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Original article

Pyridine C-region analogs of 2-(3-fluoro-4-methylsulfonylaminophenyl)propanamides as potent TRPV1 antagonists

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ABSTRACT

A series of pyridine derivatives in the C-region of *N*-((6-trifluoromethyl-pyridin-3-yl)methyl) 2-(3-fluoro-4-methylsulfonylaminophenyl)propanamides were investigated as *h*TRPV1 antagonists. The SAR analysis indicated that 6-difluorochloromethyl pyridine derivatives were the best surrogates of the C-region for previous leads. Among them, compound **31** showed excellent antagonism to capsaicin as well as to multiple *h*TRPV1 activators. It demonstrated strong analgesic activity in the formalin test in mice with full efficacy and it blocked capsaicin-induced hypothermia *in vivo*.

1. Introduction

The vanilloid receptor TRPV1 has emerged as a promising therapeutic target for treatment of neuropathic and inflammatory pain as well as for a wide range of other conditions [1–3]. TRPV1 functions as a nociceptor activated by elevated temperature, by low pH, and by both endogenous endovanilloids and exogenous agents such as capsaicin [1,2,4–6]. It is further subject to extensive regulation through signaling pathways, such as protein kinase C or protein kinase D, positioning TRPV1 to integrate the multitude of influences impacting the cell [7,8]. In view of the potential of TRPV1 as a therapeutic target, intense effort by many groups has been directed at the development of potent TRPV1 antagonists [9–17] and at understanding of the structural basis for potent ligand binding [18]. We have used the ultrapotent TRPV1 ligand resiniferatoxin as a structural lead for antagonist development [19]. Using this approach, we have developed antagonists with activities in the low nM to sub-nM range [20–24]. Here, we report our recent efforts at further optimization of our lead structures.

Recently, we reported a series of *N*-{(6-trifluoromethyl-pyridin-3-yl)methyl} 2-(3-fluoro-4-methylsulfonylaminophenyl)propanamides, designed by a pharmacophoric combination approach, which proved to be potent hTRPV1 antagonists (Fig. 1) [20–24]. Structurally the antagonistic template can be divided into three pharmacophoric parts which were designated as the A-region (3-fluoro-4-methylsulfonylaminophenyl), the B-region (propanamide), and the C-region ((6-trifluoromethyl-pyridin-3-yl) methyl). The structure activity relationships of the 2-substituent in the pyridine C-region have been investigated extensively by incorporating various functional groups, including amino [20], oxy [21], thio [22], alkyl [23] and aryl [24] groups. In these series, multiple compounds showed highly potent and stereospecific antagonism to *h*TRPV1 activators including capsaicin, pH, heat (45 °C) and NADA. In addition, selected compounds were evaluated for antinociceptive activity in neuropathic pain models, where they showed potent activity and were found to block capsaicin-induced

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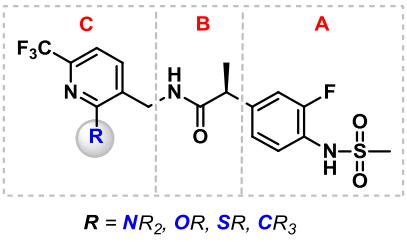


Fig. 1. Chemical structure of previously reported TRPV1 antagonist.

hypothermia, consistent with their *in vitro* mechanism of action. Finally, modeling analysis using our established *h*TRPV1 homology model [18,20] indicated that, among the receptor binding interactions, the two hydrophobic interactions by the 6-trifluoromethyl group and the 2-substituents in the C-region were critical for their potent antagonism and were made with the hydrophobic pockets composed of Leu547/Thr550 and Met514/Leu515, respectively.

In continuation of our effort to further optimize the pyridine C-region, we herein have investigated the structure activity relationships for *h*TRPV1 antagonism of various pyridine derivatives, including isosteres of the 6-trifluoromethyl moiety and isomers of pyridine. In this investigation, 2-substituents in the pyridine were fixed, using selected representative groups which had provided high potency in previous reports. With selected potent antagonists in the series, we have further characterized their analgesic activity and inhibition of capsaicin-induced hypothermia in animal models.

2. Result and discussion

2.1. Chemistry

A series of 6-substituted pyridine C-regions (**6**, **8**), containing R^1 = CH_3, CF_2H, CF_2Cl, cyclopropyl, and Ph-4-F, were synthesized starting from the corresponding anhydride **1** by a modification of the previously reported procedure (Scheme 1) [20]. For the synthesis of the 2-amino (R^2 = NR_2) and 2-thio (R^2 = SR) analogs, 3-cyanopyridone **3** was prepared and then converted into the corresponding 2-chloropyridine **4**, which was condensed with a library of amines and thiols and then reduced to provide the C-region amines **6**. For the synthesis of 2-oxy analogs, 3-amidopyridone **7** was synthesized instead and then converted to the C-region amines **8** via an O-alkylation/reduction sequence. A series of 5-methylpyridine C-regions **11** were synthesized from **9**, a β-methylated form of **2**, employing the same sequences described in Scheme 1 (Scheme 2).

