the 2-substituent in the pyridine C-region. Whereas the α -*o*-tolyl derivatives (**32**, **33**) exhibited significant reductions (>140 fold) in potency, the α -*p*-tolyl derivatives (**36**, **37**) displayed moderate loss (3–8 fold) in potency compared to the parents **1** and **2**, respectively. Surprisingly, the α -*m*-tolyl derivative **34** with a 4-methylpiperidinyl group showed exceptionally excellent antagonism with $K_{i(\text{CAP})}$ = 0.1 nM, which was even 3-fold more potent than the parent **1**. Contrary to this finding, the α -*m*-tolyl derivative **35** with a cyclohexylthio group was found to be 60-fold less potent than the parent **2**. The results indicated that the 2-substituent in the pyridine C-region contributed to the appropriate orientation of α -substituent in the B-region for optimal binding with the receptor.

Compound **34** was found to be the only antagonist in this series which showed better potency than the corresponding parent and was the most potent antagonist in the series of 2-(3-fluoro-4-methylsulfonamidophenyl)propanamide antagonists reported so far.

Next we investigated the SAR of α -benzyl derivatives (**38–43**). Unfortunately, all of them showed much weaker potencies than the corresponding α -phenyl derivatives, indicating that α -benzyl substituents appear to provide unfavorable steric interactions with the receptor.

Finally, we examined α,α' -dialkyl derivatives (**44–49**). They were found to be weak antagonist. The SAR analysis indicated that their antagonistic potencies became progressively poorer as the size of α,α' -dialkyl groups increased.

In order to examine its in vitro activity as an antagonist for multiple *h*TRPV1 activators, compound **34**, the most potent antagonist in this series, was evaluated for antagonism of TRPV1 activation by capsaicin, pH, heat (45 °C) and *N*-arachidonoyl dopamine (NADA). Inhibitory potencies were compared to the respective values for the parent **1** (Table 2). Whereas compound **34** showed 3-fold better potency for antagonism of CAP, it exhibited 5–15 fold weaker potency toward the other activators compared to **1**.

Using our *h*TRPV1 model¹³ built based on our *r*TRPV1 homology model⁹, we performed a flexible docking study of compound **34**. Its predicted binding interactions are shown in Figure 2.²¹ Since the active stereoisomer of the propanamide B-region in this template was found to be the (*S*)-configuration in all cases in our previous reports, we performed the docking study with the (*S*)-**34**.

The A-region, 3-fluoro-4-methylsulfonamidophenyl group, occupied the deep bottom hole and formed hydrophobic interactions with Tyr511, Ile564, Ile569, and Met572. The sulfonamide S=O participated in hydrogen bonding with the backbone amide of Lys571, and the phenyl ring engaged in π - π stacking with the phenyl ring of Tyr511. In the B-region, the amide group formed hydrogen bonds with the side chains of Tyr511 and Lys571, contributing to the appropriate positioning of the C-region for its hydrophobic interactions. In addition, the *m*-tolyl ring as an α -substituent nicely fitted into the hydrophobic pocket composed of Tyr554, Tyr555, Ile564, and Ile569, contributing for the high potency of **34**. In the C-region, the 3-trifluoromethyl pyridine ring extended toward the upper hydrophobic area consisting of Met514 and Tyr511. Furthermore, the 4-methylpiperidine ring was involved in hydrophobic interactions with Thr550, Tyr554, and Phe587 of the adjacent monomer.

In conclusion, the structure activity relationship for *h*TRPV1 antagonism by α -substituted acetamide derivatives in the B-region of the 2-(3-fluoro-4-methylsulfonamidophenyl) propanamide template was investigated. Steric repulsion of the α -substituent emerged as a key determinant of antagonistic potency. In this series, compound **34** showed excellent antagonism with $K_{i(\text{CAP})} = 0.1$ nM. Compound **34** was thus 3-fold more potent than the parent **1**, which we previously had described as potent antagonist, and compound **34** is the most potent antagonist reported using this antagonistic template. Detailed in vitro analysis of antagonism by **34** of the response to multiple TRPV1 activators indicated that **34** exhibited

preferential antagonism for capsaicin, with weaker potency for the other activators. The docking study of **34** indicated that its high potency might be attributed to a specific hydrophobic interaction of the m-tolyl group with the receptor.

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21. The 3D structure of the 34 was generated with Concord and energy minimized with a MMFF94s force field and MMFF94 charge until the rms of the Powell gradient was 0.05 kcal mol⁻¹ Å⁻¹ in SYBYL-X 2.0 (Tripos Int., St. Louis, MO, USA). The flexible docking study on our hTRPV1 model was carried out using GOLD v.5.2 (Cambridge Crystallographic Data Centre, Cambridge, UK), which uses a genetic algorithm (GA) and allows for full ligand flexibility and partial protein flexibility. The binding site was specified as 8 Å around the capsaicin complexed in the hTRPV1 model. The side chains of the nine residues which are important for ligand binding, (i.e., Tyr511, Ser512, Met514, Leu515, Leu518, Phe543, Leu547, Thr550, and Asn551) were allowed to be flexible with 'crystal mode' in GOLD. 34 was docked with the GoldScore scoring function, and the other parameters remained as default. All the computation calculations were undertaken on an Intel® Xeon™ Quad-core 2.5 GHz workstation with Linux Cent OS release 5.5.

