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2-(3-Fluoro-4-methylsulfonylaminophenyl)propanamides as Potent Transient Receptor Potential Vanilloid 1 (TRPV1) Antagonists: Structure−Activity Relationships of 2-Amino Derivatives in the *N*-(6-Trifluoromethylpyridin-3-ylmethyl) C-Region

Myeong Seop Kim,[†] HyungChul Ryu,[†] Dong Wook Kang,[†] Seong-Hee Cho,[†] Sejin Seo,[†] Young Soo Park,[†] Mi-Yeon Kim,[†] Eun Joo Kwak,[†] Yong Soo Kim,[†] Rahul S. Bhondwe,[†] Ho Shin Kim,[†] Seul-gi Park,[‡] Karam Son,[‡] Sun Choi,[‡] Ian A. DeAndrea-Lazarus,[§] Larry V. Pearce,[§] Peter M. Blumberg,[§] Robert Frank,[∥] Gregor Bahrenberg,[∥] Hannelore Stockhausen,[∥] Babette Y. Kögel,[∥] Klaus Schiene,[∥] Thomas Christoph,[∥] and Jeewoo Lee*[,†]

[†]Laboratory of Medicinal Chemistry, Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, Korea

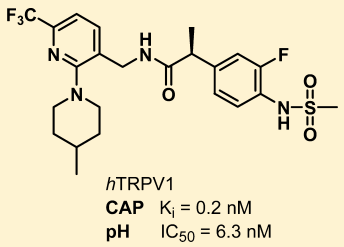
[‡]College of Pharmacy, Division of Life and Pharmaceutical Sciences, and National Core Research Center for Cell Signaling and Drug Discovery Research, Ewha Womans University, Seoul 120-750, Korea

[§]Laboratory of Cancer Biology and Genetics, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, United States

[∥]Grunenthal Innovation, Grunenthal GmbH, D-52078 Aachen, Germany

Supporting Information

ABSTRACT: A series of *N*-(2-amino-6-trifluoromethylpyridin-3-ylmethyl)-2-(3-fluoro-4-methylsulfonylaminophenyl)propanamides were designed combining previously identified pharmacophoric elements and evaluated as hTRPV1 antagonists. The SAR analysis indicated that specific hydrophobic interactions of the 2-amino substituents in the C-region of the ligand were critical for high hTRPV1 binding potency. In particular, compound **49*S*** was an excellent TRPV1 antagonist ($K_{i(CAP)}$ = 0.2 nM; $IC_{50(pH)}$ = 6.3 nM) and was thus approximately 100- and 20-fold more potent, respectively, than the parent compounds **2** and **3** for capsaicin antagonism. Furthermore, it demonstrated strong analgesic activity in the rat neuropathic model superior to **2** with almost no side effects. Compound **49*S*** antagonized capsaicin induced hypothermia in mice but showed TRPV1-related hyperthermia. The basis for the high potency of **49*S*** compared to **2** is suggested by docking analysis with our hTRPV1 homology model in which the 4-methylpiperidinyl group in the C-region of **49*S*** made additional hydrophobic interactions with the hydrophobic region.



INTRODUCTION

The transient receptor potential V1 (TRPV1) receptor is a molecular integrator of nociceptive stimuli, located predominantly in primary sensory neurons.[1] The receptor functions as a ligand-gated and nonselective cation channel with high Ca^{2+} permeability, activated by endogenous agonists including protons,[2] noxious heat,[3] inflammatory lipid mediators such as anandamide,[4] and lipoxygenase products,[5] as well as by natural products such as capsaicin (CAP)[6] and resiniferatoxin (RTX).[7]

The increase in intracellular Ca^{2+} upon TRPV1 activation causes excitation of the primary sensory neurons and the consequent central perception of pain. TRPV1 antagonists inhibit this transmission of nociceptive signaling from the periphery to the CNS as well as block other pathological states associated with this receptor. In recent years a number of TRPV1 antagonists have been developed as novel analgesic and antiinflammatory agents, particularly for the treatment of chronic pain and inflammatory hyperalgesia.[8] The clinical development and therapeutic potential of TRPV1 antagonists have been extensively reviewed.[9−13]

Previously, we identified a potent and stereospecific antagonist, (*S*)-*N*-(4-*tert*-butylbenzyl)-2-(3-fluoro-4-methanesulfonylaminophenyl)propanamide (**2**),[14] which exhibited better binding affinity and more potent antagonism for both rTRPV1 and hTRPV1 in CHO cells compared to the prototype thiourea antagonist **1**[15] (Figure 1). Its activity was stereospecific with marked selectivity for the (*S*)-configuration, whereas the (*S*)-isomer was ∼2-fold more potent than the racemate, and the (*R*)-isomer was 30- to 40-fold weaker. A docking study of **2** with our hTRPV1 homology model demonstrated a novel aspect of its binding to the receptor and identified crucial hydrogen bonds between the ligand and the receptor contributing to its stereospecific potency.[14]

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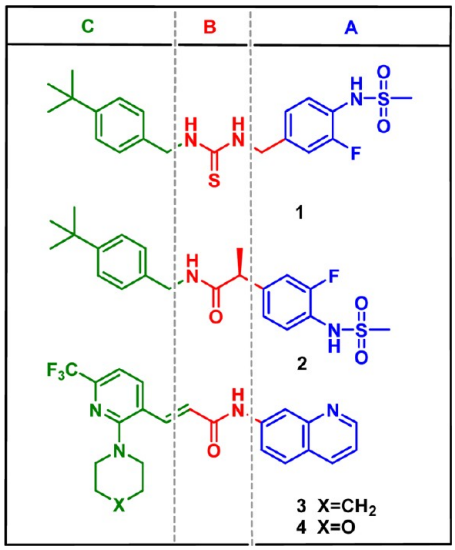


Figure 1. Lead TRPV1 antagonists.

To further optimize the antagonistic acitivity of the lead **2**, structural modifications were performed based on the three principal pharmacophores (A-region, (4-methylsulfonylamino)-phenyl; B-region, propanamide; C-region, 4-*tert*-butylbenzyl). However, none of the compounds were found to be better than **2** in terms of both binding affinity and antagonism to capsaicin activation for rTRPV1 in CHO cells.[14,16]

A series of *N*-arylcinnamides had been reported by the Amgen group[17] as potent antagonists for rat−human chimeric TRPV1 expressed in CHO cells. In that series, compounds **3** and **4** (Figure 1), which had 2-(piperidin-1-yl and morpholino)-6-(trifluoromethyl)pyridin-3-yl moieties, respectively, exhibited potent antagonism of activation of TRPV1 by capsaicin and acid and further displayed good oral bioavailability.

Combining the 2-(3-fluoro-4-methylsulfonaminophenyl)-propanamide (the A and B regions in **2**) and 2-(piperidinyl or morpholinyl)-6-trifluoromethylpyridin-3-ylmethyl groups (C-region of **3** and **4**) provided the novel designed compounds **45** and **97**. Their syntheses and biological evaluation indicated that they exhibited highly potent antagonism toward both capsaicin and pH for hTRPV1 in CHO cells, in which compounds **45** and **97** showed 46- and 15-fold enhanced potency in capsaicin antagonism compared to parent **2**, respectively. This preliminary result prompted us to investigate extensively the structure−activity relationship of this template.

In this study, we investigated the structure−activity relationships for 2-amino substituted analogues (**12**−**99**) of the template *N*-(6-trifluoromethylpyridin-3-ylmethyl)-2-(3-fluoro-4-methylsulfonylaminophenyl)propanamide (Figure 2) as hTRPV1 antagonists and we further characterized analgesic activity in a neuropathic pain model and performed molecular modeling with our hTRPV1 homology model for the most potent antagonist in the series.

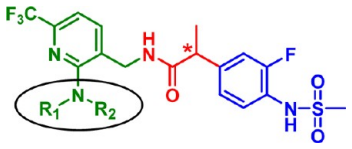
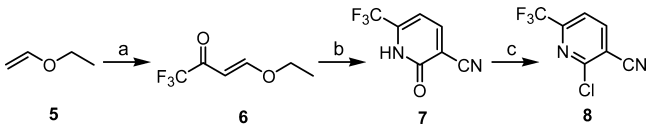


Figure 2. General structure of designed compounds.

RESULTS AND DISCUSSION

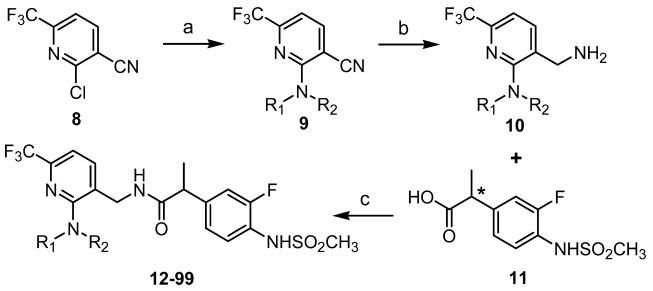
Chemistry. The key intermediate of the C-region **8** was synthesized starting from ethyl vinyl ether **5** in three steps by a modification of the previously report procedure,[18] as shown in Scheme 1. Trifluoromethyl acetylation of **5** followed by condensation with cyanoacetamide provided pyridone **7**, which was readily converted to 2-chloropyridine by $POCl_3$. The syntheses of final compounds are represented in Scheme 2.

Scheme 1. Synthesis of 2-Chloro-3-cyano-6-trifluoromethylpyridine[a]



[a]Reagents and conditions: (a) $(CF_3CO)_2O$, pyridine, $CHCl_3$; (b) $NCCH_2CONH_2$, K_2CO_3, toluene; (c) $POCl_3$.

Scheme 2. Syntheses of 2-(3-Fluoro-4-methylsulfonylaminophenyl)propanamide Analogues[a]



[a]Reagents and conditions: (a) [method A] neat NR_1R_2; [method B] NR_1R_2, $Pd(OAc)_2$, dppf, Na_2CO_3, toluene−THF (7:1); [method C] NR_1R_2, K_2CO_3, 18-crown-6, CH_3CN; [method D] DBU, CH_3CN; (b) [method A] 2 M BH_3−SMe_2 in THF; [method B] $NaBH_4$, $NiCl_2{\cdot}6H_2O$ (or $CoCl_2{\cdot}6H_2O$), MeOH; [method C] H_2, 10% Pd−C, conc HCl, MeOH; (c) EDC, HOBt, TEA, CH_3CN.

Amines were reacted with **8** to afford 2-amino substituted pyridines **9**, and then their nitrile groups were reduced to yield the corresponding primary amines **10**.[17] The amines were coupled with racemic or chiral 2-(3-fluoro-4-methylsulfonylaminophenyl)propanamide **11** to give the final compounds **12**−**99**.

Structure−Activity Relationship (SAR) Analysis. The synthesized TRPV1 ligands were evaluated in vitro for antagonism as measured by inhibition of activation by four separate stimuli (capsaicin (CAP), pH, heat, and *N*-acetyldopamine (NADA)), as indicated. The assays were conducted using a fluorometric imaging plate reader (FLIPR) with human TRPV1 heterologously expressed in Chinese hamster ovary (CHO) cells. The results are summarized in Tables 1−5, together with the potencies of the previously reported parent antagonists **1**−**4**.

The 2-unsubstituted analogue **12** was initially prepared but was found to be a weak antagonist, indicating that a hydrophobic substituent at the 2-position was necessary for substantial activity. The SAR for derivatives incorporating an amino group into the 2-position of the pyridine C-region was investigated starting from 2-acyclic amino derivatives (Table 1). The secondary amino derivatives, incorporating alkylamino

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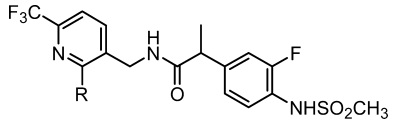
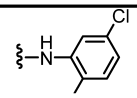
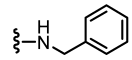
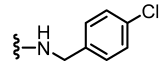
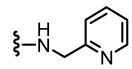
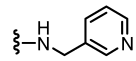
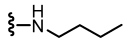
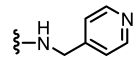
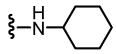
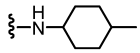
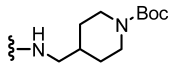
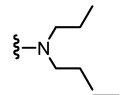
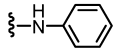
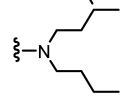
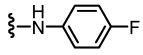
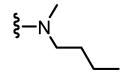
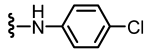
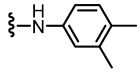
Table 1. In Vitro hTRPV1 Antagonistic Activities for 2-Acylic Amino Derivatives



	R	K_i [CAP] (nM)	IC_{50} [pH] (nM)		R	K_i [CAP] (nM)	IC_{50} [pH] (nM)
1		26	NE	**21**		14	140
2		20	1210	**22**		15.8	203
3		4.4	NE	**23**		65.7	414
4		1.2	116	**24**		WE	WE
12	H	WE	NE	**25**		NE	NE
13		9.1	293	**26**		WE	WE
14		20.4	260	**27**		19.1	2390
15		27.2	1130	**28**		3.5	148
16		19.3	1140	**29**		0.2	14.7
17		59.6	1400	**30**		0.6	10.5
18		39.2	WE	**31**		0.8	21.3
19		WE	WE	**32**		0.9	57.4
20		39.3	1160				

13–**16**, arylamino **17**–**21**, and benzylamino **22**–**26** groups, showed reasonable potencies for CAP antagonism, with values for $K_{i(CAP)}$ ranging from 9.1 to 65.7 nM. Exceptions were the 4-chloroanilino **19** and pyridinylmethylamino **24**–**26** analogues, which were devoid of activity.

Tertiary acyclic amino analogues **27**–**32** were also examined. The activity was enhanced as the number of carbons in the chain increased, and the derivatives exhibited much better potencies than the corresponding secondary amino surrogates (for example, **13** vs **28** and **14** vs **29**). In particular, the dipropylamino analogue **29** and the dibutylamino analogue **30** displayed excellent antagonism toward both CAP and pH (for **29**, $K_{i(CAP)}$ = 0.2 nM, $IC_{50(pH)}$ = 14.7 nM; for **30**, $K_{i(CAP)}$ = 0.6 nM, $IC_{50(pH)}$ = 10.5 nM). The methylbutylamino analogue **31** (five carbons) showed a potency between those of **28** (four carbons) and **29** (six carbons), suggesting that the lipophilicity is a contributor to the activity. The methylphenylamino analogue **32** showed a significant increase in potency (75-fold for CAP, 25-fold for pH) compared to the phenylamino analogue **17**, indicating that an NH in a secondary amino group was detrimental to antagonism.

Next, we sought to evaluate the SAR for monoazacyclic rings. The introduction of unsubstituted azacyclic rings, including 1-pyrrolidinyl **34**, 1-piperidinyl **45**, 1-azepanyl **79**, and 1-azocanyl **80** analogues, provided potent antagonists with subnanomolar activities ($K_{i(CAP)}$ = 0.43–1.3 nM) regardless of ring size.