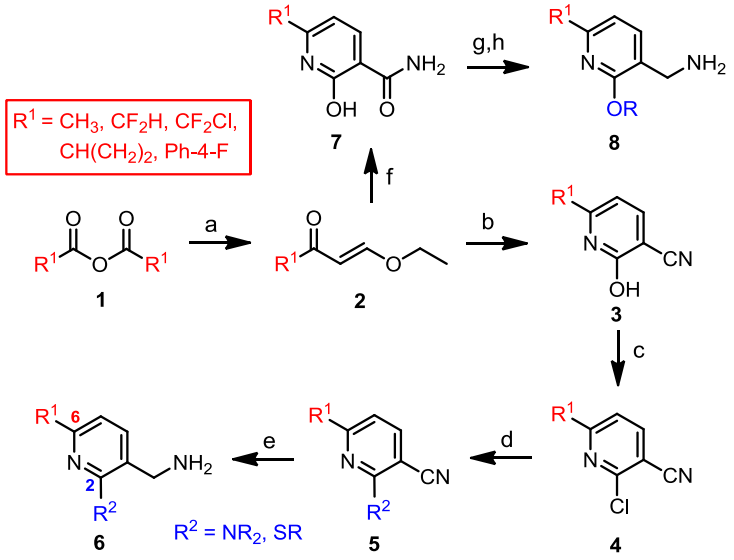
The two regioisomers of the 6-CF_3 pyridine C-region, **15** (5-CF_3-pyridine) and **19** (2-CF_3-pyridine), were synthesized efficiently from appropriate starting material (Scheme 3). For the synthesis of the 5-CF_3-pyridine C-region **15**, 2,3-dichloropyridine **12** was subjected to 2-cyano substitution followed by a Buchwald–Hartwig amination/reduction sequence to provide **15**. For the 2-CF_3-pyridine C-region, only one example **19** (R = Ph) was prepared. Pyridone **17** was synthesized from the ketal **16** in 2 conventional steps and then converted to the corresponding 2-chloropyridine **18**, which was reduced to the amine **19**, concomitant with dehalogenation.

The synthesized amines (**6**, **8**, **11**, **15**, **19**) were coupled with propionic acid [20] as previously reported to afford the final compounds **20**–**46** (Scheme 4).

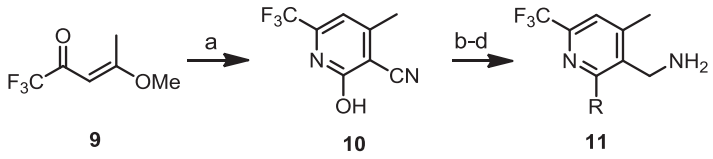
2.2. In vitro activity

The synthesized TRPV1 ligands were evaluated *in vitro* for antagonism as measured by inhibition of activation by capsaicin (CAP). The assays were conducted using a fluorometric imaging plate reader (FLIPR) with human TRPV1 heterologously expressed in Chinese hamster ovary (CHO) cells [20]. The results are summarized in Tables 1–4, together with the potencies of the previously reported antagonists **I**–**V** [20–24].

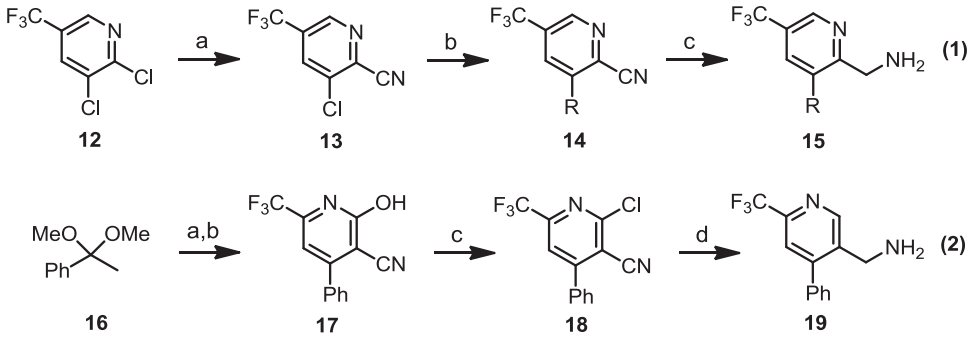
First, we investigated the SAR of the 6-position (R^1) in the pyridine C-region by substituting the 6-trifluoromethyl moiety with the corresponding isosteres, including the methyl, difluoromethyl, difluorochloromethyl, cyclopropyl and 4-fluorophenyl groups (Table 1). The 2-position (R^2) in the pyridine was held fixed as either the 4-methylpiperidinyl or the piperidinyl group, which had provided high antagonistic potency as reference compounds **I** and **II** from the previous report.



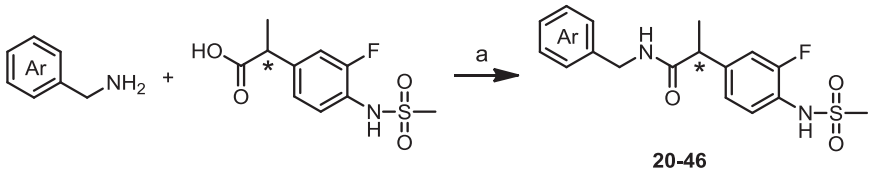
Scheme 1. Syntheses of 6-substituted pyridine C-region analogs. Reagents and conditions: (a) CH_2=CHOEt, pyridine, $CHCl_3$, 0 °C to rt; (b) 2-cyanoacetamide, NaOEt, EtOH, reflux, 3 h; (c) $POCl_3$, sealed tube, 120 °C, 6 h; (d) K_2CO_3, R–NH_2 (or R–SH), CH_3CN; (e) BH_3–SMe_2, THF, 6 h; (f) malonamide, NaOMe, MeOH, reflux, 2 h; (g) RX, K_2CO_3, 18-crown-6 ether, CH_3CN/DMF, reflux, 12 h; (h) BH_3–SMe_2, THF, reflux, 12 h.