Figure 1.
Lead TRPV1 antagonists.

Figure 2.

Docking result of (*S*)-**34** in the *h*TRPV1 model. (A) Binding mode and interactions of **34** at the binding site of *h*TRPV1. The key interacting residues are labeled and displayed as capped-stick with their carbon atoms in white color. The helices are colored by gray and the neighboring monomer helices are shown in line ribbon. Compound **34** is depicted in ball-and-stick with the carbon atoms in magenta color. The van der Waals surface representation of **34** is colored by the lipophilic potential property. Hydrogen bonds are shown in black dashed lines, and non-polar hydrogens are not shown for clarity. (B) The Fast Connolly surface representation of *h*TRPV1 and the van der Waals surface representation of the docked **34**. The molecular surface of *h*TRPV1 was generated by MOLCAD and presented with the lipophilic potential property. For clarity, the surface of *h*TRPV1 is Z-clipped and that of **34** is in magenta color. (C) 2-D representation of the interactions between **34** and *h*TRPV1. Hydrophobic, hydrogen bonding, and π - π stacking interactions are marked in light brown, red, and blue, respectively.

Scheme 1.

Synthesis of the A/B-region for **21**. Reagents and conditions: (a) diethyl malonate, NaH, DMSO, 15 °C, 52%; (b) EtI, TBAF, DMF, rt, overnight, 65%; (c) Pd/C, H₂, EtOH, 80%; (d) MsCl, pyridine, CH₂Cl₂, 0 °C–rt, 1 h, 86%; (e) 2 N NaOH, EtOH, reflux, 8 h, 99%.

Scheme 2.

Synthesis of the A/B-region for **22–43**. Reagents and conditions: (a) 1-fluoro-2-nitrobenzene, KOtBu, DMF, -5 to 0 °C; (b) H_2 , Pd/C, EtOH–EtOAc (1:1); (c) MsCl, pyridine, CH_2Cl_2 , 0 °C–rt; (d) LiOH, THF– H_2O (2:1), reflux–rt.

Scheme 3.

Synthesis of the A/B-region for **44–47**. Reagents and conditions: (a) NaH, MeI (or EtI), DMF, 0 °C; (b) Pd/C, H₂, EtOH, 1 h, 82–90%; (c) MsCl, pyridine, CH₂Cl₂, 0 °C–rt, 1 h, 78–89%; (d) LiOH, THF–H₂O, 45 °C, 2 h.

Scheme 4.

Synthesis of the A/B-region for **48–49**. Reagents and conditions: (a) HNO₃, H₂SO₄, 0 °C, 2 h; (b) NaH, 1,4-dibromobutane, THF, rt, 30 min; (c) Pd/C, H₂, EtOH, 1 h, 92%; (d) MsCl, pyridine, CH₂Cl₂, 0 °C–rt, 1 h, 82%; (e) LiOH, THF–H₂O, 45 °C, 2 h.

Scheme 5.

Syntheses of α -substituted derivatives. Reagents and conditions: (a) RCO_2H (**7**, **12**, **15**, **18**), TBTU, HOBt, DIPEA, THF-DMF, rt, 70–90%

Table 1In vitro *h*TRPV1 antagonistic activities for α -substituted acetamide derivatives

Chemical structure of the general compound: A pyridine ring substituted with a trifluoromethyl group (F_3C) at position 6, a substituent R at position 3, and a (2-(4-(2-fluoro-4-methylsulfonylphenyl)acetamido)ethyl) group at position 4. The connection between the acetamide carbonyl and the phenyl ring is marked with a red 'X'.

Definitions of R and X :

$\text{R} =$ $K_{\text{i}(\text{CAP})}$ (nM) $\text{R} =$ $K_{\text{i}(\text{CAP})}$ (nM)

X	1	$K_{\text{i}(\text{CAP})}$ (nM)	2	$K_{\text{i}(\text{CAP})}$ (nM)
1	0.3	2	0.9
21	1.6		NS
22	88.7	23	103
24	31.1	25	WE

26	1.3	27	62.5
28	2.4	29	48.7
30	3.4	31	37.6
32	42.9	33	WE

	34	0.1	35	56.4
	36	1.1	37	7.5
	38	65.9	39	WE
	40	49.3	41	WE

42	WE	43	40.3
44	49	45	65.7
46	124	47	WE
48	WE	49	WE

WE: weakly active, NS: not synthesize.

Table 2In vitro *h*TRPV1 antagonistic activities of **34** for multiple activators

Activators, parameter	1	34
CAP (f) K_i (nM)	0.3 (± 0.07)	0.1 (± 0.03)
pH, IC ₅₀ (nM)	15.8 (± 3.68)	86.7 (± 10.6)
Heat 45 °C, IC ₅₀ (nM)	2.56 (± 0.45)	36.2 (± 5.58)
NADA (f) K_i (nM)	0.02 (± 0.01)	0.25 (± 0.05)