In the SAR of substituted 1-pyrrolidinyl analogues (Table 2), we restricted our syntheses to the chiral propionic acid **11*S*** at the condensation step because the substituted pyrrolidines were themselves chiral. The 2-methylpyrrolidine analogue **34**

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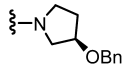
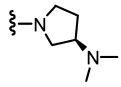
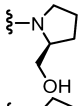
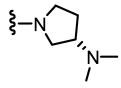
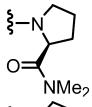
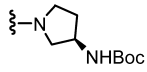
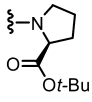
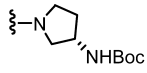
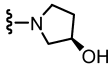
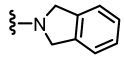
Table 2. In Vitro hTRPV1 Antagonistic Activities for 2-Pyrrolidinyl Derivatives[a]

	R	K_i [CAP] (nM)	IC_{50} [pH] (nM)		R	K_i [CAP] (nM)	IC_{50} [pH] (nM)
33		0.6	211	**39***		1.4	50
34*		0.3	WE	**40***		101	WE
35*		WE	NE	**41***		103	WE
36*		WE	NE	**42***		11	757
37*		2.6	209	**43***		7.1	737
38*		WE	WE	**44**		5	192

[a]The asterisk (*) indicates (*S*)-propanamide.

showed enhanced antagonism to CAP but markedly reduced antagonism to pH compared to the pyrrolidine analogue **33**. The structural modification of the 2- or 3-position of pyrrolidine resulted in a similar SAR pattern in which the hydrophilic substituents (**35**, **36**, **38**, **40**, **41**) led to the loss of activity whereas the hydrophobic ones (**37**, **39**, **42**–**44**) retained potency. The stereochemistry of the substituents did not affect the antagonism (**40** vs **41** and **42** vs **43**).

The SAR of six-membered azacyclic analogues was investigated next (Table 3). The 1-piperidinyl analogue **45** exhibited excellent antagonism; its activity was stereospecific, with the (*S*)-isomer **45*S*** ($K_{i(CAP)}$ = 0.3 nM) representing the active configuration. The potency of **45*S*** was ~15-fold higher than that of the lead **3**, which has the same C-region, indicating that the 2-(3-fluoro-4-methylsulfonaminophenyl)propanamide template for the A and B regions was superior to that of the arylcinnamide for antagonism. The tetrahydropyridinyl analogue **46** was highly potent like **45**. The methylpiperidinyl derivatives **47**–**49** were examined, and the 4-methyl-1-piperidinyl analogue **49** exhibited stereospecific, potent antagonism toward both CAP and pH. The active isomer **49*S*** was found to be the most potent antagonist in this study with $K_{i(CAP)}$ = 0.2 nM and $IC_{50(pH)}$ = 6.3 nM. Its potency was thus 100-fold and 200-fold better than the reference propamide **2** for CAP and pH antagonism, respectively. The structural analysis comparing **2** and **49*S*** indicated that the additional 4-methylpiperidine moiety in **49*S*** provided a new hydrophobic interaction with the receptor, which could explain the enhanced potency of **49*S***. The docking analysis using our hTRPV1 homology model will be described in the next section.

The impressive potency of **49** prompted us to investigate a variety of 4-substituted piperidinyl analogues, **50**–**78**. As demonstrated in the SAR of the pyrrolidines, most hydrophobic 4-substituents provided significant antagonism. Conversely, incorporation of hydrophilic substituents, such as the 4-keto **64**, the 4-hydroxyl **65**, the 4-pyrrolidinyl **75**, and the 4-piperidinyl **76**, caused a dramatic loss of activity. Interestingly, a series of 4-benzyl-1-piperidinyl analogues, **57**–**60**, exhibited great potencies, suggesting that there is a large hydrophobic pocket in TRPV1 sufficient to accommodate the benzyl group.

As piperidine surrogates, the 4-substituted piperazinyl **81**–**96** and morpholinyl **97**–**99** analogues were examined (Table 4). The 1-piperazinyl analogue **81** proved to be a weak antagonist; the 4-methylpiperidyl analogue **82** showed slightly better activity. The introduction of a more lipophilic group into the 4-position of piperazine led to an improvement in the antagonism except in the case of the 4-pyridyl analogue **92**, confirming that a hydrophobic substituent at the 4-position is a determinant for high potency. Among the morpholinyl analogues, the 1-morpholinyl analogue **97** and the 2,6-dimethylmorpholinyl analogue **98** exhibited potent antagonism; the 4,4-dioxothiomorpholinyl analogue **99** showed only weak potency reflecting its polar sulfonyl group.

Detailed in vitro activity of **49*S***, the most potent antagonist in this study, was investigated for multiple TRPV1 activators including capsaicin, pH, heat (45 °C), and NADA and compared to the activity of lead **2** (Table 5). Compound **49*S*** showed excellent antagonism of all four TRPV1 activators and was approximately 140- to 660-fold more potent than **2**.

Selectivity of compound **49*S*** was assessed at 10 μM against a panel of 135 other receptors and enzymes (CEREP). Even at this concentration, which was 4 orders of magnitude higher than its K_i for capsaicin, **49*S*** was negative for all but seven targets and gave greater than 50% inhibition for only three. While detailed mechanistic studies were not carried out, we confirmed that **49*S*** inhibited [^{3}H]resiniferatoxin binding to human TRPV1 (data not shown), as has been repeatedly observed for structurally related TRPV1 antagonists. [^{3}H]-Resiniferatoxin binding provides a convenient measure for ligand interaction at the capsaicin binding site on TRPV1. We conclude that **49*S*** is exerting its antagonistic activity, as fully expected, at the capsaicin binding site rather than as a channel blocker.

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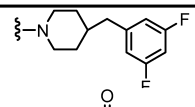
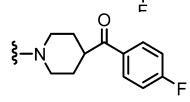
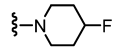
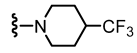
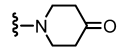
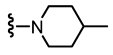
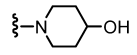
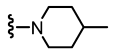
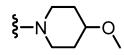
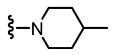
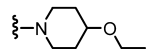
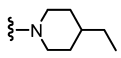
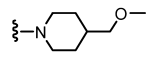
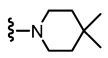
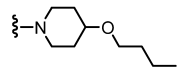
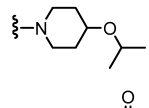
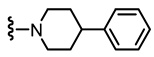
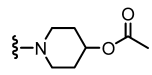
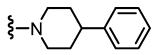
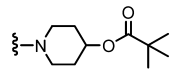
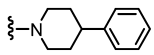
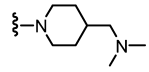
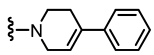
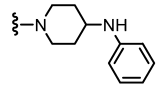
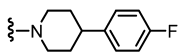
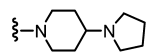
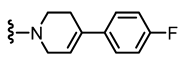
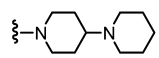
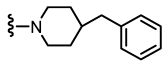
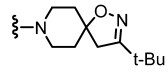
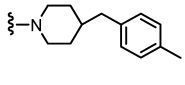
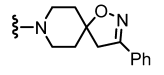
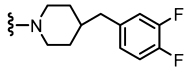
Table 3. In Vitro hTRPV1 Antagonistic Activities for 2-Piperidinyl Derivatives

	R	K_i [CAP] (nM)	IC_{50} [pH] (nM)		R	K_i [CAP] (nM)	IC_{50} [pH] (nM)
45		0.43	218	**60**		0.4	24
45*S*		0.3	46.7	**61**		3.9	990
46		0.3	12.1	**62**		0.5	19.8
47		2.9	WE	**63**		0.6	31
48		0.8	WE	**64**		91	WE
49		0.3	8.4	**65**		NE	NE
49*S*		0.2	6.3	**66**		2.4	280
49*R*		9.8	254	**67**		1.3	612
50		1.5	97.3	**68**		1.4	31
51		0.3	8	**69**		3.5	28.2
52		0.7	25.6	**70**		3	WE
53		0.6	25	**71**		4	139
53*S*		0.3	17.4	**72**		2	45.2
53*R*		7.5	2350	**73**		2.1	426
54		1.7	43	**74**		4.4	480
55		4.2	81	**75**		WE	WE
56		2.4	159	**76**		WE	WE
57		0.2	40	**77**		3.8	281
58		0.2	17	**78**		6.1	289
59		0.2	30				

In Vivo Activity. As part of the initial in vivo characterization of **49*S***, analgesic activity of **49*S*** was evaluated orally in the rat Bennett model[19] as a neuropathic pain model and its activity was compared to that of parent **2** (Figure 3). The

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Table 4. In Vitro hTRPV1 Antagonistic Activities for 2-Piperazinylpyridines and 2-Morpholinylpyridines

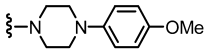
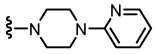
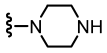
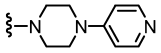
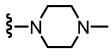
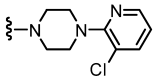
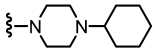
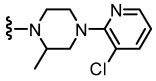
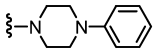
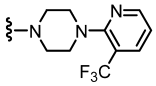
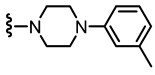
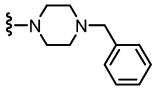
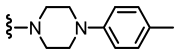
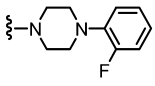
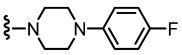
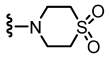
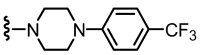
	R	K_i [CAP] (nM)	IC_{50} [pH] (nM)		R	K_i [CAP] (nM)	IC_{50} [pH] (nM)
79		0.8	25.1	**90**		2.5	44.5
80		0.7	27.8	**91**		1.4	108
81		WE	WE	**92**		WE	WE
82		146	WE	**93**		2.1	117
83		21.6	WE	**94**		4.1	WE
84		0.7	30.7	**95**		2.8	WE
85		3.4	39.2	**96**		8.8	634
86		3.3	38.3	**97**		1.3	169
87		2.4	34	**98**		1.43	1090
88		1.1	23.1	**99**		WE	WE
89		2.3	117				

Table 5. In Vitro hTRPV1 Antagonistic Activities for 2 and 49S to Multiple Activators

activator, parameter	2	**49S** (nM)
CAP, (f)K_i (nM)	28	0.2
pH, IC_{50} (nM)	1281	6.3
heat 45 °C, IC_{50} (nM)	174	0.8
NADA, (f)K_i (nM)	6.6	0.01

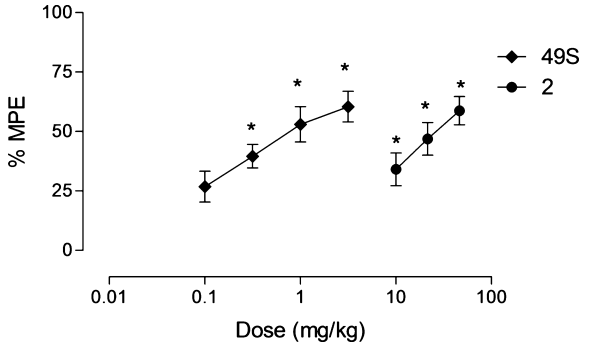


Figure 3. Effect of compound **2** (30 min after po administration) and compound **49S** (45 min after po administration) on CCI-induced cold allodynia in rats. Data are presented as the mean ± SEM; (*) $p < 0.05$ vs vehicle.

analgesic potency of **49S** demonstrated dose-dependent efficacy with ED_{50} = 0.9 mg/kg po (max 60% at 3.16 mg/kg) and was superior to that of **2**. Side effects like sedation or decreased locomotion were not observed.

Consistent with its in vitro mechanism of action, in vivo **49S** also blocked response to capsaicin (Figure 4A). The intraperitoneal injection of 3 mg/kg capsaicin resulted in a decrease of body temperature as expected,[20,21] with a reduction from 37.1 ± 0.1 to 34.0 ± 0.1 °C (15 min after capsaicin injection; $p < 0.05$). By 30 min body temperature had returned to normal. The oral administration of 0.3 mg/kg **49S** (15 min before capsaicin injection) completely inhibited the effect of capsaicin on body temperature (Figure 4A).

Furthermore, we could demonstrate that **49S** targeted TRPV1 in vivo. Although TRPV1 knockout mice show normal body temperature,[22] induction of modest hyperthermia is a common acute side effect of administration of TRPV1 antagonists.[23] We therefore examined the effect on body temperature of administration of **49S**. As illustrated in Figure 4, oral administration of 1 mg/kg **49S** in C57BL/6J wild type mice induced a body temperature increase of about 1.3 ± 0.1 °C 45 and 60 min after substance administration. In TRPV1 knockout mice (Figure 4B) this hyperthermic response was completely absent.

Molecular Modeling. Compound **49S** was identified as a highly potent antagonist showing ~100-fold greater potency compared to prototype **2**, with this enhancement in potency

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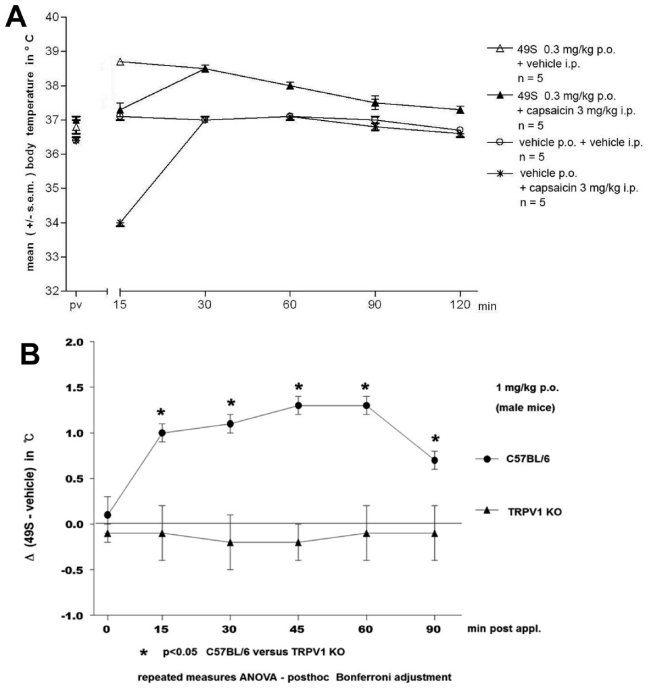

Figure 4. Effect of compound **49S** on body temperature: (A) effect of **49S** on capsaicin-induced hypothermia; (B) effect of **49S** on body temperature in wild-type and TRPV1 KO mice.

being attributable to an additional 4-methylpiperidinyl group in **49S**. To clarify the basis for this enhanced activity, we used our hTRPV1 homology model to compare the binding interactions of **49S** and **2**.

We constructed the tetramer homology model of human TRPV1 (hTRPV1) based on our rat TRPV1 model[24] through in silico mutation and refinement by energy minimization. Using our hTRPV1 model, we then performed the flexible docking study of **2** and **49S** to investigate their binding interactions and found compound **2** fitted well into the binding site (Figure 5). The sulfonylaminobenzyl group (A-region) occupied the deep bottom hole and was involved in a hydrophobic interaction with Tyr511. An oxygen atom and NH of the sulfonamide group participated in hydrogen bonding with Ser512 and Ile564. The amide group (B-region) made a hydrogen bond with Tyr511 and also contributed to the appropriate positioning of the C-region for the hydrophobic interaction. The hydrophobic 4-*tert*-butylbenzyl group (C-region) was oriented toward the upper hydrophobic region of the binding site and formed the hydrophobic interaction with Leu547.

As we expected, compound **49S** showed an excellent fit to the binding site and its 4-methylpiperidine ring in the C-region made an additional hydrophobic interaction with hTRPV1 (Figure 6). The sulfonylaminophenyl and amide moieties (A and B regions) made tight interactions with the binding site residues via the hydrophobic interaction with Tyr511 and hydrogen bonding with Tyr511, Ser512, and Ile564 as shown for **2**. The 3-trifluoromethyl group in the C-region, like the 4-*tert*-butylbenzyl group of **2**, formed a hydrophobic interaction with Leu547. Furthermore, the 4-methylpiperidinyl group in the C-region made an additional hydrophobic interaction with the hydrophobic region composed of Met514 and Leu515. That might explain why the activity of **49S** was dramatically increased, compared with that of **2**.