Scheme 2. Syntheses of 4-methyl-6-CF_3 pyridine C-region analogs. Reagents and conditions: (a) 2-cyanoacetamide, NaOEt, EtOH, reflux, overnight; (b) $POCl_3$, sealed tube, 120 °C, 6 h; (c) K_2CO_3, R–NH_2, CH_3CN or K_2CO_3, R–OH, CH_3CN; (d) Pd/C (10%), H_2 (1 atm), MeOH, rt, 8 h or BH_3–SMe_2, THF, 6 h.

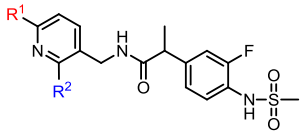


Scheme 3. Syntheses of regioisomeric pyridine C-region analogs. Reagents and conditions: (**1**) (a) NaCN, DMF, 120 °C, 8 h; (b) $PdCl_2$, DPPF, t-BuONa, R–NH_2, toluene; (c) Pd/C (10%), H_2 (1 atm), MeOH, rt, 8 h or BH_3–SMe_2, THF, 6 h; (**2**) (a) TFAA, Py, $CHCl_3$, rt; (b) NaOEt, EtOH, 2-cyanoacetamide; (c) $POCl_3$, sealed tube, 120 °C, 6 h; (d) Pd/C (10%), H_2 (1 atm), MeOH, rt, 8 h or BH_3–SMe_2, THF, 6 h.



Scheme 4. Syntheses of final compounds. Reagents and conditions: (a) HOBt, EDC, CH_3CN, rt.

Table 1
*In vitro h*TRPV1 antagonistic activities for 2,6-substituted pyridine C-region derivatives.



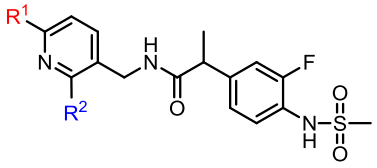
	R^1	R^2	$K_{i\ [CAP]}$ (nM)
I	–CF_3		0.3[a]
20	–CH_3		72.1
21	–CF_2H		1
22	–CF_2Cl		0.3
23			0.9
24			59
II	–CF_3		0.43[a]
25	–CF_2Cl		1.3
26			1.1

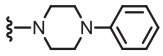
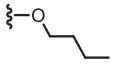
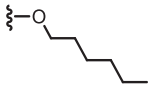
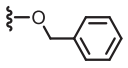
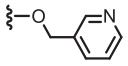
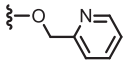
[a] Values from Ref. [20].

The 6-methyl derivative **20** showed a ca. 240-fold reduction in activity compared to the parent **I**. However, the 6-difluoromethyl **21** and the 6-difluorochloromethyl **22** derivatives retained high potency, with **22** showing the same potency as **I**. To investigate the halogen effect of the above two substituents, a cyclopropyl group, which has a lipophilicity similar to that of trifluoromethyl group, was incorporated into the 6-position. The 6-cyclopropyl derivative **23** still exhibited potent antagonism comparable to **21**. We conclude that the lipophilicity of the 6-substituent was more important for potent antagonism than was its electronic effect. This result is likewise predicted from our molecular modeling, in which the 6-trifluoromethyl group in the pyridine C-region made a hydrophobic interaction with a pocket composed of Leu547 and Thr550. To confirm the relative potency of the 6-difluorochloromethyl and cyclopropyl groups, their 2-piperidinyl derivatives **25** and **26** were also prepared and compared to their parent compound **II**. As was the case with **22** and **23**, compounds **25** and **26** showed potent and comparable antagonism to their parent compound (**II**), indicating that the lipophilicity of the substituent at the 6-position was critical for potent antagonism.

Since the 6-difluorochloromethyl group proved to be a promising bioisostere of the 6-trifluoromethyl group above, we explored the SAR of 2-substituents in the 6-difluorochloromethyl pyridine C-region to further evaluate its activity. The various 2-substituents including amino, oxy and thio groups were selected based on their potencies in previous reports [20–24] and activities were compared to those for the corresponding 6-trifluoromethyl surrogates (Table 2). As expected, most 6-difluorochloromethyl pyridine

Table 2
*In vitro h*TRPV1 antagonistic activities for 6-difluorochloropyridine derivatives.



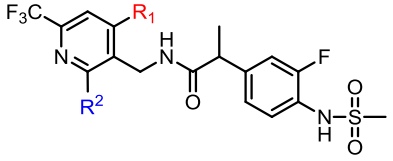
	R^2	$R^1 = CF_2C1$ $K_{i\ [CAP]}$ (nM)	$R^1 = CF_3$ $K_{i\ [CAP]}$ (nM)[a]
27		0.6	0.2
28[b]		1	0.7
29		0.5	0.8
30		0.8	0.7
31		0.3	0.3
32		0.8	0.4
33		0.6	0.5
34		0.5	0.9
35		0.9	1.3
36		0.9	0.5
37		14.6	142
38		4.2	29.5
39		3.0	0.9

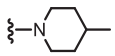
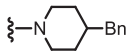
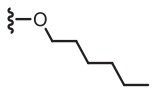
[a] Values from Refs. [20–22].
[b] Mixture of *cis/trans*.

derivatives exhibited very potent antagonism in the low nanomolar range comparable with those of the corresponding 6-trifluoromethyl surrogates. Among them, compounds **31** and **34** with 2-butyloxy and 2-cyclopentyloxy groups were selected as the most promising antagonists for further study.

Next, we sought to evaluate 4-methyl derivatives of selected 6-trifluoromethyl pyridine antagonists to investigate their steric tolerance by the receptor. The four representative derivatives **40**–**43** were synthesized and compared to their corresponding

Table 3
*In vitro h*TRPV1 antagonistic activities for 4-methylpyridine derivatives.



