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2-Aryl substituted pyridine C-region analogues of 2-(3-fluoro-4-methylsulfonylaminophenyl)propanamides as highly potent TRPV1 antagonists

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Abstract

A series of 2-aryl pyridine C-region derivatives of 2-(3-fluoro-4-methylsulfonylaminophenyl)propanamides were investigated as *h*TRPV1 antagonists. Multiple compounds showed highly potent TRPV1 antagonism toward capsaicin comparable to previous lead **7**. Among them, compound **9** demonstrated anti-allodynia in a mouse neuropathic pain model and blocked capsaicin-induced hypothermia in a dose-dependent manner. Docking analysis of **9** with our *h*TRPV1 homology model provided insight into its specific binding mode.

Keywords

Vanilloid receptor 1; TRPV1 antagonist; Capsaicin; Resiniferatoxin; Molecular modeling

TRPV1 (transient receptor potential vanilloid 1) has emerged as a promising therapeutic target for neuropathic pain as well as a broad range of other indications.¹ Located predominantly in C-fiber sensory afferent neurons, TRPV1 is a nociceptor which integrates stimuli from exogenous compounds such as capsaicin, endogenous endovanilloids, heat and acidity.^{2–7} TRPV1 is further co-regulated by the signaling milieu of the cell, as reflected in the activity of kinases such as protein kinase C, protein kinase A, or the level of phosphatidylinositol-4,5-bisphosphate. Development of antagonists represents the leading

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therapeutic strategy, while defunctionalization/desensitization subsequent to agonist stimulation also holds promise.⁸ Starting with capsaicin as the lead structure, intense research efforts have generated substantial insights into vanilloid structure activity relations and are yielding potent, orally active antagonists.^{9–15}

We previously reported a series of 2-(3-fluoro-4-methylsulfonylamino-phenyl)propanamides as potent *h*TRPV1 antagonists in which the three pharmacophores were designated as A-region (3-fluoro-4-methylsulfonylamino-phenyl), B-region (propanamide) and C-region ((6-trifluoromethyl-pyridin-3-yl)methyl), respectively (Fig. 1).¹⁶ The structure activity relationships of the 2-substituent in the pyridine C-region have been investigated extensively by introducing various groups, including amino,¹⁶ oxy,¹⁷ thio¹⁸ and alkyl¹⁹ groups. In the series, a number of compounds showed highly potent and stereospecific antagonism to multiple TRPV1 activators including capsaicin, pH, heat (45 °C) and *N*-arachidonoyl dopamine (NADA). In addition, the selected compounds demonstrated strong analgesic activity in the mouse neuropathic pain model and blocked capsaicin-induced hypothermia, consistent with its in vitro mechanism of action. The modeling analysis using our *h*TRPV1 homology model indicated that a new hydrophobic interaction by the 2-substituents with the receptor in addition to that by the 6-trifluoromethyl group in the C-region was critical for their potent antagonism.¹⁶

In continuation of our effort to further optimize the 2-substituent in the *N*-(6-trifluoromethyl-pyridin-3-ylmethyl) C-region, here we have investigated the structure activity relationships of 2-aryl type derivatives as *h*TRPV1 antagonists. With a selected potent antagonist in the series, we have further characterized its analgesic activity and inhibition of capsaicin-induced hypothermia in animal models and we also have performed molecular modeling with our *h*TRPV1 homology model.

The synthesis of the 2-aryl derivatives was accomplished by two methods. First, a series of aryl boronic acids were reacted with 2-chloropyridine **1**¹⁶ to afford 2-aryl pyridines **2** by a Suzuki reaction. The 2-(1-imidazolyl)pyridine derivative of **2** was an exception in that it was prepared by the direct condensation of imidazole with **1**. The nitrile groups of **2** were reduced to yield the corresponding primary amines **3**. The amines of **3** were coupled with racemic propanoic acid **4**²⁰ to provide the corresponding final compounds (Scheme 1, Method A). Alternatively, some 2-aryl analogues were synthesized directly from the 2-chloro derivatives of the final compound **6** under Suzuki conditions (Scheme 2, Method B).