CONCLUSION

A variety of 2-amino substituted 6-trifluoromethylpyridin-3-ylmethylamines as the C-region were coupled with the well characterized A,B-region TRPV1 antagonistic template 2-(3-

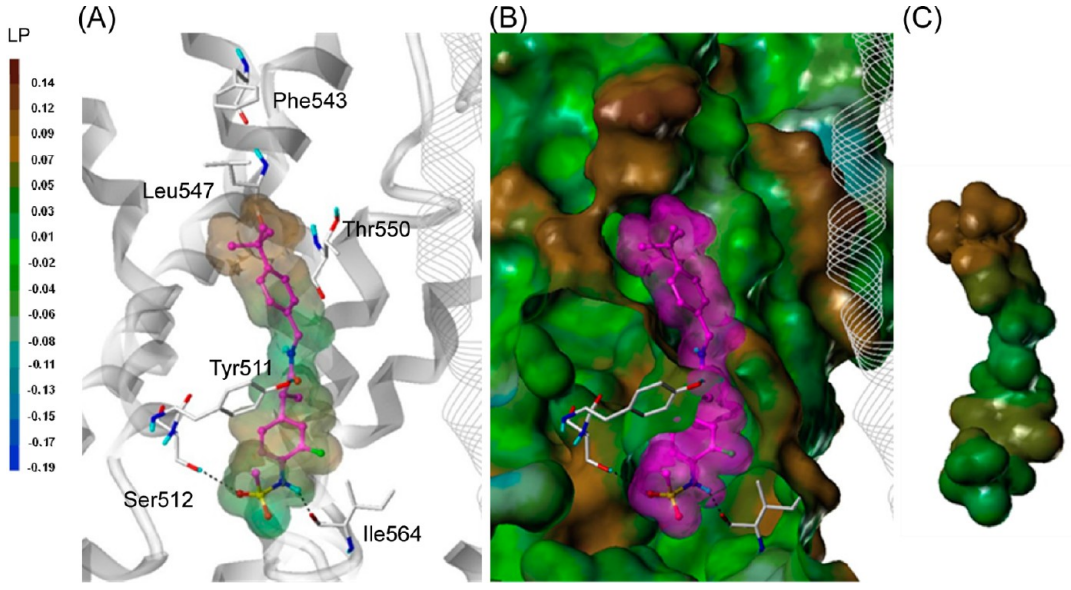

Figure 5. Flexible docking of **2** in the hTRPV1 model. (A) Binding mode of **2**. The key residues are marked and displayed as capped-stick with carbon atoms in white. The helices are colored in gray, and the helices of the adjacent monomer are displayed in line ribbon. The ligand is depicted as ball-and-stick with carbon atoms in magenta. The van der Waals surface of the ligand is presented with its lipophilic potential property. Hydrogen bonds are shown as black dashed lines, and nonpolar hydrogens are undisplayed for clarity. (B) Surface representations of the docked ligand and hTRPV1. The fast Connolly surface of hTRPV1 was generated by MOLCAD and colored by the lipophilic potential property. The surface of hTRPV1 is Z-clipped and that of the ligand is in its carbon color for clarity. (C) van der Waals surface of the ligand colored by its lipophilic potential property.

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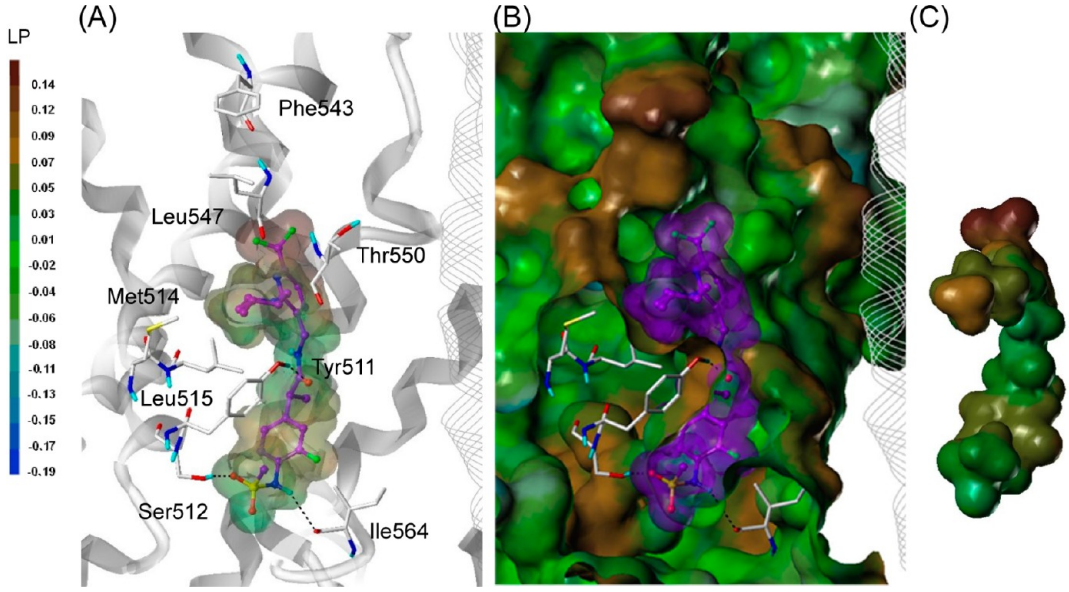

Figure 6. Flexible docking of **49*S*** in the hTRPV1 model: (A) binding mode of **49*S***; (B) surface representations of the docked ligand and hTRPV1; (C) van dar Waals surface of the ligand colored by its lipophilic potential property. The ligand is depicted as ball-and-stick with carbon atoms in purple. The details are the same as in Figure 5.

fluoro-4-methylsulfonylaminophenyl)propionic acid to provide a series of propanamides for evaluation as potent TRPV1 antagonists (**12**–**99**). The analysis of structure–activity relationship indicated that a critical feature of the 2-amino substituents for activity was that they support a hydrophobic interaction with the receptor. Among the compounds of the series, compound **49*S*** showed the best TRPV1 antagonism, blocking the activations by capsaicin, pH, heat, and NADA. It was selective, and it demonstrated strong analgesic activity in a neuropathic pain model with almost no side effects. Consistent with its action in vivo being through TRPV1, compound **49*S*** blocked capsacin-induced hypothermia but caused modest TRPV1-related hyperthermia in mice. The docking study with our homology model indicated that **49*S*** showed an excellent fit to the binding site in which the sulfonylaminobenzyl and propanamide moieties interacted with Tyr511, Ser512, and Ile564 as found in **2** and the 4-methylpiperidinyl group in the C-region made an additional hydrophobic interaction with the hydrophobic region composed of Met514 and Leu515, resulting in its high potency.

EXPERIMENTAL SECTION

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 instrument and a 6460 Triple Quad LC–MS instrument. 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 Agilent TC-C18 column (4.6 mm × 250 mm, 5 μm), Agilent Eclipse Plus C18 column (4.6 mm × 250 mm, 5 μm), and Daicel Chiralcel OD-H column (4.6 mm × 250 mm, 5 μm). Optical rotations were measured in a JASCO DIP-2000 digital polarimeter.

2-Hydroxy-6-(trifluoromethyl)nicotinonitrile (7). To an ice cold solution of ethyl vinyl ether (9.58 mL, 0.1 mol) and pyridine (8.1 mL, 0.1 mmol) in $CHCl_3$ (anhydrous 100 mL) was added trifluoroacetic anhydride (21 g, 0.1 mol) at 0 °C. The reaction mixture was gradually stirred at 0 °C to room temperature for 5 h. The mixture was concentrated in vacuo to obtain crude (*E*)-4-ethoxy-1,1,1-trifluorobut-3-en-2-one **6** (10.92 g, 65%) as a light yellow liquid, which was directly used for the next step without further purification.

A mixture of **6** (10 g, 59.5 mmol), 2-cyanoacetamide (5 g, 59.5 mmol), and K_2CO_3 (10.37 g, 75 mmol) in toluene (200 mL) was refluxed under a Dean–Stark trap for 9 h. The progress of the reaction was monitored by TLC (40% EtOAc/hexane, $R_f \approx 0.2$). After the starting material disappeared, the reaction mixture was concentrated in vacuo to give the residue which was purified by flash column chromatography on silica gel using EtOAc/hexane (1:25) as eluant to yield compound **7** (8.6 g, 77%) as a yellow solid, mp 210–215 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 8.02 (d, J = 7.5 Hz, 1H), 6.97 (d, J = 7.68 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ 169.27, 151.01–149.98 (q, J = 34.3), 147.42, 127.00–118.83 (q, J = 272.4), 118.16, 109.05, 102.67; IR (KBr) 3408, 2825, 2704, 2231, 1827, 1680, 1590, 1562, 1484, 1437, 1348, 1312, 1290, 1218, 1199, 1180, 1153, 1116, 1095 cm^{-1}; LC–MS (ESI) m/z 189 (MH^+).

2-Chloro-6-(trifluoromethyl)nicotinonitrile (8). A mixture of **7** (7.52 g, 40 mmol) and $POCl_3$ (26 mL, 280 mmol) was refluxed for 3 h. The reaction mixture was cooled and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexane (1:20) as eluant to yield compound **8** (4.28 g, 52%) as a white solid, mp 37–40 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.22 (d, J = 7.9 Hz, 1H), 7.77 (d, J = 7.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.19, 151.23–150.13 (q, J = 36.8), 144.42, 123.87–115.67 (q, J = 273.5), 119.11–119.04 (q, J = 2.7), 113.94, 113.47; IR (KBr) 3177, 3084, 3036, 2921, 2832, 2241, 1946, 1833, 1709, 1590, 1567, 1457, 1364, 1337, 1250, 1226, 1192, 1155, 1109, 1077 cm^{-1}; LC–MS (ESI) m/z 207 (MH^+).

General Procedure for Amination (9). *Method A (for Compounds **13**–**16**, **22**, **27**–**31**, **44**).* A mixture of 2-chloro-6-trifluoromethyl-nicotinonitrile (1.0 mmol) was dissolved in amine (10.0 mmol). The mixture was stirred for 16 h at room temperature. The aqueous portion was extracted with EtOAc (30 mL) twice. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexane (1:15) as eluant.

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*Method B (for Compounds **17–21, 23, 32, 46**).* A mixture of 2-chloro-6-trifluoromethylnicotinonitrile (1.0 mmol), bis-(triphenylphosphine)palladium(II) dichloride (0.10 mmol), and copper iodide (0.20 mmol) was dissolved in 1-methyl-2-pyrrolidinone (10 mL). After the mixture was stirred for 10 min, alkyene and *N*,*N*-diisopropylethylamine (2.0 mmol) were added to the mixture. The mixture was stirred for 12 h at 90–110 °C. The mixture was filtered over Celite, and the aqueous portion was extracted with ether twice. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexane (1:15) as eluant.

*Method C (for Compounds **24–26, 33–43, 54, 57–61, 73–99**).* A mixture of 6-*tert*-butyl-2-hydroxynicotinonitrile (1.0 mmol) and 1-bromopentane (2.0 mmol), 18-crown-6-ether (cat.), and K_2CO_3 (4.0 mmol) was dissolved in CH_3CN/DMF (1:2) solution. The mixture was refluxed for 12 h and then cooled to room temperature. The aqueous portion was extracted with EtOAc (30 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexane (1:1) as eluant.

*Method D (for Compounds **45, 47–53, 55, 56, 62–72**).* A mixture of 2-chloro-6-trifluoromethylnicotinonitrile (1.0 mmol), piperidine (2.0 mmol), and DBU (2.0 mmol) was dissolved in acetonitrile (10 mL). The mixture was stirred for 12 h at room temperature. The aqueous portion was extracted with EtOAc (30 mL) twice. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexane (1:15) as eluant.

General Procedure for Reduction (10). *Method A (for Compounds **17–21, 23–26, 32–35, 37–53, 57–81, 83, 85, 86, 89, 90, 93–99**).* To a stirred solution of nitrile (2.00 mmol) in THF (anhydrous, 10 mL) was added 2 M $BH_3{\cdot}SMe_2$ in THF (3 mL, 3 equiv) at room temperature. After being refluxed for 8 h, the mixture was cooled to room temperature and 2 M HCl solution was added. The mixture was then refluxed for 30 min. After cooling to room temperature, the mixture was neutralized by 2 M NaOH solution and the aqueous portion extracted with EtOAc several times. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH (10:1) as eluant.

*Method B (for Compounds **13–16, 22, 27–31, 36, 56**).* Nitrile (1.0 mmol) and $NiCl_2{\cdot}6H_2O$ (2.0 mmol) were dissolved in MeOH (8 mL). Sodium borohydride (4.0 mmol) was added slowly to the mixture. The mixture was refluxed for 12 h and then cooled to room temperature. The solution was filtered through Celite and dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH (10:1) as eluant.

*Method C (for Compounds **54, 55, 82, 84, 87, 88, 91, 92**).* A suspension of nitrile compounds (5.0 mmol), 10% Pd/C (500 mg), and concentrated HCl (3 mL) in MeOH (30 mL) was hydrogenated under a balloon of hydrogen for 6 h at room temperature and filtered through Celite. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography on silica gel using EtOAc as eluant.

General Procedure for Coupling (12–99). A mixture of acid (10.0 mmol), amine (12.0 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (12.0 mmol) in DMF (20 mL) was stirred for 12 h at room temperature. The aqueous portion was extracted with EtOAc (50 mL). The aqueous phase was saturated with NaCl and extracted again with EtOAc (25 mL). The combined organic extracts were washed with 1 M HCl (25 mL) and brine (25 mL), dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexane (1:2) as eluant.

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (12). Yield 12%, white solid, mp 70 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.63 (br t, 1H), 8.56 (s, 1H), 7.82 (t, 2H), 7.32 (t, J = 8.28 Hz, 1H), 7.20 (d, J = 12.12 Hz, 1H), 7.12 (d, J = 8.34 Hz, 1H), 4.37 (m, 2H), 3.69 (q, J = 6.78 Hz, 1H), 3.00 (s, 3H), 1.36 (d, J = 6.78 Hz, 3H). MS (FAB) m/z 420 (MH^+).

***N*-((2-(Butylamino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (13).** Yield 71%, white solid, mp 65–67 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (dd, J = 8.4 Hz, 1H), 7.22 (d, J = 7.3 Hz, 1H), 7.15 (dd, J = 11.2 Hz, 1H), 7.06 (d, 1H), 6.76 (d, J = 7.3 Hz, 1H), 6.51 (br s, 1H), 5.95 (br t, 1H), 5.71 (br t, 1H), 4.42–4.43 (m, 2H), 3.53–3.33 (m, 3H), 3.03 (s, 3H), 1.59–1.50 (m, 2H) 1.51 (d, J = 7.1 Hz, 3H), 1.45–1.33 (m, 2H), 0.95 (t, 3H). MS (FAB) m/z 491 (MH^+).

***N*-((2-(Cyclohexylamino)-6-(trifluoromethyl)pyridin-3-yl)-methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)-propanamide (14).** Yield 93%, white solid, mp 82–84 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (t, 1H), 7.22 (d, J = 5.8 Hz, 1H), 7.18 (dd, J = 8.9, 1.5 Hz, 1H), 7.08 (d, J = 6.6 Hz, 1H), 6.74 (d, J = 5.8 Hz, 1H), 6.47 (br s, 1H), 5.84 (br d, 1H), 5.67 (br t, 1H), 4.32 (m, 2H), 3.91 (m, 1H), 3.48 (q, J = 5.7 Hz, 1H), 3.03 (s, 3H), 1.98–1.61 (m, 5H), 1.52 (d, J = 5.7 Hz, 3H), 1.42–1.07 (m, 5H). MS (FAB) m/z 517 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-((4-methylcyclohexyl)amino)-6-(trifluoromethyl)pyridin-3-yl)-methyl)propanamide (15). Yield 25%, yellowish solid, mp 82 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.57 (br t, 1H), 7.40 (dd, J = 33.18, 6.78 Hz, 1H), 7.31 (t, J = 8.28 Hz, 1H), 7.21 (d, J = 11.34 Hz, 1H), 7.12 (d, J = 8.28 Hz, 1H), 6.86 (dd, J = 18.12, 6.78 Hz, 1H), 6.30 (dd, J = 75.54, 6.78 Hz, 1H), 4.15 (m, 2H), 3.67 (m, 2H), 2.99 (s, 3H), 1.66 (m, 2H), 1.52 (m, 2H), 1.44 (m, 1H), 1.36 (d, J = 6.84 Hz, 3H), 1.17 (m, 2H), 0.98 (m, 2H), 0.88 (d, J = 6.06 Hz, 3H). MS (FAB) m/z 531 (MH^+).