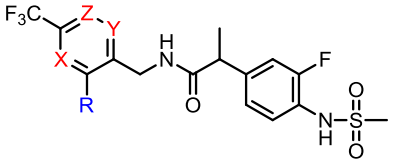
	R^2	$R^1 = CH_3$ $K_{i\ [CAP]}$ (nM)	$R^1 = H$ $K_{i\ [CAP]}$ (nM)[a]
40		17.5	0.3
41		9.2	0.2
42		100	0.4
43		44	0.9

[a] Values from Refs. [20,21].

parents (Table 3). The incorporation of a 4-methyl group to the pyridine led to ca. 50–250 fold reductions in potency compared to those of the corresponding parent antagonists, indicating that the 4-methyl group was poorly tolerated probably by inducing steric repulsion with the receptor or unfavorable conformation of B/C-regions.

Finally, the SAR of pyridine isomers in the C-region was investigated. Due to limitations of synthetic accessibility, only a few representative compounds were prepared and compared to the parents (Table 4). Relative to the parent compounds **III** and **IV**

Table 4
*In vitro h*TRPV1 antagonistic activities for 2 (or 5)-trifluoromethylpyridine derivatives.



	R	X	Y	Z	$K_{i\ [CAP]}$ (nM)
III		N	CH	CH	0.6[a]
44		CH	N	CH	WE
IV		N	CH	CH	0.43[a]
45		CH	N	CH	28.6
V		N	CH	CH	6.4[a]
46		CH	CH	N	32.7

[a] Values from Refs. [20,24].

Table 5
In vitro hTRPV1 antagonistic activities of **31** and **34** for multiple activators.

Activators, parameter	I	31	34
CAP (f)K_i (nM)	0.3	0.3	0.5
pH, IC_{50} (nM)	15.8	8.3	18.5
Heat 45 °C, IC_{50} (nM)	2.56	7.0	19.3
NADA (f)K_i (nM)	0.02	0.02	0.04

(X = N, Y = Z = H), the shift of a nitrogen to the 4-position led to a dramatic reduction in antagonism, with **44** and **45** (Y = N, X = Z = H) proving to be weak and moderate antagonists, respectively. Likewise, relative to the parent compound **V**, the shift of a nitrogen to the 5-position to provide **46** (Z = N, X = Y = H) caused a 5-fold reduction in potency. These results indicate that among the pyridine isomers the 6-trifluoromethyl pyridine was optimal for antagonism.

Detailed *in vitro* activities of **31** and **34**, the most potent antagonists in this study, were investigated for multiple TRPV1 activators, namely capsaicin, pH, heat (45 °C) and *N*-arachidonoyl dopamine (NADA), and were compared to the activity of lead compound **I** (Table 5). Compound **31** showed excellent antagonism toward all activators comparable to **I**, but compound **34** exhibited 2–3 fold less potency than **31**.

2.3. In vivo activity

Consistent with its *in vitro* mechanism of action as an *h*TRPV1 antagonist, *in vivo* **31** and **34** likewise blocked response to capsaicin (Table 6). Compounds **31** and **34** were administered orally at a dose 0.3 mg/kg 15 min before intraperitoneal injection of 3 mg/kg capsaicin, following the procedure described previously [20]. This dose of **31** and **34** inhibited the hypothermic response to capsaicin, assayed 30 min after capsaicin injection, by 43% and 41%, respectively. Compound **34** showed a dose-dependent inhibition in capsaicin-induced hypothermia and almost completely blocked the response at a dose of 10 mg/kg.

We evaluated the *in vivo* analgesic activities of the two selected antagonists, **31** and **34**, in the formalin test [25] in mice upon oral administration (Table 7). Compound **31** showed a significant antinociceptive effect, with 56% and 73% inhibition of response at the doses of 0.1 and 0.3 mg/kg, respectively. Since we had observed that TRPV1 knock-out mice showed approximately 50% of the magnitude of response in the formalin test as was seen in wild-type mice (unpublished observations), the inhibition of the formalin response that we found for this compound would correspond to the expected result for full TRPV1 blockade. Compound **34** showed weaker activity than that of **31** in the formalin assay, inhibiting the nociceptive response by 29% and 15% at the doses of 0.3 and 1.0 mg/kg, respectively.

Table 6
Effect of compounds **31** and **34** on capsaicin-induced hypothermia in mice.

Dose (mpk)	0.1	0.3	1	10
	% Inhibition			
31		43		
34	23	41	60	92

Table 7
Analgesic activity of compound **31** and **34** on formalin model after oral administration in mice.

Dose (mpk)	0.1	0.3	1
	MPE		
31	56	73	
34		29	15

3. Conclusion

The structure activity relationship of pyridine derivatives in the C-region of *N*-(6-trifluoromethyl-pyridin-3-ylmethyl) 2-(3-fluoro-4-methylsulfonylaminophenyl)propanamides was investigated for hTRPV1 antagonism. The analysis indicated that among the current series the 6-difluorochloromethyl pyridine C-region was the most adequate surrogate of the C-region for the leads described previously. The two selected antagonists **31** and **34** showed excellent antagonism to multiple *h*TRPV1 activators and blocked capsaicin-induced hypothermia, consistent with their actions *in vitro* being through TRPV1. Compound **31** demonstrated strong analgesic activity in the formalin test in mice with full TRPV1 efficacy.

