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Analysis of structure–activity relationships for the 'A-region' of *N*-(4-*t*-butylbenzyl)-*N*′-[4-(methylsulfonylamino)benzyl]thiourea analogues as TRPV1 antagonists

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Abstract—The structure–activity relationships for the 'A-region' of *N*-(4-*t*-butylbenzyl)-*N*′-[4-(methylsulfonylamino)benzyl]thiourea analogues have been investigated as TRPV1 receptor antagonists. The 2-halogen analogues showed enhanced antagonism compared to the prototype antagonist.

1. Introduction

The vanilloid receptor subtype 1 (VR1 or TRPV1)[1] belongs to the transient receptor potential (TRP) superfamily. Members of this family are nonvoltage activated cation channel proteins and share a structural characteristic of six transmembrane segments.[2,3] The receptor has been cloned from dorsal root ganglia (DRG) of the rat,[4] the human,[5] the chicken,[6] the guinea pig,[7] and the rabbit.[8] Other vanilloid receptor homologues were also cloned recently from different organisms but are not believed to be sensitive to vanilloids.[2] TRPV1 is a molecular integrator of nociceptive stimuli expressed predominantly on unmyelinated pain-sensing nerve fibers (C-fibers) and small Aδ fibers in the dorsal root, trigeminal, and nodose ganglia.

TRPV1 is activated by protons,[9] heat,[4] endogenous substances such as anandamide[10] and the lipoxygenase products,[11] and natural ligands such as capsaicin (CAP)[12] and resiniferatoxin (RTX).[13] Since TRPV1 functions as a nonselective cation channel with high Ca^{2+} permeability, its activation by these agents leads to an increase in intracellular Ca^{2+} that results in excitation of primary sensory neurons and ultimately the central perception of pain. The involvement of this receptor in both pathological and physiological conditions suggests that the blocking of this receptor activation, by desensitization or antagonism, would have considerable therapeutic utility. Among its therapeutic targets, pain is of particular interest. The validation of TRPV1 as a molecular target for the treatment of chronic pain was confirmed using transgenic mice lacking functional TRPV1 receptors. These mice exhibited impairment in the perception of thermal and inflammatory pain.[14]

TRPV1 antagonists have attracted much attention as promising drug candidates to inhibit the transmission of painful signals from the periphery to the CNS and to block other pathological states associated with this

Keywords: TRPV1 antagonist; Vanilloid receptor 1 antagonist; Capsaicin; Resiniferatoxin.
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receptor. The therapeutical advantage of TRPV1 antagonism over agonism is that it lacks the initial excitatory effect preceding the desensitization. The initial acute pain associated with capsaicin treatment has proven to be the limiting toxicity.

Previously, we have demonstrated that isosteric replacement of the phenolic hydroxyl group in potent vanilloid receptor agonists with the alkylsulfonamido group generated compounds which were effective antagonists of the action of capsaicin on rat TRPV1.[15,16] As shown in Figure 1, substitution of 4-hydroxy-3-methoxyphenyl in the potent agonist **1**[17] with 4-(methylsulfonylamino)phenyl led to a prototype antagonist **2**,[15] which showed high binding affinity and potent antagonism (K_i = 63 nM and $K_{i(ant)}$ = 54 nM in rTRPV1/CHO). We have further described that 3-substitutents in the A-region affected the extent of agonism/antagonism. Thus, the 3-fluoro derivative **3** (K_i = 53.5 nM, $K_{i(ant)}$ = 9.16 nM in rTRPV1/CHO[15]; IC_{50} = 37 nM in rTRPV1/DRG[18]) was a potent antagonist not only of capsaicin stimulation of rTRPV1 but also of stimulation by temperature and pH. Conversely, the 3-methoxy derivative **4** showed a shift to partial agonism (K_i = 50.4 nM, 17.4% agonism and 84.1% antagonism in rTRPV1/CHO).[19]