***tert*-Butyl 4-(((3-((2-(3-Fluoro-4-(methylsulfonamido)-phenyl)propanamido)methyl)-6-(trifluoromethyl)pyridin-2-yl)-amino)methyl)piperidine-1-carboxylate (16).** Yield 55%, white solid, mp 83–85 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (dd, J = 8.2, 8.2 Hz, 1H), 7.25 (d, 1H), 7.16 (d, 1H), 7.06 (d, 1H), 6.77 (d, J = 7.3 Hz, 1H), 6.21 (br s, 1H), 5.93 (br s, 1H), 4.32 (m, 2H), 4.06 (m, 2H), 3.49 (q, J = 7.3 Hz, 1H), 3.32 (m, 2H), 2.66 (m, 2H), 1.76 (m, 2H), 1.51 (d, J = 7.0 Hz, 3H), 1.46 (s, 9H). MS (FAB) m/z 632 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(phenylamino)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (17). Yield 58%, white solid, mp 189–192 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.67 (m, 2H), 7.59 (d, J = 8.2 Hz, 1H), 7.34 (dd, J = 8.2, 8.2 Hz, 1H), 7.19 (dd, J = 10.9, 1.9 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 7.06 (d, J = 7.7 Hz, 1H), 7.02–6.95 (m, 3H), 4.45 (m, 2H), 3.67 (q, J = 7.1 Hz, 1H), 2.89 (s, 3H), 1.47 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 511 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-((4-fluorophenyl)amino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-propanamide (18). Yield 58%, white solid, mp 158 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.90 (m, 2H), 7.59 (d, J = 7.5 Hz, 1H), 7.33 (dd, J = 8.3, 8.3 Hz, 1H), 7.24 (m, 1H), 7.21 (dd, J = 11.4, 1.8 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 7.06 (d, J = 7.5 Hz, 1H), 6.59 (m, 1H), 4.46 (m, 2H), 3.68 (q, J = 7.1 Hz, 1H), 2.87 (s, 3H), 1.47 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 529 (MH^+).

***N*-((2-((4-Chlorophenyl)amino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)-propanamide (19).** Yield 58%, white solid, mp 196 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.10 (d, J = 9.0 Hz, 2H) 7.62 (d, J = 7.5 Hz, 1H), 7.34 (dd, J = 8.3, 8.3 Hz, 1H), 7.23 (d, J = 9.0 Hz, 2H), 7.19 (dd, J = 11.7, 2.0 Hz, 1H), 7.10 (d, J = 8.3 Hz, 1H), 7.09 (d, J = 7.5 Hz, 1H), 4.45 (m, 2H), 3.67 (q, J = 7.0 Hz, 1H), 2.88 (s, 3H), 1.47 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 545 (MH^+).

***N*-((2-((3,4-Dimethylphenyl)amino)-6-(trifluoromethyl)-pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)-phenyl)propanamide (20).** Yield 58%, white solid, mp 176 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (m, 2H), 7.43 (dd, J = 8.4, 8.4 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.14 (dd, J = 11.0, 2.2 Hz, 1H), 7.06 (d, J = 8.7 Hz, 1H), 7.03 (d, J = 7.7 Hz, 1H), 6.95 (d, J = 7.5 Hz, 1H), 6.41(br s, 1H), 5.85 (br t, 1H), 4.47 (d, J = 6.4 Hz, 2H), 3.52 (q, J = 7.1 Hz, 1H), 2.96 (s, 3H), 2.27 (s, 3H), 2.23 (s, 3H), 1.52 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 539 (MH^+).

***N*-((2-((5-Chloro-2-methylphenyl)amino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (21).** Yield 58%, pale yellow solid, mp 169 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (d, J = 2.2 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.42 (dd, J = 8.3, 8.3 Hz, 1H), 7.11 (d, J = 7.7 Hz, 1H), 7.08 (dd, J = 9.0, 2.2 Hz, 1H), 7.03−7.00 (m, 3H), 6.43 (br s, 1H), 5.87 (br t, 1H), 4.49 (m, 2H), 3.51 (q, J = 7.1 Hz, 1H), 3.02 (s, 3H), 2.25 (s, 3H), 1.48 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 559 (MH^+).

***N*-((2-(Benzylamino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (22).** Yield 68%, white solid, mp 154−157 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.42−7.14 (m, 8H), 6.82 (d, J = 7.2 Hz, 1H), 6.69 (br t, 1H), 4.68−4.44 (m, 2H), 4.25 (m, 2H), 3.62 (q, J = 7.1 Hz, 1H), 2.94 (s, 3H), 1.37 (d, J = 7.3 Hz, 3H). MS (FAB) m/z 525 (MH^+).

***N*-((2-((4-Chlorobenzyl)amino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (23).** Yield 58%, white solid, mp 145 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (dd, J = 8.3, 8.3 Hz, 1H), 7.33 (d, J = 8.6 Hz, 2H), 7.25 (d, J = 8.6 Hz, 2H), 7.24 (d, J = 7.5 Hz, 1H), 7.07 (dd, J = 11.2, 2.0 Hz, 1H), 6.99 (d, J = 8.4 Hz, 1H), 6.81 (d, J = 7.5 Hz, 1H), 6.71 (br t, 1H), 6.47 (br s, 1H), 5.72 (br s, 1H), 4.58 (m, 2H), 4.32 (m, 2H), 3.44 (q, J = 7.1 Hz, 1H), 3.03 (s, 3H), 1.42 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 559 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-((pyridin-2-ylmethyl)amino)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (24). Yield 62%, white solid, mp 155−165 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 8.44 (m, 1H), 7.68 (td, J = 1.65, 7.68 Hz, 1H), 7.32−7.43 (m, 3H), 7.09−7.26 (m, 3H), 6.85 (d, J = 7.53 Hz, 1H), 4.72 (m, 2H), 4.33 (s, 2H), 3.63 (q, J = 7.14 Hz, 1H), 2.99 (s, 3H), 1.45 (d, J = 7.14 Hz, 3H). MS (FAB) m/z 526 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-((pyridin-3-ylmethyl)amino)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (25). Yield 65%, white solid, mp 80−100 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44−8.56 (m, 2H), 7.77 (m, 1H), 7.48 (m, 1H), 7.25 (m, 1H), 6.98−7.12 (m, 2H), 6.80−6.87 (m, 2H), 4.62 (m, 2H), 4.33 (m, 2H), 3.48 (m, 1H), 3.04 (s, 3H), 1.44 (d, J = 6.78 Hz, 3H). MS (FAB) m/z 526 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-((pyridin-4-ylmethyl)amino)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (26). Yield 68%, white solid, mp 80−100 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.46−8.49 (m, 2H), 7.47 (t, J = 8.24 Hz, 1H), 7.24−7.31 (m, 3H), 7.02−7.09 (m, 2H), 6.84 (m, 1H), 5.92 (m, 1H), 4.64 (m, 2H), 4.38 (m, 2H), 3.46 (m, 1H), 3.03 (s, 3H), 1.45 (d, J = 7.14 Hz, 3H). MS (FAB) m/z 526 (MH^+).

***N*-((2-(Dimethylamino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (27).** Yield 75%, white solid, mp 65−67 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46−7.40 (m, 2H), 7.20−7.00 (m, 3H), 6.60 (br t, 1H), 4.50 (br d, 2H), 3.60 (m, 1H), 3.00 (s, 3H), 2.80 (s, 6H), 1.49 (d, J = 7.0 Hz, 3H). MS (FAB) m/z 463 (MH^+).

***N*-((2-(Diethylamino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (28).** Yield 74%, white solid, mp 58−60 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (dd, J = 8.0, 8.0 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.20−7.00 (m, 3H), 6.50 (br s, 1H), 6.30 (br s, 1H), 4.50 (m, 2H), 3.50 (q, J = 7.0 Hz, 1H), 3.20 (m, 2H), 3.00 (s, 3H), 1.50 (d, J = 7.0 Hz, 3H), 0.99 (t, J = 7.2 Hz, 6H). MS (FAB) m/z 491 (MH^+).

***N*-((2-(Dipropylamino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (29).** Yield 58%, white solid, mp 96−98 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54−7.44 (m, 2H), 7.18−7.07 (m, 3H), 6.63 (br s, 1H), 6.20 (br t, 1H), 4.44 (m, 2H), 3.55 (q, J = 7.3 Hz, 1H), 3.12−3.07 (m, 4H), 3.02 (s, 3H), 1.54−1.40 (m, 4H), 0.83 (t, J = 7.3 Hz, 6H). MS (FAB) m/z 519 (MH^+).

***N*-((2-(Dibutylamino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (30).** Yield 70%, white solid, mp 102−104 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51−7.43 (m, 2H), 7.19−7.07 (m, 3H), 6.96 (br s, 1H), 6.40 (br t, 1H), 4.50 (m, 2H), 3.56 (q, J = 7.1 Hz, 1H), 3.13 (m, 4H), 3.02 (s, 3H), 1.52 (d, J = 7.1 Hz, 3H) 1.50 (m, 4H), 1.31−1.10 (m, 4H), 0.87 (t, J = 7.1 Hz, 6H). MS (FAB) m/z 547 (MH^+).

***N*-((2-(Butyl(methyl)amino)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (31).** Yield 60%, white solid, mp 66 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (dd, J = 8.3, 8.3 Hz, 1H), 7.45 (d, J = 7.9 Hz, 1H), 7.05−7.19 (m, 3H), 6.52 (br s, 1H), 6.13 (br t, 1H), 4.46 (d, J = 5.9 Hz, 2H), 3.56 (q, J = 7.1 Hz, 1H), 3.05−3.12 (m, 2H), 3.04 (s, 3H), 2.80 (s, 3H), 1.42−1.58 (m, 5H), 1.20−1.38 (m, 2H), 0.90 (t, J = 7.3 Hz, 3H). MS (FAB) m/z 505 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(methyl(phenyl)amino)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (32). Yield 83%, white solid, mp 76−84 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, J = 7.9 Hz, 1H), 7.53 (dd, J = 8.2, 8.2 Hz, 1H) 7.29−7.23 (m, 3H), 7.10−7.01 (m, 3H), 6.83 (m, 2H), 6.48(br s, 1H), 5.42 (br t, 1H), 3.88 (d, J = 6.0 Hz, 2H), 3.38 (s, 3H), 3.37 (q, J = 7.1 Hz, 1H), 3.04 (s, 3H), 1.43 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 525 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(pyrrolidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (33). Yield 80%, white solid, mp 130−135 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (dd, J = 8.1, 8.1 Hz, 1H), 7.38 (d, J = 7.5 Hz, 2H), 7.13 (dd, J = 11.1, 2.0 Hz, 1H), 7.07 (dd, J = 7.8, 1.8 Hz, 1H), 6.94 (d, J = 7.5 Hz, 1H), 5.72 (br t, 1H), 4.47 (d, J = 5.3 Hz, 2H), 3.52 (q, J = 6.9 Hz, 1H), 3.42−3.46 (m, 4H), 3.02 (s, 3H), 1.82−1.89 (m, 4H), 1.50 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 489 (MH^+).

(2*S*)-2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(2-methylpyrrolidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (34). Yield 75%, white solid, mp 80−85 °C. $[\alpha]_D^{25}$ −12.740 (c 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53−7.35 (m, 2H), 7.17−6.98 (m, 3H), 6.52 (br s, 1H), 5.85 (br s, 1H), 4.57 (m, 1H), 4.28 (m, 2H), 3.53 (m, 3H), 3.14 (m, 1H), 3.02 (d, J = 3.66 Hz, 3H), 2.14 (m, 1H), 1.90 (m, 2H), 1.59 (m, 1H), 1.50 (d, J = 1.65 Hz, 3H), 1.10 (d, J = 6.03 Hz, 3H). MS (FAB) m/z 503 (MH^+).

(*S*)-2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-((*S*)-2-(hydroxymethyl)pyrrolidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (35). Yield 42%, white solid, mp 123−129 °C. $[\alpha]_D^{25}$ −45.620 (c 0.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (m, 2H), 7.13−7.00 (m, 3H), 6.68 (br s, 1H), 6.17 (br s, 1H), 4.59 (m, 2H), 4.35 (m, 1H), 3.78 (m, 1H), 3.66−3.45 (m, 4H), 3.19 (m, 2H), 3.10 (s, 3H), 2.04−1.72 (m, 4H), 1.50 (d, J = 7.14 Hz, 3H). MS (FAB) m/z 519 (MH^+).

(*S*)-1-(3-(((*S*)-2-(3-Fluoro-4-(methylsulfonamido)phenyl)propanamido)methyl)-6-(trifluoromethyl)pyridin-2-yl)-*N*,*N*-dimethylpyrrolidine-2-carboxamide (36). Yield 55%, white solid, mp 90−100 °C. $[\alpha]_D^{25}$ −170.019 (c 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (m, 1H), 7.46 (d, J = 7.32 Hz, 1H), 7.40 (t, J = 8.25 Hz, 1H), 7.26 (m, 1H), 7.18 (m, 1H), 6.99 (d, J = 7.50 Hz, 1H), 5.40 (t, J = 7.35 Hz, 1H), 4.52 (dd, J = 15.03, 6.42 Hz, 1H), 4.35 (d, J = 14.82 Hz, 1H), 3.75 (m, 2H), 3.23 (s, 3H), 2.98 (s, 3H), 2.95 (s, 3H), 2.35 (m, 1H), 2.15 (m, 1H), 2.04 (m, 1H), 1.89 (m, 1H), 1.50 (d, J = 7.14 Hz, 3H). MS (FAB) m/z 560 (MH^+).

(*S*)-*tert*-Butyl 1-(3-(((*S*)-2-(3-Fluoro-4-(methylsulfonamido)phenyl)propanamido)methyl)-6-(trifluoromethyl)pyridin-2-yl)pyrrolidine-2-carboxylate (37). Yield 62%, white solid, mp 80−95 °C. $[\alpha]_D^{25}$ −33.2 (c 0.15, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.83−7.56 (m, 5H), 6.58 (s, 1H), 4.69 (s, 1H), 4.43 (m, 2H), 3.91 (m, 1H), 3.58 (m, 4H), 3.00 (s, 3H), 1.85 (m, 2H), 1.50 (d, J = 7.1 Hz, 3H), 1.44 (s, 9H). MS (FAB) m/z 589 (MH^+).

(*S*)-2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-((*R*)-3-hydroxypyrrolidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (38). Yield 62%, white solid, mp 88−90 °C. $[\alpha]_D^{25}$ −1.6 (c 0.05, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (m, 2H), 7.13−7.05 (m, 2H), 6.99 (d, J = 7.5 Hz, 1H), 5.97 (br s, 1H), 4.56−4.37 (m, 2H), 3.75−3.36 (m, 6H), 3.03 (s, 3H), 2.04−1.97 (m, 1H), 1.83−1.74 (m, 2H), 1.50 (d, J = 7.0 Hz, 3H). MS (FAB) m/z 505 (MH^+).