4. Experimental

4.1. Chemistry

4.1.1. General

All chemical reagents were commercially available. Melting points were determined on a Büchi Melting Point B-540 apparatus and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230–400 mesh, Merck. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were recorded on JEOL JNM-LA 300 [300 MHz (^{1}H), 75 MHz (^{13}C)] and Bruker Avance 400 MHz FT-NMR [400 MHz (^{1}H), 100 MHz (^{13}C)] spectrometers. Chemical shifts are reported in ppm units with Me_4Si as a reference standard. Infrared (IR) spectra were recorded on a JASCO FT/IR-4200 spectrometer. Mass spectra were recorded on a VG Trio-2 GC-MS and 6460 Triple Quad LC/MS. All final compounds were purified to >95% purity, as determined by high-performance liquid chromatography (HPLC). HPLC was performed on an Agilent 1120 Compact LC (G4288A) instrument using an Agilent Eclipse Plus C18 column (4.6 × 250 mm, 5 μm) and a Daicel Chiralcel OD-H column (4.6 × 250 mm, 5 μm).

4.1.2. General procedure for chemistry

The general synthetic procedure for the syntheses of the final compounds was described in previous reports [20–22].

*4.1.2.1. N-((6-Methyl-2-(4-methylpiperidin-1-yl)pyridin-3-yl) methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**20**).* 75% yield, white solid, mp = 78–80 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (t, 1H, *J* = 8.2 Hz), 7.13 (d, 1H, *J* = 8.6 Hz), 7.07 (d, 2H, *J* = 8.0 Hz), 6.76 (s, 1H), 6.74 (s, 1H), 6.46 (brs, 1H), 4.41 (t, 2H, *J* = 4.4 Hz), 3.51 (q, 1H), 3.18 (m, 2H), 3.02 (s, 3H), 2.78 (m, 2H), 2.42 (s, 3H), 1.73 (m, 2H), 1.50 (d, 3H, *J* = 7.1 Hz), 1.26 (m, 2H), 0.97 (d, 3H, *J* = 6.4 Hz); MS (FAB) *m*/*z* 463 (M+H).

*4.1.2.2. N-((6-(Difluoromethyl)-2-(4-methylpiperidin-1-yl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**21**).* 48% yield, white solid, mp = 105–107 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (t, 2H, *J* = 4.7 Hz), 7.17 (d, 1H, *J* = 8.1 Hz), 7.09 (d, 1H, *J* = 6.0 Hz), 6.48 (s, 1H), 6.42 (brs, 1H), 4.46 (brs, 1H), 3.55 (q, 1H), 3.26 (t, 2H, *J* = 13.2 Hz), 3.02 (s, 3H), 2.79 (t, 2H, *J* = 11.9 Hz), 1.71 (brs, 2H), 1.52 (d, 3H, *J* = 6.6 Hz), 1.19 (m, 2H), 0.97 (d, 3H, *J* = 6.0 Hz); MS (FAB) *m*/*z* 499 (M+H).

*4.1.2.3. N-((6-(Chlorodifluoromethyl)-2-(4-methylpiperidin-1-yl) pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl) propanamide (**22**).* 77% yield, white solid, mp = 175–176 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.53 (m, 2H), 7.07–7.18 (m, 3H), 6.72

(bs, 1H), 6.37 (bt, 1H), 4.46 (d, 2H, $J = 5.7$ Hz), 3.56 (q, 1H, $J = 6.9$ Hz), 3.32 (m, 2H), 3.02 (s, 3H), 2.82 (m, 2H), 1.71 (m, 2H), 1.53 (d, 3H, $J = 7.5$ Hz), 1.23 (m, 3H), 0.97 (d, 3H, $J = 6.9$ Hz); MS (FAB) *m/z* 534 (M+H).

*4.1.2.4. N-((6-Cyclopropyl-2-(4-methylpiperidin-1-yl)pyridin-3-yl) methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**23**).* 58% yield, white solid, mp = 84 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (t, 1H, $J = 8.3$ Hz), 7.23–7.06 (m, 3H), 6.74 (d, 2H, $J = 7.5$ Hz), 6.47 (bs, 1H), 4.40 (t, 2H, $J = 5.9$ Hz), 3.50 (q, 1H, J = 7.1 Hz), 3.18 (m, 2H), 3.01 (s, 3H), 2.72 (m, 2H), 1.90 (m, 1H), 1.69 (m, 2H), 1.50 (d, 3H, $J = 7.1$ Hz), 1.26–1.13 (m, 3H), 0.95 (d, 3H, $J = 6.4$ Hz); MS (FAB) *m/z* 489 (M+H).

*4.1.2.5. N-((6-(4-Fluorophenyl)-2-(4-methylpiperidin-1-yl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**24**).* 52% yield, white solid, mp = 154–156 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49–7.54 (m, 2H), 7.51 (dd, 1H, $J = 8.1$ and 8.1 Hz), 7.43 (d, 1H, 7.8 Hz), 7.29 (d, 1H, $J = 7.8$ Hz), 7.09–7.17 (m, 4H), 6.64 (bt, 1H), 4.48 (d, 2H, $J = 5.7$ Hz), 3.52 (q, 1H, $J = 6.9$ Hz), 3.30 (m, 2H), 3.03 (s, 3H), 2.88 (m. 2H), 1.76 (m, 2H), 1.51 (d, 3H, $J = 6.9$ Hz), 1.24 (m, 3H), 0.99 (d, 3H, $J = 6.6$ Hz); MS (FAB) *m/z* 543 (M+H).