The synthesized TRPV1 ligands were evaluated in vitro for antagonism as measured by inhibition of TRPV1 activation by capsaicin (100 nM). The assays were conducted using a fluorometric imaging plate reader (FLIPR) with human TRPV1 heterologously expressed in Chinese hamster ovary (CHO) cells.¹⁶ The results are summarized in Tables 1–3, together with the potencies of the previously reported **7** ($K_{i(\text{CAP})} = 0.3 \text{ nM}$) as a reference.¹⁶

To investigate the SAR for 2-aryl derivatives of the pyridine C-region, we began with the 2-(substituted phenyl)pyridine derivatives (Table 1). First, we examined the 2-phenyl derivative **8**, which showed a reasonable antagonism and was found to be ca. 10-fold less potent than the corresponding saturated surrogate, the 2-cyclohexyl derivative ($K_{i(\text{CAP})} = 0.6$

nM) previously reported.¹⁹ However, the incorporation of a 4-fluoro atom into **8** led to very potent antagonist **9** with $K_{i(\text{CAP})} = 0.4$ nM, which exhibited a 16-fold increase in potency compared to **8**. Whereas the 3-fluorophenyl derivative **10** also exhibited a 5-fold increase in potency compared to **8**, the 2-fluorophenyl derivative **11** displayed instead a 10-fold reduction in potency, probably due to conformational distortion between the pyridine and phenyl rings by the polar 2-fluoro atom. A similar SAR pattern was seen with the chlorophenyl derivatives.

The 4-chlorophenyl and 3-chlorophenyl derivatives, **12** and **13**, displayed 9- and 13-fold increases in potency, respectively, compared to **8**. We also examined the phenyl derivatives with electron-donating groups. The 4-methoxyphenyl **14**, 4-(dimethylamino)phenyl **15**, and 4-(*t*-butyl)phenyl **16** derivatives exhibited 6-, 2- and 13-fold better potency, respectively, than the parent **8**. The 3-methylsulfonylaminophenyl derivative **17** showed lack of activity. The analysis in the mono-substituted phenyl series indicated that lipophilicity of the substituent at the 3- or 4-position on the phenyl was critical for potency.

Next, we sought to evaluate the SAR of disubstituted-phenyl derivatives. Since 4-fluorophenyl and 3-chlorophenyl derivatives showed excellent antagonism as described above, the combined derivative, 3-chloro-4-fluorophenyl derivative **18**, was designed and proved to be an extremely potent antagonist with $K_{i(\text{CAP})} = 0.2$ nM, which was ca. 30-fold more potent than the parent **8**. Other disubstituted phenyl derivatives, **19–25**, were also examined. Whereas the more lipophilic derivatives **19** and **20** retained high potencies, the less lipophilic derivatives **21–25** showed lower potencies for antagonism than did **8**. These results confirmed that the lipophilicity at the 3- and 4-positions of the phenyl ring was a key determinant for antagonistic potency in the series of 2-phenyl pyridine C-region derivatives.

We next explored the SAR of 2-heterocyclic pyridine C-region derivatives (Table 2). Among 2-heteromonocyclic derivatives, the thiophen-2-yl derivative **26** exhibited excellent antagonism with $K_{i(\text{CAP})} = 0.3$ nM, indicating ca. 20-fold stronger potency than that of the phenyl surrogate **8**. The thiophen-3-yl derivative **27** was also a potent antagonist, but less potent than **26**. The furan-2 and 3-yl derivatives **28** and **29** showed lower potency compared to the corresponding thiophenyl derivatives but still retained higher potency than phenyl derivative **8**. The imidazol-1-yl derivative **30** was found to be a weak antagonist, probably due to its low lipophilicity. The analysis indicated that the antagonism in the 2-heteromonocyclic derivatives correlated with the corresponding lipophilicities of the heterocyclic rings (thiophene > furan > imidazole). Several 2-heterobicyclic derivatives, **31–34**, were also investigated. However, except for benzothiophene derivative **34**, they were only moderate to weak antagonists.