(*S*)-*N*-((2-((*R*)-3-(Benzyloxy)pyrrolidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (39). Yield 62%, white solid, mp 109−112 °C. $[\alpha]_D^{25}$ −31.108 (c 1.0, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (dd, J = 8.1 Hz, 2H), 7.33−7,28 (m,

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5H), 7.10 (dd, J = 11 Hz, 1H), 6.97 (dd, J = 7.8 Hz, 2H), 6.72 (s, 1H), 5.98 (br s, 1H), 4.54 (d, 2H), 4.20 (br s, 1H), 3.75−3.39 (m, 5H), 2.97 (s, 1H), 2.16−2.11 (m, 1H), 2.05−1.94 (m, 1H), 1.76 (s, 1H), 1.45 (d, J = 7.0 Hz, 3H). MS (FAB) m/z 595 (MH^+).

***tert*-Butyl ((*R*)-1-(3-(((*S*)-2-(3-Fluoro-4-(methylsulfonamido)-phenyl)propanamido)methyl)-6-(trifluoromethyl)pyridin-2-yl)-pyrrolidin-3-yl)carbamate (40).** Yield 65%, white solid, mp 78−79 °C. $[\alpha]_D^{25}$ 10.8925 (c 0.8, MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 7.48−7.41 (dd, J = 8.1 Hz, 2H), 7.22−7.14 (dd, J = 8.1 Hz, 2H), 6.97 (d, J = 7.68 Hz, 1H), 4.42 (q, 2H), 4.07 (q, 1H), 3.70 (m, 2H), 3.52 (m, 2H), 3.02 (s, 3H), 2.06 (m, 1H), 1.82 (m, 1H), 1.44 (m, 12H). MS (FAB) m/z 604 (MH^+).

***tert*-Butyl ((*S*)-1-(3-(((*S*)-2-(3-Fluoro-4-(methylsulfonamido)-phenyl)propanamido)methyl)-6-(trifluoromethyl)pyridin-2-yl)-pyrrolidin-3-yl)carbamate (41).** Yield 58%, white solid, mp 78−80 °C. $[\alpha]_D^{25}$ −5.62 (c 0.1, MeOH). ^{1}H NMR (300 MHz, CD_3OD) δ 7.43 (d, J = 8.22 Hz, 2H), 7.41 (s, 1H), 7.22−7.13 (dd, J = 8.1 Hz, 2H), 6.97 (d, J = 7.5 Hz, 1H), 4.60 (q, 2H), 4.50−4.32 (m, 2H), 3.72 (m, 2H), 3.57−3.48 (m, 1H), 3.02 (s, 3H), 2.15−2.06 (m, 1H), 1.89−1.82 (m, 1H), 1.44 (m, 12H). MS (FAB) m/z 604 (MH^+).

(*S*)-*N*-((2-((*R*)-3-(Dimethylamino)pyrrolidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (42). Yield 67%, white solid, mp 75−77 °C. $[\alpha]_D^{25}$ 16.294 (c 1.0, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (dd, J = 8.1 Hz, 1H), 7.42 (d, J = 8.1 Hz, 1H), 7.16 (dd, J = 10.5 Hz, 1H), 7.06 (d, J = 9.5 Hz, 1H), 6.97 (d, J = 6.9 Hz, 1H), 5.98 (br s, 1H), 4.79 (s, 3H), 4.55 (dd, J = 6.9 Hz, 1H), 4.40 (dd, J = 6.9 Hz, 1H), 3.64−3.48 (m, 4H), 3.36 (t, 1H), 3.02 (s, 3H), 2.71−2.66 (m, 4H), 2.29 (s, 6H), 2.11−2.05 (m, 1H), 1.83−1.76 (m, 2H), 1.49 (d, J = 7.0 Hz, 3H). MS (FAB) m/z 532 (MH^+).

(*S*)-*N*-((2-((*S*)-3-(Dimethylamino)pyrrolidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (43). Yield 65%, white solid, mp 78−79 °C. $[\alpha]_D^{25}$ −28.355 (c 0.6, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (dd, J = 8.1 Hz, 1H), 7.42 (d, J = 8.1 Hz, 1H), 7.13 (dd, J = 10.5 Hz, 1H), 7.08 (d, J = 9.5 Hz, 1H), 6.98 (d, J = 6.9 Hz, 1H), 5.98 (br s, 1H), 4.55 (dd, J = 6.9 Hz, 1H), 4.42 (dd, J = 6.9 Hz, 1H), 3.66−3.45 (m, 4H), 3.36 (t, 1H), 3.02, (s, 3H), 2.69 (m, 1H), 2.28 (s, 6H), 2.11−2.05 (m, 1H), 1.83−1.74(m, 2H), 1.49 (d, J = 7.0 Hz, 3H). MS (FAB) m/z 532 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(isoindolin-2-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (44). Yield 55%, white solid, mp 175−177 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, J = 7.5 Hz, 1H), 7.25−7.37 (m, 5H), 7.01−7.10 (m, 3H), 6.24 (br s, 1H), 5.75 (br t, 1H), 4.84 (s, 4H), 4.59 (d, J = 5.7 Hz, 2H), 3.52 (q, J = 7.2 Hz, 1H), 2.94 (s, 3H), 1.49 (d, J = 7.2 Hz, 3H). MS (FAB) m/z 537 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (45). Yield 88%, white solid, mp 75−79 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47−7.55 (m, 2H), 7.07−7.22 (m, 3H), 6.33 (br t, 1H), 4.47 (d, J = 5.7 Hz, 2H), 3.54 (q, J = 6.9 Hz, 1H), 3.00−3.05 (m, 7H), 1.61 (m, 6H), 1.52 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 503 (MH^+).

(*S*)-2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (45*S*). Yield 65%, white solid, mp 75−79 °C. $[\alpha]_D^{25}$ −1.39 (c 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47−7.55 (m, 2H), 7.07−7.22 (m, 3H), 6.33 (br t, 1H), 4.47 (d, J = 5.7 Hz, 2H), 3.54 (q, J = 6.9 Hz, 1H), 3.00−3.05(m, 7H), 1.52 (d, J = 6.9 Hz, 3H), 1.61 (m, 6H). MS (FAB) m/z 503 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((6′-(trifluoromethyl)-3,6-dihydro-2*H*-[1,2′-bipyridin]-3′-yl)methyl)-propanamide (46). Yield 60%, white solid, mp 82−85 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47−7.52 (m, 2H), 7.06−7.22 (m, 4H), 6.68 (br s, 1H), 6.40 (br t, 1H), 5.79−5.83 (m, 2H), 4.49 (d, J = 5.7 Hz, 2H), 3.69 (m, 2H), 3.56 (q, J = 7.2 Hz, 1H), 3.21 (m, 2H), 3.02 (s, 3H), 2.27 (m, 2H), 1.52 (d, J = 7.2 Hz, 3H). MS (FAB) m/z 501 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(2-methylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-propanamide (47). Yield 60%, white solid, mp 78−80 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62−7.48 (m, 2H), 7.30 (m, 1H), 7.18−7.07 (m, 2H), 6.71 (br t, 1H), 6.58 (br s, 1H), 4.67−4.57 (m, 1H), 4.35 (m, 1H), 3.56−3.46 (m, 2H), 3.02 and 3.01 (s, 3H), 3.01−2.95 (m, 1H), 2.79 (m, 1H), 1.80−1.50 (m, 9H), 0.90 and 0.85 (d, 3H). MS (FAB) m/z 517 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(3-methylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-propanamide (48). Yield 82%, white solid, mp 67−69 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54−7.47 (m, 2H), 7.21 (d, J = 7.7 Hz, 1H), 7.15−7.07 (m, 2H), 6.64 (br s, 1H), 6.34 (br t, 1H), 4.48 (d, J = 5.9 Hz, 2H), 3.56 (q, J = 7.0 Hz, 1H), 3.32−3.17 (m, 2H), 3.03 (s, 3H), 2.74 (m, 1H), 2.46 (m, 1H), 1.82−1.61 (m, 4H), 1.53 (d, J = 7.1 Hz, 3H), 1.13−1.01 (m, 1H), 0.91 (m, 3H). MS (FAB) m/z 517 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-methylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-propanamide (49). Yield 75%, white solid, mp 85 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47−7.55 (m, 2H), 7.07−7.22 (m, 3H), 6.29 (br t, 1H), 4.47 (d, J = 5.7 Hz, 2H), 3.54 (q, J = 6.9 Hz, 1H), 3.30 (m, 2H), 3.03 (s, 3H), 2.82 (m, 2H), 1.71 (m, 2H), 1.52 (d, J = 6.9 Hz, 3H), 1.24 (m, 3H), 0.97 (d, J = 6.6 Hz, 3H). MS (FAB) m/z 517 (MH^+).

(*S*)-2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-methylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-propanamide (49*S*). Yield 86%, white solid, mp 75−77 °C. $[\alpha]_D^{20}$ −2.73 (c 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, J = 8.25 Hz, 1H), 7.48 (d, J = 8.43 Hz, 1H), 7.20 (d, J = 7.89 Hz, 1H), 7.15−7.07 (m, 2H), 6.44 (br s, 1H), 6.27 (br t, 1H), 4.47 (d, J = 5.49 Hz, 2H), 3.55 (q, J = 6.93 Hz, 1H), 3.30 (t, 2H), 3.03 (s, 3H), 2.82 (tt, 2H), 1.73 (m, 2H), 1.53 (d, J = 7.14 Hz, 3H), 1.23 (m, 3H), 0.97 (d, J = 6.39 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.48, 161.14, 155.55−153.10 (d, J = 244.6), 145.47−144.44 (q, J = 34.1), 140.25−140.18 (d, J = 6.6), 137.64, 128.03, 124.16, 124.11−124.08 (d, J = 3.4), 123.68−123.56 (d, J = 12.7), 125.51−117.34 (q, J = 272.1), 114.91−114.71 (d, J = 20), 114.05−114.02 (d, J = 2.8), 50.49−50.42 (d, J = 6.3), 46.39, 39.84, 39.82, 34.34−34.30 (d, J = 3.8), 30.60, 21.81, 18.53. MS (FAB) m/z 517 (MH^+); HRMS calcd for $C_{23}H_{29}F_4N_4O_3S$ (M + H), 517.1897, found 517.1904.

(*R*)-2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-methylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-propanamide (49*R*). Yield 50%, white solid, mp 78−80 °C. $[\alpha]_D^{20}$ −2.30 (c 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, J = 8.25 Hz, 1H), 7.48 (d, J = 8.43 Hz, 1H), 7.20 (d, J = 7.89 Hz, 1H), 7.15−7.07 (m, 2H), 6.44 (br s, 1H), 6.27 (br t, 1H), 4.47 (d, J = 5.49 Hz, 2H), 3.55 (q, J = 6.93 Hz, 1H), 3.30 (t, 2H), 3.03 (s, 3H), 2.82 (tt, 2H), 1.73 (m, 2H), 1.53 (d, J = 7.14 Hz, 3H), 1.23 (m, 3H), 0.97 (d, J = 6.39 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.48, 161.14, 155.55−153.10 (d, J = 244.6), 145.47−144.44 (q, J = 34.1), 140.25−140.18 (d, J = 6.6), 137.64, 128.03, 124.16, 124.11−124.08 (d, J = 3.4), 123.68−123.56 (d, J = 12.7), 125.51−117.34 (q, J = 272.1), 114.91−114.71 (d, J = 20), 114.05−114.02 (d, J = 2.8), 50.49−50.42 (d, J = 6.3), 46.39, 39.84, 39.82, 34.34−34.30 (d, J = 3.8), 30.60, 21.81, 18.53. MS (FAB) m/z 517 (MH^+).

***N*-((2-(4-Ethylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)-methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)-propanamide (50).** Yield 61%, white solid, mp 78−80 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.47−7.52 (m, 2H), 7.19 (d, J = 7.8 Hz, 1H), 7.06−7.14 (m, 2H), 6.69 (br s, 1H), 6.37 (br t, 1H), 4.47 (d, J = 5.7 Hz, 2H), 3.56 (q, J = 6.9 Hz, 1H), 3.33 (m, 2H), 3.02 (s, 3H), 2.80 (m, 2H), 1.76 (m, 2H), 1.52 (d, J = 6.9 Hz, 3H), 1.21−1.32 (m, 5H), 0.91 (t, J = 7.2 Hz, 3H). MS (FAB) m/z 531 (MH^+).

***N*-((2-(4,4-Dimethylpiperidin-1-yl)-6-(trifluoromethyl)-pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)-phenyl)propanamide (51).** Yield 80%, white solid, mp 128 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (dd, J = 8.4, 8.4 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.21 (d, J = 7.5 Hz, 1H), 7.14 (dd, J = 11.4, 1.9 Hz, 1H), 7.09 (d, J = 8.8 Hz, 1H), 6.47 (br s, 1H), 6.26 (br t, 1H), 4.47 (d, J = 5.0 Hz, 2H), 3.56 (q, J = 7.1 Hz, 1H), 3.08−3.04 (m, 4H), 3.03 (s, 3H), 1.53 (d, J = 7.1 Hz, 3H), 1.48−1.43 (m, 4H), 0.99 (s, 6H). MS (FAB) m/z 531 (MH^+).

***N*-((2-(3,5-Dimethylpiperidin-1-yl)-6-(trifluoromethyl)-pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)-phenyl)propanamide (52).** Yield 68%, white solid, mp 175 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47−7.54 (m, 2H), 7.21 (d, J = 7.8 Hz,

1H), 7.13 (dd, J = 8.1, 1.8 Hz, 1H), 7.07 (d, J = 8.1 Hz, 1H), 6.48 (br s, 1H), 6.28 (br t, 1H), 4.47 (d, J = 5.7 Hz, 2H), 3.54 (q, J = 6.9 Hz, 1H), 3.23 (m, 2H), 3.03 (s, 3H), 2.35 (m, 2H), 1.54−1.76 (m, 2H), 1.52 (d, J = 6.9 Hz, 3H), 0.90 (d, J = 5.7 Hz, 3H), 0.88 (d, J = 5.7 Hz, 3H). MS (FAB) m/z 531 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-phenylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (53). Yield 75%, white solid, mp 138−141 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48−7.51 (m, 2H), 7.08−7.36 (m, 8H), 6.52 (s, 1H), 6.23 (br s, 1H), 4.53 (d, J = 5.1 Hz, 2H), 3.56 (q, J = 7.2 Hz, 1H), 3.46 (m, 2H), 2.95−3.00 (m, 5H), 2.03 (m, 2H), 1.82 (m, 2H), 1.54 (d, J = 7.2 Hz, 3H). MS (FAB) m/z 579 (MH^+).

(*S*)-2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-phenylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (53*S*). Yield 85%, white solid, mp 135 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.58 (br t, 1H), 7.55 (d, J = 7.56 Hz, 1H), 7.37 (d, J = 7.56 Hz, 1H), 7.29 (m, 5H), 7.21 (m, 2H), 7.15 (d, J = 7.56 Hz, 1H), 4.32 (m, 2H), 3.72 (q, J = 7.56 Hz, 1H), 3.51 (m, 2H), 2.99 (s, 3H), 2.90 (q, 2H), 2.71 (m, 1H), 1.81 (m, 4H), 1.38 (d, J = 7.56 Hz, 3H). MS (FAB) m/z 579 (MH^+).