*4.1.2.6. N-((6-(Chlorodifluoromethyl)-2-(piperidin-1-yl)pyridin-3-yl) methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**25**).* 70% yield, pale yellow solid, mp = 63–65 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.52 (m, 2H), 7.06–7.18 (m, 3H), 6.46 (bt, 1H), 4.47 (d, 2H, $J = 5.1$ Hz), 3.58 (q, 1H, $J = 6.9$ Hz), 3.05 (m, 4H), 3.00 (s, 3H), 1.61 (m, 6H), 1.53 (d, 3H, $J = 7.1$ Hz); MS (FAB) *m/z* 520 (M+H).

*4.1.2.7. N-((6-Cyclopropyl-2-(piperidin-1-yl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**26**).* 57% yield, white solid, mp = 65–75 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (t, 1H, $J = 8.4$ Hz), 7.24–7.06 (m, 3H), 6.74 (d, 2H, $J = 7.5$ Hz), 6.47 (bs, 1H), 4.39 (t, 2H, $J = 5.3$ Hz), 3.50 (q, 1H, $J = 7.0$ Hz), 3.01 (s, 3H), 2.92 (m, 4H), 1.90 (m, 1H), 1.55 (m, 2H), 1.50 (d, 3H, $J = 7.1$ Hz), 1.01–0.86 (m, 3H); MS (FAB) *m/z* 475 (M+H).

*4.1.2.8. N-((6-(Chlorodifluoromethyl)-2-(dipropylamino)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**27**).* 58% yield, white solid, mp = 101–103 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (dd, 1H, $J = 8.1$ and 8.1 Hz), 7.43 (d, 1H, $J = 7.8$ Hz), 7.16 (dd, 1H, $J = 2.1$ and 10.8 Hz), 7.08–7.12 (m, 2H), 6.46 (bs, 1H), 6.15 (bt, 1H), 4.44 (d, 2H, $J = 5.7$ Hz), 3.53 (q, 1H, $J = 6.9$ Hz), 3.10 (m, 4H), 3.02 (s, 3H), 1.44–1.54 (m, 4H), 0.83 (t, 6H, $J = 7.2$ Hz), MS (FAB) *m/z* 535 (M+H).

*4.1.2.9. N-((6-(Chlorodifluoromethyl)-2-(3,5-(cis/trans)-dimethylpiperidin-1-yl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**28**).* 58% yield, colorless oil, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45–7.54 (m, 2H), 7.06–7.15 (m, 3H), 6.62 (bs, 1H), 6.31 (bt, 1H), 4.46 (d, 2H, $J = 5.7$ Hz), 3.54 (q, 1H, $J = 7.2$ Hz), 3.25 (m, 2H), 3.02 (s, 3H), 2.36 (m, 2H), 2.03 (m, 1H), 1.53–1.65 (m, 3H), 1.52 (d, 3H, $J = 7.2$ Hz) 0.92 (d, 3H, $J = 6.6$ Hz), 0.88 (d, 3H, $J = 6.6$ Hz); MS (FAB) *m/z* 548 (M+H).

*4.1.2.10. N-((2-(Azepan-1-yl)-6-(chlorodifluoromethyl)pyridin-3-yl) methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**29**).* 58% yield, white solid, mp = 59–61 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (dd, 1H, $J = 8.1$ and 8.1 Hz), 7.38 (d, 1H, $J = 7.5$ Hz), 7.17 (dd, 1H, $J = 1.8$ and 11.1 Hz), 7.08 (d, 1H, $J = 8.1$ Hz), 6.99 (d, 1H, $J = 7.5$ Hz), 6.57 (bs, 1H), 5.87 (bt, 1H), 4.42 (d, 2H, $J = 5.7$ Hz), 3.56 (q, 1H, $J = 6.9$ Hz), 3.39 (t, 4H, $J = 6.0$ Hz), 3.02 (s, 3H), 1.75 (m, 4H), 1.56 (m, 4H), 1.52 (d, 3H, $J = 6.9$ Hz); MS (FAB) *m/z* 534 (M+H).

*4.1.2.11. N-((6-(Chlorodifluoromethyl)-2-(4-phenylpiperazin-1-yl) pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl) propanamide (**30**).* 65% yield, white solid, mp = 108 °C, ^{1}H NMR (300 MHz, CD_3OD) δ 8.60 (m, 1H), 7.58 (d, 1H, $J = 7.5$ Hz), 7.47–7.25 (m, 4H), 7.25–7.00 (m, 4H), 4.57–4.35 (m, 2H), 3.73 (d, 1H, $J = 7.1$ Hz), 3.45–3.32 (m, 8H), 2.95 (bs, 3H), 1.47 (d, 3H, $J = 7.1$ Hz); MS (FAB) *m/z* 597 (M+H).

*4.1.2.12. N-((2-Butoxy-6-(chlorodifluoromethyl)pyridin-3-yl) methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**31**).* 70% yield, white solid, mp = 75–77 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49–7.54 (m, 2H), 7.14 (d, 1H, $J = 7.5$ Hz), 7.05–7.09 (m, 2H), 6.46 (bs, 1H), 6.02 (bt, 1H), 4.36 (m, 4H), 3.52 (q, 1H, $J = 6.9$ Hz), 3.03 (s, 2H), 1.71 (m, 2H), 1.48 (m, 5H), 0.97 (t, 3H, $J = 7.2$ Hz); MS (FAB) *m/z* 508 (M+H).