Finally, we examined 2-alkylphenyl derivatives (Table 3). The benzyl derivative **35** was found to be weak antagonist. However, its elongation by one carbon, providing the 2-phenylethyl derivative **36**, led to a dramatic increase in antagonism probably due to optimal hydrophobic interaction with the receptor. This interpretation was confirmed with other derivatives with C₂-spacer between phenyl residue and trifluoromethyl-pyridine moiety, **37–40**, which likewise showed potent antagonism in the range of $K_{i(\text{CAP})} = 0.6–2.2$ nM.

The in vitro activity of compound **9**, selected as a representative antagonist in this series, was further investigated as an antagonist for other endogenous TRPV1 activators (Table 4). Whereas compound **9** showed excellent antagonism toward capsaicin and NADA, it exhibited only moderate antagonism toward pH and heat.

In order to confirm that compound **9** also blocked response to capsaicin in vivo, consistent with its in vitro mechanism of action as an *h*TRPV1 antagonist, it was administered orally to mice 15 min before intraperitoneal injection of 3 mg/Kg capsaicin, following the procedure previously described¹⁶ (Table 5A). Compound **9** inhibited the hypothermic response to capsaicin, assayed at 30 min after capsaicin injection, in a dose-dependent manner, with 91% inhibition at the highest dose. We further evaluated the analgesic activity of compound **9** administered orally in the mouse chronic constriction injury (CCI) model²¹ for neuropathic pain (Table 5B). We found that compound **9** showed excellent anti-allodynic potency with ED₅₀ = 0.01 mg/Kg po, but with limited efficacy (53% MPE).

To develop insight into the nature of the binding interactions of **9** with *h*TRPV1, we carried out a flexible docking study using our human TRPV1 (*h*TRPV1) model,¹⁶ constructed on the basis of our rat TRPV1 (*r*TRPV1) model.²² Results are shown in Figure 2. In the A-region, the 4-methylsulfonylaminophenyl group occupied the deep bottom hole, making hydrophobic interactions with Tyr511, Ile564, and Ile569. The sulfonamide NH participated in hydrogen bonding with Tyr565, and the phenyl ring in the A-region engaged in π - π stacking with the phenyl ring of Tyr511. In the B-region, the amide group was able to hydrogen bond with Tyr511 and contributed to the proper positioning of the C-region. In the C-region, the 3-trifluoromethyl group oriented toward the hydrophobic pocket composed of Leu547 and Thr550 as previously examined.¹⁶ The pyridine ring formed a hydrophobic interaction with Phe587 on the adjacent monomer, and the fluorine atom of the 3-trifluoromethyl group made a hydrogen bond with Asn551. Finally, the 4-fluorophenyl group, representing the 2-substituent of pyridine, was involved in the crucial hydrophobic interactions with Met514 and Leu515.

In summary, we investigated the structure activity relationships of 2-aryl pyridine C-region derivatives of the previous lead as *h*TRPV1 antagonists. Several compounds (**9**, **18**, **19**, **26**) in the series showed excellent TRPV1 antagonism with $K_{i(CAP)} = 0.2$ – 0.4 nM comparable to reference compound **7**. The SAR analysis of 2-aryl groups indicated that the lipophilicity of the substituent in the phenyl and heterocyclic derivatives was critical for antagonistic activity. Among the potent derivatives in the series, compound **9** was selected for further

study. It was shown to antagonize capsaicin-induced hypothermia in a dose-dependent manner, consistent with its action in vivo being through TRPV1, and it demonstrated anti-allodynia in a mouse neuropathic pain model. Docking analysis of **9** with our *h*TRPV1 homology model indicated that **9** showed a binding mode similar to that of reference compound **7** previously reported.¹⁶

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Figure 1.
Lead TRPV1 antagonist template.

Figure 2.