(*R*)-2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-phenylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (53*R*). Yield 82%, white solid, mp 142 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.58 (br t, 1H), 7.55 (d, J = 7.56 Hz, 1H), 7.37 (d, J = 7.56 Hz, 1H), 7.29 (m, 5H), 7.21 (m, 2H), 7.15 (d, J = 7.56 Hz, 1H), 4.32 (m, 2H), 3.72 (q, J = 7.56 Hz, 1H), 3.51 (m, 2H), 2.99 (s, 3H), 2.90 (q, 2H), 2.71 (m, 1H), 1.81 (m, 4H), 1.38 (d, J = 7.56 Hz, 3H). MS (FAB) m/z 579 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((4-phenyl-6′-(trifluoromethyl)-3,6-dihydro-2*H*-[1,2′-bipyridin]-3′-yl)methyl)propanamide (54). Yield 65%, white solid, mp 71−73 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54−7.02 (m, 10H), 6.64 (br s, 1H), 6.34 (m, 1H), 6.18 (br s, 1H), 4.52 (m, 2H), 3.88 (d, J = 2.73 Hz, 2H), 3.59−3.36 (m, 4H), 2.99 (m, 5H), 2.67 (m, 2H), 1.51 (d, J = 7.14 Hz, 3H). MS (FAB) m/z 577 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(4-fluorophenyl)piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (55). Yield 66%, white solid, mp 117−119 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46−7.51 (m, 2H), 6.97−7.25 (m, 7H), 6.72 (br s, 1H), 6.24 (br t, 1H), 4.50 (d, J = 5.7 Hz, 2H), 3.59 (q, J = 6.9 Hz, 1H), 3.45 (m, 2H), 3.00 (s, 3H), 2.93 (m, 2H), 1.92 (m, 2H), 1.76 (m, 3H), 1.51 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 597 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((4-(4-fluorophenyl)-6′-(trifluoromethyl)-3,6-dihydro-2*H*-[1,2′-bipyridin]-3′-yl)methyl)propanamide (56). Yield 55%, white solid, mp =83−85 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54−7.36 (m, 4H), 7.23 (d, J = 7.71 Hz, 1H), 7.13−7.02 (m, 3H), 6.21 (m, 1H), 6.12 (m, 1H), 4.53 (d, J = 5.49 Hz, 2H), 3.87 (m, 2H), 3.55 (q, J = 6.96 Hz, 1H), 3.37 (t, J = 5.67 Hz, 2H), 3.00 (s, 3H), 2.65 (m, 2H), 1.51 (d, J = 7.14 Hz, 3H). MS (FAB) m/z 595 (MH^+).

***N*-((2-(4-Benzylpiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (57).** Yield 78%, white solid, mp 81−83 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (dd, J = 8.2, 8.2 Hz, 1 H), 7.48 (d, J = 7.9 Hz, 1H), 7.29−7.14 (m, 7H), 7.07 (d, J = 8.1 Hz, 1H), 6.49 (br s, 1H), 6.23 (br t, 1H), 4.46 (d, J = 5.7 Hz, 2H), 3.54 (q, J = 7.0 Hz, 1H), 3.31 (m, 2H), 3.02 (s, 3H), 2.78 (m, 2H), 2.59 (d, J = 6.6 Hz, 2H), 1.78−1.71 (m, 3H), 1.52 (d, J = 7.1 Hz, 3H), 1.30 (m, 2H). MS (FAB) m/z 593 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(4-methylbenzyl)piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (58). Yield 64%, white solid, mp 110−130 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45−7.53 (m, 2H), 7.18 (m, 1H), 6.98−7.12 (m, 6H), 6.41 (s, 1H), 6.19 (t, J = 3.87 Hz, 1H), 4.44 (d, J = 4.11 Hz, 2H), 3.51 (q, J = 5.28 Hz, 1H), 3.28 (m, 2H), 3.00 (s, 3H), 2.76 (t, J = 9.21 Hz, 2H), 2.51 (d, J = 5.01 Hz, 2H), 2.31 (s, 3H), 1.66 (m, 3H), 1.50 (m, 3H), 1.23 (m, 2H). MS (FAB) m/z 607 (MH^+).

***N*-((2-(4-(3,4-Difluorobenzyl)piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (59).** Yield 59%, white solid, mp 100−120 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43−7.48 (m, 2H), 6.92−7.28 (m, 6H), 6.86 (m, 1H), 6.51 (m, 1H), 4.45 (d, J = 5.49 Hz, 2H), 3.60 (q, J = 6.96 Hz, 1H), 3.33 (t, J = 11.60 Hz, 2H), 3.01 (s, 3H), 2.78 (t, J = 11.72 Hz, 2H), 2.53 (d, J = 6.60 Hz, 2H), 1.63−1.72 (m, 3H), 1.50 (d, J = 7.23 Hz, 3H), 1.28 (m, 2H). MS (FAB) m/z 629 (MH^+).

***N*-((2-(4-(3,5-Difluorobenzyl)piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (60).** Yield 61%, white solid, mp 70 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46−7.53 (m, 2H), 7.07−7.27 (m, 3H), 6.08 (s, 1H), 6.61−6.69 (m, 3H), 6.28 (t, J = 5.60 Hz, 1H), 4.46 (d, J = 5.49 Hz, 2H), 3.58 (q, J = 7.14 Hz, 1H), 3.31 (m, 2H), 3.05 (s, 3H), 2.80 (m, 2H), 2.56 (d, J = 6.78 Hz, 2H), 1.70−1.74 (m, 3H), 1.53 (d, J = 7.14 Hz, 3H), 1.32 (m, 2H). MS (FAB) m/z 629 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(4-fluorobenzoyl)piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (61). Yield 65%, white solid, mp 100−115 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (m, 2H), 7.23−7.03 (m, 7H), 6.54 (br s, 1H), 6.22 (m, 1H), 4.45 (m, 2H), 3.54 (q, J = 6.96 Hz, 1H), 3.37 (m, 2H), 3.04 (s, 3H), 2.80 (m, 2H), 2.01 (m, 2H), 1.75 (m, 1H), 1.51 (d, J = 6.96 Hz, 3H), 1.42 (m, 3H). MS (FAB) m/z 625 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-fluoropiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (62). Yield 55%, white solid, mp 207−208 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.50 (d, J = 8.1 Hz, 1H), 7.43 (t, J = 8.1 Hz, 1H), 7.14−7.26 (m, 3H), 4.75 (d, J = 5.0 Hz, 1H), 4.38 (d, J = 5.7 Hz, 2H) 3.71 (q, J = 7.2 Hz, 1H), 3.30 (m, 2H), 3.03 (m, 2H), 2.96 (s, 3H), 1.88 (m, 4H), 1.46 (d, J = 7.2 Hz, 3H). MS (FAB) m/z 521 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((6-(trifluoromethyl)-2-(4-(trifluoromethyl)piperidin-1-yl)pyridin-3-yl)methyl)propanamide (63). Yield 75%, white solid, mp 82−84 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47−7.51 (m, 2H), 7.25 (d, J = 7.8 Hz, 1H), 7.08−7.15 (m, 2H), 6.34 (br s, 1H), 6.04 (br t, 1H), 4.47 (d, J = 5.7 Hz, 2H), 3.61 (q, J = 6.9 Hz, 1H), 3.43 (m, 2H), 3.01 (s, 3H), 2.84 (t, J = 11.1 Hz, 2H), 1.95 (m, 2H), 1.66 (m, 1H), 1.53 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 571 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-oxopiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (64). Yield 44%, white solid, mp 86−88 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55−7.49 (m, 2H), 7.29 (d, J = 7.9 Hz, 1H), 7.17 (dd, J = 11.2, 2.0 Hz, 1H), 7.11 (d, J = 8.6 Hz, 1H), 6.70 (br s, 1H), 6.04 (br t, 1H), 4.54 (d, J = 5.7 Hz, 2H), 3.61 (q, J = 7.0 Hz, 1H), 3.49 (t, J = 6.0 Hz, 4H), 3.04 (s, 3H), 2.55 (t, J = 6.1 Hz, 4H), 1.55 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 517 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-hydroxypiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (65). Yield 87%, white solid, mp 81−83 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54−7.49 (m, 2H), 7.23 (d, J = 7.7 Hz, 1H), 7.16−7.09 (m, 2H), 6.69 (br s, 1H), 6.25 (br t, 1H), 4.48 (m, 2H), 3.84 (m, 1H), 3.58 (q, J = 7.3 Hz, 1H), 3.38−3.26 (m, 2H), 3.04 (s, 3H), 2.97−2.88 (m, 2H), 2.02−1.92 (m, 2H), 1.75 (s, 1H), 1.53 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 519 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-methoxypiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (66). Yield 50%, white solid, mp 65−67 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53−7.47 (m, 2H), 7.22 (d, J = 7.7 Hz, 1H), 7.15−7.07 (m, 2H), 6.77 (br s, 1H), 6.32 (br t, 1H), 4.47 (d, J = 5.7 Hz, 2H), 3.58 (q, J = 7.1 Hz, 1H), 3.40−3.25 (m, 3H), 3.37 (s, 3H), 3.03 (s, 3H), 2.95−2.86 (m, 2H), 2.04−1.95 (m, 2H), 1.63−1.50 (m, 2H), 1.53 (d, J = 7.0 Hz, 3H). MS (FAB) m/z 533 (MH^+).

***N*-((2-(4-Ethoxypiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (67).** Yield 59%, white solid, mp 69−71 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55−7.48 (m, 2H), 7.23 (d, J = 7.7 Hz, 1H), 7.15−7.08 (m, 2H), 6.54 (br s, 1H), 6.23 (br t, 1H), 4.48 (d, 2H), 3.58−3.23 (m, 6H), 3.04 (s, 3H), 2.94−2.86 (m, 2H), 2.05−1.95 (m, 2H), 1.63−1.50 (m, 2H), 1.53 (d, J = 7.1 Hz, 3H), 1.24 (t, J = 7.0 Hz, 3H). MS (FAB) m/z 547 (MH^+).

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2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(methoxymethyl)piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (68). Yield 60%, white solid, mp 117−119 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46−7.52 (m, 2H), 7.20 (d, J = 7.8 Hz, 1H), 7.07−7.15 (m, 2H), 6.82 (br s, 1H), 6.37 (br t, 1H), 4.46 (d, J = 5.7 Hz, 2H), 3.58 (q, J = 6.9 Hz, 1H), 3.26−3.38 (m, 5H), 3.02 (s, 3H), 2.82 (m, 2H), 1.79 (m, 3H), 1.51 (d, J = 6.9 Hz, 3H), 1.25−1.30 (m, 4H). MS (FAB) m/z 547 (MH^+).

***N*-((2-(4-Butoxypiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)-propanamide (69).** Yield 74%, white solid, mp 70−72 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54−7.48 (m, 2H), 7.21 (d, J = 7.5 Hz, 1H), 7.14−7.07 (m, 2H), 6.64 (br s, 1H), 6.26 (br t, 1H), 4.47 (d, J = 5.7 Hz, 2H), 3.57 (q, J = 7.1 Hz, 1H), 3.50−3.26 (m, 5H), 3.03 (s, 3H), 2.94−2.86 (m, 2H), 2.02−1.95 (m, 2H), 1.62−1.50 (m, 7H), 1.45−1.33 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H). MS (FAB) m/z 575 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-isopropoxypiperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-propanamide (70). Yield 44%, white solid, mp 77−79 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55−7.48 (m, 2H), 7.22 (d, J = 7.7 Hz, 1H), 7.15−7.08 (m, 2H), 6.56 (br s, 1H), 6.23 (br t, 1H), 4.47 (d, J = 5.9 Hz, 2H), 3.74 (m, 1H), 3.60−3.45 (m, 2H), 3.37−3.33 (m, 2H), 3.04 (s, 3H), 2.94−2.85 (m, 2H), 1.98−1.90 (m, 2H), 1.62−1.50 (m, 2H), 1.53 (d, J = 7.0 Hz, 3H), 1.18 (d, J = 6.1 Hz, 6H). MS (FAB) m/z 561 (MH^+).

1-(3-((2-(3-Fluoro-4-(methylsulfonamido)phenyl)-propanamido)methyl)-6-(trifluoromethyl)pyridin-2-yl)-piperidin-4-yl Acetate (71). Yield 55%, white solid, mp 115−117 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57−7.48 (m, 2H), 7.24 (d, J = 8.1 Hz, 1H), 7.17−7.09 (m, 2H), 6.47 (br s, 1H), 6.05 (br t, 1H), 4.93 (m, 1H), 4.47 (d, J = 5.7 Hz, 2H), 3.57 (q, J = 7.0 Hz, 1H), 3.35−3.25 (m, 2H), 3.07−2.97 (m, 2H), 3.04 (s, 3H), 2.08 (s, 3H), 2.02−1.92 (m, 2H), 1.80−1.70 (m, 2H), 1.54 (d, J = 7.3 Hz, 3H). MS (FAB) m/z 561 (MH^+).

1-(3-((2-(3-Fluoro-4-(methylsulfonamido)phenyl)-propanamido)methyl)-6-(trifluoromethyl)pyridin-2-yl)-piperidin-4-yl Pivalate (72). Yield 52%, white solid, mp 86−88 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54−7.47 (m, 2H), 7.22 (d, J = 7.7 Hz, 1H), 7.15 (dd, J = 11.0, 1.8 Hz, 1H), 7.10 (m, 1H), 6.49 (br s, 1H), 6.01 (br t, 1H), 4.94 (m, 1H), 4.47 (d, J = 6.0 Hz, 2H), 3.58 (q, J = 7.0 Hz, 1H), 3.32−3.22 (m, 2H), 3.13−3.03 (m, 2H), 3.04 (s, 3H), 2.00−1.90 (m, 2H), 1.82−1.70 (m, 2H), 1.55 (d, J = 7.1 Hz, 3H), 1.21 (s, 9H). MS (FAB) m/z 603 (MH^+).

***N*-((2-(4-((Dimethylamino)methyl)piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (73).** Yield 60%, white solid, mp 85−130 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44−7.49 (m, 2H), 7.05−7.24 (m, 3H), 6.22 (br t, 1H), 4.42 (d, J = 5.7 Hz, 2H), 3.55 (q, J = 7.2 Hz, 1H), 3.28 (m, 2H), 3.00 (s, 3H), 2.90 (m, 2H), 2.63 (d, J = 4.2 Hz, 2H), 2.58 (s, 6H), 1.89−2.13 (m, 4H), 1.49 (d, J = 6.9 Hz, 3H), 1.36 (m, 2H). MS (FAB) m/z 560 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(phenylamino)piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)-methyl)propanamide (74). Yield 48%, white solid, mp 67−69 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49−7.54 (m, 2H), 7.08−7.25 (m, 6H), 6. 72 (t, J = 7.2 Hz, 1H), 6.63 (d, J = 8.1 Hz, 2H), 6.21 (br t, 1H), 4.48 (d, J = 5.7 Hz, 2H), 3.57 (q, J = 6.9 Hz, 1H), 3.35−3.46 (m, 3H), 3.01−3.04 (m, 5H), 2.60 (m, 2H), 2.17 (m, 2H), 1.52 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 594 (MH^+).

***N*-((2-([1,4′-Bipiperidin]-1′-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)-propanamide (75).** Yield 30%, white solid, mp 103 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.51 (d, J = 7.7 Hz, 1H), 7.42 (dd, J = 8.2, 8.2 Hz, 1H), 7.53 (d, J = 7.7 Hz, 1H), 7.13−7.21 (m, 2H), 4.30−4.47 (m, 2H), 3.71 (q, J = 7.0 Hz, 1H), 3.48−3.52 (m, 2H), 2.97 (s, 3H), 2.80−2.84 (m, 2H), 2.55−2.75 (m, 5H), 1.88−2.00 (m, 2H), 1.60−1.75 (m, 6H), 1.50−1.55 (m, 2H), 1.46 (d, J = 7.0 Hz, 3H). MS (FAB) m/z 586 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(pyrrolidin-1-yl)piperidin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)-methyl)propanamide (76). Yield 37%, white solid, mp 144 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.50 (d, J = 7.7 Hz, 1H), 7.42 (dd, J = 8.3, 8.3 Hz, 1H), 7.25 (d, J = 7.7 Hz, 1H), 7.10−7.22 (m, 2H), 4.29−4.45 (m, 2H), 3.72 (q, J = 7.1 Hz, 1H), 3.40−3.50 (m, 2H), 2.70−2.92 (m, 6H), 2.40 (m, 1H), 1.95−2.10 (m, 2H), 1.81−2.10 (m, 4H), 1.57−1.74 (m, 2H), 1.46 (d, J = 7.0 Hz, 3H). MS (FAB) m/z 572 (MH^+).