*4.1.2.13. N-((6-(Chlorodifluoromethyl)-2-(pentyloxy)pyridin-3-yl) methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**32**).* 48% yield, white solid, mp = 86 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58–7.47 (m, 2H), 7.17–7.03 (m, 3H), 6.50 (bs, 1H), 5.98 (bt, 1H), 4.43–4.25 (m, 4H), 3.51 (q, 1H, $J = 8.6$ Hz), 3.03 (s, 3H), 1.78–1.67 (m, 2H), 1.49 (d, 3H, $J = 6.8$ Hz), 1.46–1.27 (m, 6H), 0.94–0.87 (m, 3H); MS (FAB) *m/z* 522 (M+H).

*4.1.2.14. N-((6-(Chlorodifluoromethyl)-2-isobutoxypyridin-3-yl) methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**33**).* 57% yield, white solid, mp = 105 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58–7.48 (m, 2H), 7.16 (d, 1H, $J = 7.5$ Hz), 7.11 (m, 1H), 7.06 (m, 1H), 6.46 (m, 1H), 5.95 (bt, 1H), 4.44–4.32 (m, 2H), 4.19–4.06 (m, 2H), 3.51 (q, 1H, $J = 7.1$ Hz), 3.04 (s, 3H), 2.05 (m, 1H), 1.49 (d, 3H, $J = 7.1$ Hz), 0.99 (d, 6H, $J = 6.8$ Hz); MS (FAB) *m/z* 509 (M+H).

*4.1.2.15. N-((6-(Chlorodifluoromethyl)-2-(cyclopentyloxy)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**34**).* 71% yield, white solid, mp = 106 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57–7.48 (m, 2H), 7.15–7.03 (m, 3H), 6.56 (bs, 1H), 6.01–5.90 (bt, 1H), 5.46 (m, 1H), 4.42–4.27 (m, 2H), 3.52 (q, 1H, $J = 7.1$ Hz), 3.03 (s, 3H), 2.08–1.91 (m, 2H), 1.78–1.56 (m, 6H), 1.49 (d, 3H, $J = 7.1$ Hz); MS (FAB) *m/z* 520 (M+H).

*4.1.2.16. N-((6-(Chlorodifluoromethyl)-2-(cyclohexyloxy)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**35**).* 58% yield, white solid, mp = 97 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59–7.48 (m, 2H), 7.14–7.02 (m, 3H), 6.49 (bs, 1H), 6.01 (bt, 1H), 5.13 (m, 1H), 4.47–4.29 (m, 2H), 3.52 (q, 1H, $J = 7.3$ Hz), 3.03 (s, 3H), 1.99–1.85 (m, 2H), 1.77–1.62 (m, 2H), 1.52–1.38 (m, 9H); MS (FAB) *m/z* 534 (M+H).

*4.1.2.17. N-((2-(Benzyloxy)-6-(chlorodifluoromethyl)pyridin-3-yl) methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**36**).* 52% yield, white solid, mp = 62 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (d, 1H, $J = 7.3$ Hz), 7.50–7.30 (m, 6H), 7.20 (d, 1H, $J = 7.8$ Hz), 7.05 (dd, 1H, $J = 11.2$ and 2.0 Hz), 6.97 (d, 1H, $J = 7.9$ Hz), 6.52 (bs, 1H), 6.00 (bt, 1H), 5.49–5.36 (m, 2H), 4.46–4.30 (m, 2H), 3.42 (q, 1H, $J = 7.1$ Hz), 3.00 (bs, 3H), 1.43 (d, 3H, $J = 7.1$ Hz); MS (FAB) *m/z* 542 (M+H).

*4.1.2.18. N-((6-(Chlorodifluoromethyl)-2-(pyridin-3-ylmethoxy)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**37**).* 61% yield, white solid, mp = 78 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61–8.56 (m, 2H), 7.77 (m, 1H), 7.62 (d, 1H, $J = 7.6$ Hz), 7.48 (dd, 1H, $J = 8.0$ and 8.0 Hz), 7.31 (m, 1H), 7.21 (d, 1H, $J = 7.6$ Hz), 7.09–6.92 (m, 2H), 5.88 (bt, 1H), 5.47–5.37 (m, 2H),

4.43–4.30 (m, 2H), 3.49 (q, 1H, J = 6.8 Hz), 3.03 (s, 3H), 1.28 (d, 3H, J = 6.8 Hz); MS (FAB) m/z 543 (M+H).

*4.1.2.19. N-((6-(Chlorodifluoromethyl)-2-(pyridin-2-ylmethoxy)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**38**).* 52% yield, white solid, mp = 67 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, 1H, J = 4.4 Hz), 7.75 (dd, 1H, J = 7.6 and 7.6 Hz), 7.67 (d, 1H, J = 7.2 Hz), 7.51–7.41 (m, 2H), 7.27–7.19 (m, 2H), 7.13 (dd, 1H, J = 11.2 and 1.6 Hz), 7.04 (d, 1H, J = 8.4 Hz), 6.50 (bs, 1H), 5.63–5.48 (m, 2H), 4.61–4.40 (m, 2H), 3.60 (q, 1H, J = 7.2 Hz), 3.05 (s, 3H), 1.49 (d, 3H, J = 7.2 Hz); MS (FAB) m/z 543 (M+H).

*4.1.2.20. N-((6-(Chlorodifluoromethyl)-2-(cyclohexylthio)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**39**).* 66% yield, white solid, mp = 70–73 °C, ^{1}H NMR (300 MHz, CD_3OD) δ 7.35–7.48 (m, 3H), 7.26 (d, 1H, J = 7.8 Hz), 7.16 (dd, 1H, J = 1.8 and 11.1 Hz), 7.10 (d, 1H, J = 8.4 Hz), 6.13 (bs, 1H), 4.35 (d, 2H, J = 5.7 Hz), 3.82 (m, 1H), 3.56 (q, 1H, J = 7.2 Hz), 3.02 (s, 3H), 2.06 (m, 2H), 1.75 (m, 2H), 1.49 (d, 3H, J = 7.2 Hz), 1.26–1.33 (m, 6H); MS (FAB) m/z 550 (M+H).