Docking study of **9** in the *h*TRPV1 model. (A) Binding mode and interactions of **9** at the binding site of *h*TRPV1. The important interacting residues are labeled and depicted as capped-stick with their carbon atoms in white color. The secondary structure of *h*TRPV1 is in gray color, and the neighboring monomer helices are shown in line ribbon. Compound **9** is displayed in ball-and-stick with the carbon atoms in magenta color. The van der Waals surface representation of **9** is colored by the lipophilic potential property. Hydrogen bonds are drawn in black dashed lines, and non-polar hydrogens are not displayed for clarity. (B) The Fast Connolly surface representation of *h*TRPV1 and the van der Waals surface representation of the docked **9**. The molecular surface of *h*TRPV1 was generated by MOLCAD and presented with the lipophilic potential property. The surface of *h*TRPV1 is Z-clipped for clarity, and that of **9** is in magenta color. (C) Van der Waals surface of the docked **9** colored by the lipophilic potential property.

Scheme 1.

Syntheses of 2-aryl-6-trifluoromethyl pyridine C-region derivatives (Method A). Reagents and conditions: (a) $R-B(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3 , toluene/1,4-dioxane, reflux, overnight, 55–90%, for 1-imidazole, imidazole, CH_3CN , reflux, overnight, 50%; (b) BH_3-SMe_2 , THF, reflux, overnight, 40–90%; (c) **4**, TBTU, HOBt, DIPEA, THF-DMF, 70–90%.

Scheme 2.

Syntheses of 2-aryl-6-trifluoromethyl pyridine C-region derivatives (Method B). Reagents and conditions: (a) $\text{BH}_3\text{-SMe}_2$, THF, reflux, overnight, 90%; (b) **4**, TBTU, HOBT, DIPEA, THF–DMF, 92%; (c) R-B(OH)_2 , $\text{Pd(PPh}_3)_4$, Na_2CO_3 , toluene–EtOH, 100 °C, microwave, 1 h, 32–98%.

Table 1In vitro *h*TRPV1 antagonistic activities for 2-phenyl derivatives

	R	K_i [CAP] (nM)		R	K_i [CAP] (nM)
7		0.3	17		NE
8		6.4	18		0.2
9		0.4	19		0.4
10		1.3	20		0.9
11		63.3	21		4.2
12		0.7	22		25.6
13		0.5	23		58
14		1	24		5
15		3.2	25		3.4
16		0.5			

Table 2In vitro *h*TRPV1 antagonistic activities for 2-heterocyclic derivatives

	R	K_i [CAP] (nM)		R	K_i [CAP] (nM)
8		6.4	30		36.1
26		0.3	31		WE
27		2	32		WE
28		5.8	33		20.3
29		1.4	34		1

Table 3In vitro *h*TRPV1 antagonistic activities for 2-Ar(alk/alken/alkyn)yl derivatives

	R	K_i [CAP] (nM)		R	K_i [CAP] (nM)
8		6.4	38		0.8
35		141	39		0.6
36		1.4	40		1.8
37		2.2			

Table 4In vitro *h*TRPV1 antagonistic activities of **9** for multiple activators

Activators, parameter	9
CAP, K_i (nM)	0.4
pH, IC_{50} (nM)	130.8
Heat 45 °C, IC_{50} (nM)	25.9
NADA, K_i (nM)	0.84

Table 5

(A) Effect of compound **9** on capsaicin-induced hypothermia in mice (B) analgesic activity of compound **9** on CCI-induced cold allodynia after oral administration in mouse

(A) CAP-induced hypothermia				
Dose (mpk)	0.1	0.3	1	10
Inhibition %	25	38	60	91
(B) CCI model				
Dose (mpk)	0.0001	0.001	0.01	0.1
MPE	21.9 ($\pm$ 4.2)	30.5 ($\pm$ 5.9)	52.9 ($\pm$ 5.6)	47.1 ($\pm$ 8.0)

Data, $n = 10$, mean $\pm$ SEM, * $p < 0.05$ versus vehicle. MPE, maximal possible effect. mpk, mg per Kg.