***N*-((2-(3-(*tert*-Butyl)-1-oxa-2,8-diazaspiro[4.5]dec-2-en-8-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (77).** Yield 20%, beige solid, mp 124 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.56 (br t, 1H), 7.55 (d, J = 7.56 Hz, 1H), 7.37 (d, J = 7.56 Hz, 1H), 7.32 (t, J = 8.34 Hz, 1H), 7.22 (d, J = 9.84 Hz, 1H), 7.14 (d, J = 8.34 Hz, 1H), 4.28 (m, 2H), 3.70 (q, J = 6.78 Hz, 1H), 3.19 (m, 2H), 3.08 (m, 2H), 3.00 (s, 3H), 2.83 (s, 2H), 1.76 (m, 4H), 1.37 (d, J = 6.78 Hz, 3H), 1.13 (s, 9H). MS (FAB) m/z 615 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(3-phenyl-1-oxa-2,8-diazaspiro[4.5]dec-2-en-8-yl)-6-(trifluoromethyl)-pyridin-3-yl)methyl)propanamide (78). Yield 20%, yellowish solid, mp 131 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.58 (br t, 1H), 7.68 (m, 2H), 7.56 (d, J = 8.34 Hz, 1H), 7.44 (m, 3H), 7.39 (d, J = 7.56 Hz, 1H), 7.33 (t, J = 8.34 Hz, 1H), 7.22 (d, J = 10.56 Hz, 1H), 7.15 (d, J = 7.56 Hz, 1H), 4.32 (m, 2H), 3.71 (q, J = 6.84 Hz, 1H), 3.28 (m, 2H), 3.14 (m, 2H), 3.00 (s, 3H), 1.90 (m, 4H), 1.37 (d, J = 6.84 Hz, 3H). MS (FAB) m/z 634 (MH^+).

***N*-((2-(Azepan-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (79).** Yield 78%, white solid, mp 126−130 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (dd, J = 8.1, 8.1 Hz, 1H), 7.40 (d, J = 7.5 Hz, 1H), 7.14 (dd, J = 8.1, 1.8 Hz, 1H), 7.08 (d, J = 8.1 Hz, 1H), 7.03 (d, J = 7.5 Hz, 1H), 5.86 (br t, 1H), 4.43 (d, J = 5.7 Hz, 2H), 3.54 (q, J = 6.9 Hz, 1H), 3.38 (m, 4H), 3.03 (s, 3H), 1.75 (m, 4H), 1.57 (m, 4H), 1.52 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 531 (MH^+).

***N*-((2-(Azocan-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (80).** Yield 68%, white solid, mp 138−141 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (t, J = 8.1 Hz, 1H), 7.36 (d, J = 7.8 Hz, 1H), 7.14 (dd, J = 2.1, 11.1 Hz, 1H), 7.07 (d, J = 8.1 Hz, 1H), 6.96 (d, J = 7.8 Hz, 1H), 6.94 (br s, 1H), 5.97 (br s, 1H), 4.39 (d, J = 5.1 Hz, 2H), 3.59 (q, J = 7.2 Hz, 1H), 3.46 (m, 4H), 3.01 (s, 3H), 1.68 (m, 4H), 1.51 (m, 6H). MS (FAB) m/z 531 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(piperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (81). Yield 42%, white solid, mp 44−48 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.62 (d, J = 7.5 Hz, 1H), 7.37−7.45 (m, 2H), 7.14−7.21 (m, 2H), 4.60 (s, 2H), 4.42 (q, J = 15.6 Hz, 2H) 3.73 (q, J = 6.9 Hz, 1H), 3.25 (m, 4H) 3.06 (q, J = 15.6 Hz, 2H), 2.99 (s, 3H), 1.46 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 504 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-methylpiperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-propanamide (82). Yield 68%, pale yellow solid, mp 97−99 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (dd, J = 8.4, 8.4 Hz, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.23 (d, J = 7.7 Hz, 1H), 7.14 (dd, J = 11.2, 1.9 Hz, 1H), 7.09 (d, J = 8.2 Hz, 1H), 6.21 (br t, 1H), 4.47 (m, 2H), 3.57 (q, J = 7.1 Hz, 1H), 3.19−3.15 (m, 4H), 3.04 (s, 3H), 2.53−2.49 (m, 4H), 2.34 (s, 3H), 1.54 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 518 (MH^+).

***N*-((2-(4-Cyclohexylpiperazin-1-yl)-6-(trifluoromethyl)-pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)-phenyl)propanamide (83).** Yield 72%, white solid, mp 112−114 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47−7.53 (m, 2H), 7.23 (d, J = 7.8 Hz, 1H), 7.07−7.15 (m, 2H), 6.26 (br t, 1H), 4.44 (d, J = 5.7 Hz, 2H), 3.58 (q, J = 6.9 Hz, 1H), 3.27 (m, 4H), 3.03 (s, 3H), 2.84 (m, 4H), 2.50 (m, 1H), 1.94 (m, 2H), 1.85 (m, 2H), 1.51 (d, J = 6.9 Hz, 3H), 1.25−1.30 (m, 6H). MS (FAB) m/z 586 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-phenylpiperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-propanamide (84). Yield 58%, white solid, mp 87−92 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (d, J = 7.7 Hz, 1H), 7.48 (dd, J = 8.2, 8.2 Hz, 1H), 7.31 (m, 3H), 7.13 (dd, J = 11.0, 1.8 Hz, 1H), 7.08 (d, J = 8.8 Hz, 1H), 6.96−6.89 (m, 3H), 6.33 (br s, 1H), 6.20 (br t, 1H), 4.54 (d, J = 6.0 Hz, 2H), 3.57 (q, J = 7.0 Hz, 1H), 3.32−3.29 (m, 8H), 2.99 (s, 3H), 1.53 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 580 (MH^+).

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2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(m-tolyl)piperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-propanamide (85). Yield 60%, white solid, mp 61−63 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45−7.54 (m, 2H), 7.06−7.28 (m, 4H), 6.73−6.76 (m, 3H), 6.28(br s, 1H), 6.20 (br t, 1H), 4.52 (d, J = 5.7 Hz, 2H), 3.56 (q, J = 6.9 Hz, 1H), 3.24 (m, 8H), 2.98 (s, 3H), 2.35 (s, 3H), 1.52 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 594 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(*p*-tolyl)-piperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-propanamide (86). Yield 67%, white solid, mp 83−85 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (m, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.24−7.32 (m, 2H), 6.96−7.12 (m, 4H), 6.78−6.81 (m, 3H), 4.40 (d, J = 5.7 Hz, 2H), 3.56 (q, J = 6.9 Hz, 1H), 3.12 (m, 8H), 2.86 (s, 3H), 2.18 (s, 3H), 1.40 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 594 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(2-fluorophenyl)piperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)-methyl)propanamide (87). Yield 67%, white solid, mp 178−180 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 8.1 Hz, 1H), 7.52 (dd, J = 8.3, 8.3 Hz, 1H), 7.27 (d, J = 8.0 Hz, 1H), 7.11 (m, 4H), 6.98 (m, 2H), 6.40 (br s, 1H), 6.16 (br t, 1H), 4.53 (d, J = 4.6 Hz, 2H), 3.58 (q, J = 7.3 Hz, 1H), 3.32−3.28 (m, 4H), 3.18−3.15 (m, 4H), 3.01 (s, 3H), 1.54 (d, J = 7.0 Hz, 3H). MS (FAB) m/z 598 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(4-fluorophenyl)piperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)-methyl)propanamide (88). Yield 80%, white solid, mp 87−92 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 7.5 Hz, 1H), 7.49 (dd, J = 7.9, 7.9 Hz, 1H), 7.27 (d, J = 7.5 Hz, 1H), 7.14 (d, J = 11.0 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 6.99 (m, 2H), 6.90 (m, 2H), 6.58 (br s, 1H), 6.17 (br t, 1H), 4.52 (d, J = 5.7 Hz, 2H), 3.58 (q, J = 6.8 Hz, 1H), 3.29−3.25 (m, 4H), 3.22−3.18 (m, 4H), 3.01 (s, 3H), 1.53 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 598 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((6-(trifluoro-methyl)-2-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)-pyridin-3-yl)methyl)propanamide (89). Yield 52%, white solid, mp 164−166 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.39−7.52 (m, 5H), 7.27 (d, J = 7.8 Hz, 1H), 7.11−7.20 (m, 2H), 4.46 (d, J = 5.7 Hz, 2H), 3.67 (q, J = 6.9 Hz, 1H), 3.33−3.38 (m, 8H), 3.00 (s, 3H), 1.53 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 648 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(4-methoxyphenyl)piperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (90). Yield 73%, white solid, mp 86−88 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45−7.54 (m, 2H), 7.26 (d, J = 7.5 Hz, 1H), 7.06−7.14 (m, 2H), 6.85−6.93 (m, 4H), 6.41 (br s, 1H), 6.23 (br t, 1H), 4.53 (d, J = 5.7 Hz, 2H), 3.79 (s, 3H), 3.56 (q, J = 6.9 Hz, 1H), 3.79 (m, 4H), 3,12 (m, 4H), 2.99 (s, 3H), 1.51 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 610 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(pyridin-2-yl)piperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)-methyl)propanamide (91). Yield 24%, yellowish solid, mp 133 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.51 (s, 1H), 8.60 (br t, 1H), 8.14 (d, J = 4.5 Hz, 1H), 7.57 (m, 2H), 7.41 (d, J = 8.28 Hz, 1H), 7.33 (t, J = 8.34 Hz, 1H), 7.22 (d, J = 11.34 Hz, 1H), 7.15 (d, J = 8.28 Hz, 1H), 6.86 (d, J = 8.28 Hz, 1H), 6.67 (t, J = 6.84 Hz, 1H), 4.34 (m, 2H), 3.72 (q, J = 6.78 Hz, 1H), 3.61 (s, 4H), 3.20 (s, 4H), 3.00 (s, 3H), 1.38 (d, J = 6.78 Hz, 3H). MS (FAB) m/z 582 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-(4-(pyridin-4-yl)piperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)-methyl)propanamide (92). Yield 58%, white solid, mp 132−134 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.28 (d, J = 6.4 Hz, 2H), 7.55 (d, J = 7.9 Hz, 1H), 7.50 (dd, J = 8.2, 8.2 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H), 7.14 (dd, J = 11.4, 2.0 Hz, 1H), 7.09 (d, J = 8.3 Hz, 1H), 6.69 (d, J = 6.6 Hz, 2H), 6.26 (br t, 1H), 4.52 (d, J = 5.7 Hz, 2H), 3.60 (q, J = 7.0 Hz, 1H), 3.43−3.38 (m, 4H), 3.29−3.25 (m, 4H), 3.02 (s, 3H), 1.54 (d, J = 7.1 Hz, 3H). MS (FAB) m/z 581 (MH^+).

***N*-((2-(4-(3-Chloropyridin-2-yl)piperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (93).** Yield 48%, white solid, mp 135 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.60 (br t, 1H), 8.24 (d, J = 4.5 Hz, 1H), 7.82 (d, J = 7.56 Hz, 1H), 7.58 (d, J = 7.5 Hz, 1H), 7.41 (d, J = 7.5 Hz, 1H), 7.32 (t, J = 8.34 Hz, 1H), 7.22 (d, J = 12.06 Hz, 1H), 7.14 (d, J = 8.28 Hz, 1H), 7.02 (m, 1H), 4.33 (m, 2H), 3.71 (q, J = 6.84 Hz, 1H), 3.40 (s, 4H), 3.25 (s, 4H), 2.99 (s, 3H), 1.37 (d, J = 6.78 Hz, 3H). MS (FAB) m/z 616 (MH^+).

***N*-((2-(4-(3-Chloropyridin-2-yl)-2-methylpiperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)propanamide (94).** Yield 23%, beige solid, mp 141 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.57 (d, J = 6.06 Hz, 1H), 8.24 (d, J = 4.56 Hz, 1H), 7.81 (d, J = 7.56 Hz, 1H), 7.61 (dd, J = 18.84, 7.5 Hz, 1H), 7.45 (t, J = 6.84 Hz, 1H), 7.33 (t, J = 8.28 Hz, 1H), 7.22 (d, J = 11.34 Hz, 1H), 7.15 (d, J = 8.34 Hz, 1H), 7.02 (t, 1H), 4.34 (m, 2H), 3.72 (q, J = 6.78 Hz, 1H), 3.48 (m, 2H), 3.38 (m, 4H), 3.20 (m, 1H), 3.00 (s, 3H), 1.37 (d, J = 6.78 Hz, 3H), 1.02 (d, 3H). MS (FAB) m/z 629 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((6-(trifluoro-methyl)-2-(4-(3-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)-pyridin-3-yl)methyl)propanamide (95). Yield 69%, white solid, mp 77−79 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50 (d, J = 3.6 Hz, 1H), 7.91 (dd, J = 7.8, 1.8 Hz, 1H), 7.54 (d, J = 6.9 Hz, 1H), 7.49 (dd, J = 8.1, 8.1 Hz, 1H), 7.05−7.17 (m, 4H), 6.40 (br t, 1H), 4.52 (d, J = 5.7 Hz, 2H), 3.61 (q, J = 6.9 Hz, 1H), 3.35 (m, 8H), 3.02 (s, 3H), 1.53 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 649 (MH^+).

***N*-((2-(4-Benzylpiperazin-1-yl)-6-(trifluoromethyl)pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)phenyl)-propanamide (96).** Yield 58%, white solid, mp 105−109 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48−7.54 (m, 2H), 7.21−7.34 (m, 7H), 7.11 (dd, J = 8.1, 2.1 Hz, 1H), 7.07 (d, J = 8.1 Hz, 1H), 6.24 (br t, 1H), 4.46 (d, J = 5.7 Hz, 2H), 3.51−3.58 (m, 3H), 3.14 (m, 4H) 3.02 (s, 3H), 2.54 (m, 4H), 1.52 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 594 (MH^+).

2-(3-Fluoro-4-(methylsulfonamido)phenyl)-*N*-((2-morpholino-6-(trifluoromethyl)pyridin-3-yl)methyl)propanamide (97). Yield 60%, white solid, mp 82−84 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49−7.56 (m, 2H), 7.26 (d, J = 7.5 Hz, 1H), 7.08−7.17 (m, 2H,), 6.53 (br s, 1H), 6.06 (br t, 1H), 4.48 (d, J = 5.7 Hz, 2H), 3.76 (m, 4H), 3.57 (q, J = 6.9 Hz, 1H), 3.13 (m, 4H), 3.04 (s, 3H), 1.55 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 505 (MH^+).