*4.1.2.21. N-((4-Methyl-2-(4-methylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**40**).* 66% yield, white solid, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51–7.46 (t, 1H, J = 8.0 Hz), 7.14 (s, 1H), 7.07–7.02 (m, 2H), 6.80 (brs, 1H), 4.52 (d, 2H, J = 5.3 Hz), 3.48 (q, 1H), 3.17 (d, 1H, J = 11.9 Hz), 3.02 (s, 3H), 2.78 (tt, 2H, J = 11.0 and 2.6 Hz), 2.38 (s, 3H), 1.71 (m, 2H), 1.47 (d, 3H, J = 7.0 Hz), 1.26–1.13 (m, 2H), 0.97 (d, 3H, J = 6.6 Hz), 0.88 (m, 1H); MS (FAB) m/z 531 (M+H).

*4.1.2.22. N-((2-(4-Benzylpiperidin-1-yl)-4-methyl-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**41**).* 59% yield, white solid, mp = 167 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (dd, 1H, J = 8.3 and 8.3 Hz), 7.35–7.27 (m, 2H), 7.25–7.11 (m, 4H), 7.10–6.98 (m, 2H), 6.70 (bt, 1H), 4.58–4.42 (m, 2H), 3.45 (q, 1H, J = 7.1 Hz), 3.21–3.02 (m, 2H), 2.99 (s, 3H), 2.83–2.68 (m, 2H), 2.58 (d, 2H, J = 6.6 Hz), 2.37 (s, 3H), 1.80–1.64 (m, 3H), 1.47 (d, 3H, J = 7.1 Hz), 1.32–1.78 (m, 2H); MS (FAB) m/z 607 (M+H).

*4.1.2.23. N-((2-(Hexyloxy)-4-methyl-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**42**).* 44% yield, white solid, ^{1}H NMR (300 MHz, $ClCl_3$) δ 7.50 (dd, 1H, J = 8.3 Hz), 7.03 (m, 3H), 6.03 (bt, 1H), 4.42 (m, 2H), 4.29 (m, 2H), 3.45 (q, 1H, J = 7.6 Hz), 3.02 (s, 3H), 2.47 (s, 3H), 1.69 (m, 2H), 1.45 (d, 3H, J = 7.1 Hz), 1.35 (m, 2H), 1.25 (m, 2H), 0.92 (m, 5H); MS (FAB) m/z 534 (M+H).

*4.1.2.24. N-((2-(Cyclopentyloxy)-4-methyl-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**43**).* 75% yield, white solid, mp = 92–98 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (t, 1H, J = 8.3 Hz), 7.03 (m, 3H), 5.96 (bs, 1H), 5.43 (m, 1H), 4.45–4.33 (m, 2H), 3.46 (q, 1H, J = 7.1 Hz), 3.02 (s, 3H), 2.48 (s, 3H), 1.99–1.96 (m, 2H), 1.65–1.55 (m, 6H), 1.46 (d, 3H, J = 6.9 Hz); MS (FAB) m/z 518 (M+H).

*4.1.2.25. N-((3-(Pyrrolidin-1-yl)-5-(trifluoromethyl)pyridin-2-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**44**).* 85% yield, white solid, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (s, 1H), 7.52 (t, 1H, J = 8.4 Hz), 7.41 (bs, 1H), 7.19 (t, 2H, J = 8.4 Hz), 6.41 (bs, 1H), 4.59–4.54 (dq, 2H, J = 12.1 and 4.2 Hz), 3.68 (q, 1H, J = 7.3 Hz), 3.30 (m, 4H), 3.01 (s, 3H), 1.99 (m, 4H), 1.55 (d, 3H, J = 7.1 Hz); MS (FAB) m/z 489 (M+H).

*4.1.2.26. N-((2-(4-Methylbenzylthio)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**45**).* 81% yield, white solid, ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.54–7.49 (m, 2H), 7.23–7.15 (m, 2H), 6.63 (bs, 1H), 4.67–4.52 (dq, 2H, J = 13.5 and 4.4 Hz), 3.68 (q, 1H, J = 7.5 Hz), 3.01 (s, 3H), 2.96–2.83 (m, 4H), 1.74–1.60 (m, 4H), 1.59 (m, 2H), 1.55 (d, 2H, J = 7.1 Hz); MS (FAB) m/z 503 (M+H).

*4.1.2.27. N-((4-Phenyl-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (**46**).* 54% yield, colorless oil, ^{1}H NMR (300 MHz, CD_3OD) δ 8.55 (s, 1H), 7.46–7.53 (m, 5H), 7.24–7.27 (m, 2H), 7.05 (dd, 1H, J = 11.1 and 1.8 Hz), 6.99 (d, 1H, J = 8.1 Hz), 6.62 (bs 1H), 5.67 (bt, 1H), 4.49 (d, 2H, J = 5.7 Hz), 3.45 (q, 1H, J = 6.9 Hz), 3.04 (s, 3H), 1.45 (d, 3H, J = 6.9 Hz); MS (FAB) m/z 496 (M+H).

4.2. Biological assay

The methods for *in vitro* and *in vivo* assays were reported in the previous literature [16,17]. All animal protocols were approved by the institutional review committee at Grunenthal Innovations.

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