***N*-((2-((2*S*,6*R*)-2,6-Dimethylmorpholino)-6-(trifluoromethyl)-pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)-phenyl)propanamide (98).** Yield 80%, white solid, mp 66−68 °C. $[\alpha]_D^{25}$ −1.33 (c 0.5, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48−7.52 (m, 2H), 7.10−7.25 (m, 3H), 6.52 (br s, 1H), 6.06 (br t, 1H), 4.47 (d, J = 5.7 Hz, 2H), 3.70 (m, 2H), 3.58 (q, J = 6.9 Hz, 1H), 3.16 (m, 2H), 3.04 (s, 3H), 2.64 (m, 2H), 1.55 (d, J = 6.9 Hz, 3H), 1.19 (d, J = 6.3 Hz, 6H). MS (FAB) m/z 533 (MH^+).

***N*-((2-(1,1-Dioxidothiomorpholino)-6-(trifluoromethyl)-pyridin-3-yl)methyl)-2-(3-fluoro-4-(methylsulfonamido)-phenyl)propanamide (99).** Yield 70%, white solid, mp 77−79 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50−7.56 (m, 2H), 7.33 (d, J = 7.5 Hz, 1H), 7.09−7.17 (m, 2H), 5.94 (br t, 1H), 4.46 (d, J = 5.7 Hz, 2H), 3.72 (m, 4H), 3.60 (q, J = 6.9 Hz, 1H), 3.16 (m, 4H), 3.02 (s, 3H), 1.54 (d, J = 6.9 Hz, 3H). MS (FAB) m/z 553 (MH^+).

Biological Study. Functional Investigations on the Vanilloid Receptor 1 (TRPV1). For determination of agonistic or antagonistic compound activity, the FLIPR-3 instrument (Molecular Devices Corp.) was used for the capsaicin, NADA, and pH assays.

Capsaicin Assay. CHO-K1 cells stably expressing human, rat, or mouse TRPV1 are plated on poly-D-lysine-coated black 96-well plates with a clear bottom (BD Biosciences) at a density of 20 000 cells/well in a volume of 100 μL of Ham's F12 medium with L-glutamine, 10% v/v fetal calf serum (Gibco Invitrogen), and 20 μg/mL L-proline (Sigma). The cells are then incubated overnight at 37 °C, 5% CO_2, and approximately 98% relative humidity. The following day, the cells are incubated with Fluo-4 (Molecular Probes) and 0.01 vol % Pluronic F127 (Molecular Probes) in Hank's buffered saline solution (HBSS, Gibco Invitrogen) for 30 min at 37 °C. The plates are then washed 3 times with HBSS buffer and, after a further incubation for 15 min at room temperature, used in the FLIPR assay for the Ca^{2+} measurement (wavelength λ_{ex} = 488 nm, λ_{em} = 540 nm). The quantification is carried out by measuring the highest fluorescence intensity (FC, fluorescence counts) over time.

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The FLIPR protocol consists of two additions of substances. To test for agonistic activity, the test compounds (3× concentrated in 150 μL total volume, final screening concentration of 10 μM, or dose−response curves from 0.01 to 25 μM) are added to the cells and the Ca^{2+} inflow is compared to the control (capsaicin 10 μM). To test for antagonistic activity, 100 nM capsaicin is added 6 min after test compound addition by the FLIPR pipettor, and the inflow of Ca^{2+} is determined again. Desensitizing agonists and antagonists lead to a suppression of the Ca^{2+} inflow after addition of capsaicin. The inhibition [%] compared to the maximum achievable inhibition with the reference antagonist is calculated.

To test for nonspecific inhibition of the fluorescence assay by the test compounds, plates containing wild-type CHO-K1 cells are activated with ATP (50 μL/well, 10 μM final concentration). Each assay plate contains a reference standard (e.g., BCTC) as well as vehicle controls (HBSS-DMSO) and positive controls (10 μM capsaicin). A capsaicin dose−response curve is generated on a separate plate to determine the EC_{50} concentration.

For data analysis, FLIPR raw data (fluorescence units) are transferred to Excel to determine % stimulation (compared to the response elicited by 10 μM capsaicin) in the first part of the experiment or % inhibition (of the signal elicited by 100 nM capsaicin) in the second part of the experiment for the drugs under investigation. EC_{50}/IC_{50} values are calculated using GraphPad Prism (GraphPad Software, San Diego, CA, U.S.). Functional (f)K_i values are calculated according to the modified Cheng−Prusoff equation.[21]

pH Assay. In the pH assay, human TRPV1-transfected cells are activated with HBSS containing 60 mM MES (Sigma, instead of capsaicin), resulting in a final pH of 6.0−6.3 in the assay medium. Data are recorded and processed as described above.

Temperature Assay. In the temperature assay, CHO-K1 cells expressing human TRPV1 are stimulated by a final medium temperature of 45 °C. For the indirect measurement of intracellular calcium using Fluo-4 dye, CHO-K1/TRPV1 cells are seeded in 100 μL of complete HAM's F12 nutrient mixture medium in clear bottom black 96 MTPs (Corning Cellbind surface assay plates) at 3,000 c/w 96 h before analysis. The day of the experiment, medium is replaced and cells are loaded for 90 min at 37 °C with 50 μL of buffer B. After removal of buffer B, the plate is then incubated in 50 μL of buffer A for 15 min at room temperature and protected from light. An amount of 25 μL of reference antagonist or test compound (3-fold concentrated in buffer A giving the final concentration in the wells) is added to start the experiment, followed by an incubation step at room temperature for 5 min. During this incubation period, the baseline fluorescence prior to stimulation is measured at λ_{ex} = 488 nm, λ_{em} = 540 nm using the FLX800 fluorescence reader (Biotek). After 5 min, the plate is transferred into the PCR cycler PTC200, MJ Research (flat bottom insert) and the temperature cycling program is started (using heated lid, 2 min 57 °C, 1 min 20°). Directly after this temperature stimulus resulting in a final temperature 45 °C (approximately EC_{80} of temperature stimulation response) in the wells, the fluorescence is measured again. The difference in fluorescence units before and after temperature stimulation is calculated and used for the IC_{50} determination of the antagonists. Each experiment is also conducted on CHO-K1 wild-type cells to detect unspecific drug interactions in this assay system. Buffer A consisted of HBSS + $CaCl_2$ + MgCl (Gibco) containing 2.5 mM probenecid and 10 mM HEPES, pH 7.4. Buffer B consisted of HBSS + $CaCl_2$ + MgCl (Gibco) containing 2.17 μM Fluo-4 AM (Molecular Probes), 2.5 mM probenecid, 10 mM HEPES, pH 7.4.

NADA Assay. The effect of test compounds to inhibit a *N*-arachidonoyldopamine stimulus acting on TRPV1 or to perform as agonist on calcium release in TRPV1 transfected eukaryotic cells is analyzed. NADA (*N*-arachidonoyldopamine, Tocris) is dissolved in ethanol to 10 mM stock concentration and stored at −20 °C. Working solutions are freshly prepared by dilution in buffer A. The test compounds are freshly prepared as 10 mM solutions in DMSO (Fluka) and then diluted in buffer B. To keep the DMSO concentration below 0.1%, the highest concentration of compounds used in the test is 10 μM. For indirect measurement of intracellular calcium by FLIPR3 using Fluo-4 dye, CHO-K1/TRPV1 cells are seeded in 100 μL of complete HAM's F12 nutrient mixture medium in clear-bottom black 96 MTPs (Corning Cellbind surface assay plates) at 20,000 c/w 24 h before analysis. The day of experiment, medium is replaced and cells are loaded with 50 μL of buffer C for 40 min at 37 °C. After washing twice with buffer D, 100 μL of buffer D is added to the cells followed by 15 min of incubation at room temperature in the dark. Then, in the FLIPR instrument, an amount of 50 μL of known antagonist or test compound (3-fold concentrated in buffer B, giving 0.1% BSA final concentration in the wells) is added to the cells followed by the addition of 50 μL of NADA as agonist (approximately EC_{90}, 4-fold concentrated in buffer A, final BSA concentration of 0.1%, final volume of 200 μL). Fluorescence measurements are performed for about 9 min before and for about 5 min after agonist injection. (f) K_i values are calculated according to Cheng−Prusoff.[25] Buffer A consisted of HBSS +$CaCl_2$ + MgCl (Gibco No. 14025-050) containing 2.5 mM probenecid, 0.1% BSA (PAA No. K05-013, 30% stock solution), 10 mM HEPES, pH 7.4. Buffer B consisted of HBSS +$CaCl_2$ + MgCl (Gibco No. 14025-050) containing 2.5 mM probenecid, 0.3% BSA (PAA No. K05-013, 30% stock solution), 10 mM HEPES, pH 7.4. Buffer C consisted of HBSS + $CaCl_2$ + MgCl (Gibco No. 14025-050) containing 2.17 μM Fluo-4 AM (Molecular Probes No. F14201, 50 μg), 2.5 mM probenecid, 10 mM HEPES, pH 7.4. Buffer D consisted of HBSS + $CaCl_2$ + MgCl (Gibco No. 14025-050) containing 2.5 mM probenecid, 10 mM HEPES, pH 7.4.

Animal Pain Model Assay. Adult male Sprague−Dawley rats (170−310 g, obtained from Janvier, Le Genest Saint Isle, France) were used in the study. Animals were kept under standard laboratory conditions with free access to standard laboratory food and tap water. The experiments were performed in accordance with ECC guidelines (86/609/EEC) for the use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. Animals were tested in randomized groups of 10 for each dose and vehicle controls. Although the operators performing behavioral tests were not formally "blinded" with respect to the treatment, they were not aware of the nature of the differences between the drugs and the study hypothesis. Rats underwent surgery involving four loose ligations of the sciatic nerve according to the Bennett model. Under pentobarbital anesthesia (Narcoren, 60 mg/kg ip), the right common sciatic nerve was exposed by blunt dissection at the level of mid-thigh and four loose ligatures (Softcat Chrom USP 4/0, metric 2; Braun Melsungen, Germany) were placed around the nerve, taking care not to interrupt the epineural circulation. After operation, animals were allowed to recover for 1 week. Cold allodynia was stable for several weeks and was tested on a metal plate cooled by a water bath to a constant temperature of 4 °C by means of counting the number of brisk paw withdrawals during 2 min. Animals were observed for periods of 2 min before and 15, 30, 45, and 60 min after administration of test compounds or vehicle control (for compound **2**, 10% DMSO, 10% glucose, in 0.9% NaCl; for compound **49S**, 10% DMSO, 50% PEG400, in H_2O). % MPE of each time point was calculated according to the formula $[(T_0 - T_1)/T_0] \times 100$, where T_0 and T_1 were numbers of paw withdrawal reactions before and after drug administration, respectively. Data were analyzed by means of two-factor analysis of variance (ANOVA) with repeated measures. In the case of a significant treatment effect, pairwise comparison was performed at every test time point on raw data by post hoc analysis with Bonferroni adjustment. Results were considered statistically significant if $p < 0.05$.

Care and Handling of Mice. Animals were housed under a (12 h)/(12 h) light−dark cycle (lights on at 06:00 a.m.) and with room temperature of 20−24 °C, relative air humidity of 35−70%, 15 air changes per hour, and air movement of <0.2 m/s. The animals had free access to standard laboratory food (Ssniff R/M-Haltung, Ssniff Spezialdiäten GmbH, Soest, Germany) and tap water. All animals were used only once in all pain models. There were at least 5 days between delivery of the animals and the start of experiment. Animal testing was performed in accordance with the recommendations and policies of the International Association for the Study of Pain[26] and the German Animal Welfare Law. All study protocols were approved by the local government committee for animal research, which is also an ethics

committee. Animals were assigned randomly to treatment groups. Different doses and vehicle were tested in a randomized fashion. Although the operators performing the behavioral tests were not formally "blinded" with respect to the treatment, they were not aware of the study hypothesis or the nature of differences between drugs.

Capsaicin Induced Hypothermia in Mice. The administration of the TRPV1 agonist capsaicin is known to produce a dramatic fall in body temperature.[20,21] The studies were conducted with male NMRI mice (30−35 g) supplied by a commercial breeder (Charles River, Sulzfeld, Germany). Mice were habituated to the experimental room (23−24 °C) for up to $1^1/_2$ h prior to treatment. Intraperitoneal injection of capsaicin, 3 mg/kg, was used to analyze TRPV1 agonist-induced hypothermia. The rectal temperature was measured twice before (baseline) and 15, 30, 60, 90, and 120 min after capsaicin with a thermocouple probe (connected to a digital thermometer; Thermalert TH-5, Physitemp, Clifton, NJ). Compound **49*S*** at a dose of 0.3 mg/kg or vehicle (10% DMSO, 50% PEG in H_2O) was administered alone to investigate a possible intrinsic effect on body temperature and 15 min before intraperitoneal capsaicin injection.

Analysis of Body Temperature in Wild Type and TRPV1 Knockout Mice. For the experiments on the effect of the substance on body temperature, male TRPV1 knockout mice (B6.129S4-Trpv1^{tm1Jul}/J, Jax mice, U.S.; 20−30 g) were used on congenic background and compared to male C57BL/6J wild-type mice (Charles River, Sulzfeld, Germany; 20−30 g). The rectal temperature was measured as described above, twice before (baseline) and 15, 30, 60, and 90 min after substance. Compound **49*S*** dissolved in 10% DMSO and 50% PEG in H_2O was administered at a dose of 1 mg/kg 15 min before intraperitoneal capsaicin (3 mg/kg) injection.

Molecular Modeling. The human TRPV1 (hTRPV1) tetramer homology model was constructed by the Build Mutants protocol in Discovery Studio, version 3.1 (Accelrys Inc., San Diego, CA, U.S.), using the capsaicin-docked form of our rat TRPV1 tetramer homology model[24] as a template. The 27 residues different between rat and human TRPV1 sequences were computationally mutated as Phe439Leu, Ala450Met, Glu458Asp, Tyr463Phe, Leu465Met, Lys466-Glu, Asn467Lys, Ser483Leu, Leu503Met, Ser505Thr, Ile514Met, Val518Leu, Val525Ala, Ser526Thr, Gln533His, Arg534Leu, Met547-Leu, Leu585Ile, Asn605Asp, Met609Ser, Pro613Ser, Lys615Arg, Cys616Trp, Ser619Pro, Lys622Arg, Gly624Pro, and Asn625Asp. The side chains and backbone within 4.5 Å of the mutated residues were optimized by the MODELER 9v8 program through a combination of conjugate gradient minimization and molecular dynamics with simulated annealing. Among the resulting five models, the model with the lowest probability density function (PDF) total energy was selected. Then it was energy minimized with the backbone atoms fixed, using a CHARMm force field until the rms of the conjugate gradient was lower than 0.05 kcal mol^{-1} A^{-1}. The 3D structures of the ligands were generated with Concord and energy minimized using an MMFF94s force field and an MMFF94 charge until the rms of the Powell gradient was 0.05 kcal mol^{-1} A^{-1} in SYBYL-X 1.2 (Tripos International, St. Louis, MO, U.S.). The flexible docking study on our hTRPV1 model was performed by GOLD, version 5.0.1 (Cambridge Crystallographic Data Centre, Cambridge, U.K.), which uses a genetic algorithm (GA) and allows for full ligand flexibility and partial protein flexibility. The binding site was defined as 8 Å around the capsaicin, complexed in the hTRPV1 model. The side chains of the nine residues (i.e., Tyr511, Ser512, Met514, Leu515, Leu518, Phe543, Leu547, Thr550, and Asn551), which are thought to be important for ligand binding, were set to be flexible with "crystal mode" in GOLD. The ligands were docked using the GoldScore scoring function with 30 GA runs, and other parameters were set as default. All computation calculations were undertaken on an Intel Xeon Quad-core 2.5 GHz workstation with Linux Cent OS, release 5.5.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HPLC purities of all final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 82-2-880-7846. Fax: 82-2-888-0649. E-mail: jeewoo@snu.ac.kr.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

TRPV1, transient receptor potential cation channel, subfamily V, member 1; SAR, structure−activity relationship

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