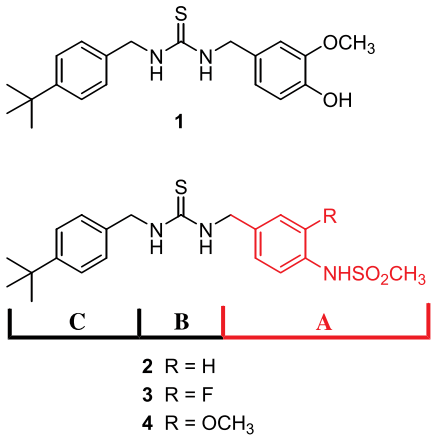
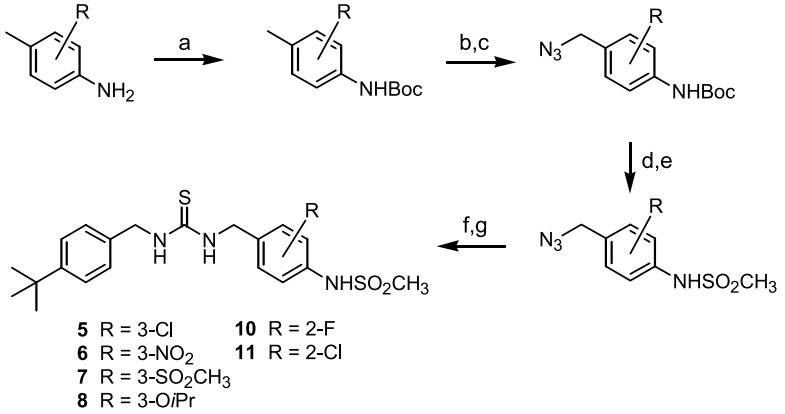


Figure 1.

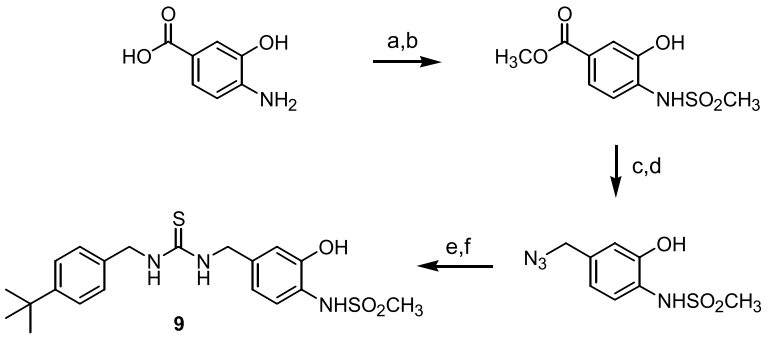
In order to optimize in vitro activities of 4-methylsulfonamide TRPV1 antagonists, we have investigated their structure–activity relationships based on the structural regions designated as A-, B- and C-regions.[20]The extensive modification of the C-region led to the finding that 4-*t*-butylbenzyl is one of the most favorable groups for high receptor binding affinity and potent antagonism of TRPV1.[21] As a continuation of our effort, we herein describe the structure–activity relationships in the A-region of the high affinity lead compounds **2**–**4** in which the B- and C-regions of the novel ligands were fixed as thiourea and 4-*t*-butylbenzyl groups, respectively.

2. Chemistry

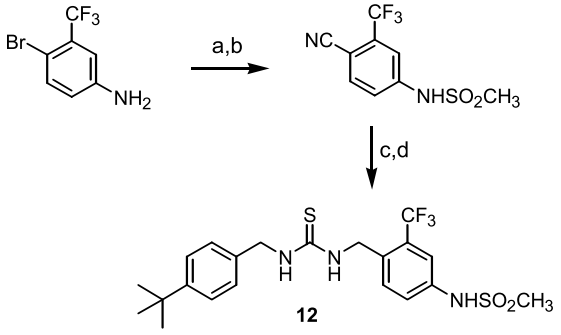
The target compounds were synthesized in general by the coupling of 4-*t*-butylbenzyl isothiocyanate with the corresponding amines of the A-region. The syntheses of 2- or 3-substituted analogues are outlined in Scheme 1. Starting from commercially available 4-methylanilines, the corresponding benzyl azides were prepared by *t*-butoxycarbonyl protection of the amines, benzylic bromination (or hydroxylation), followed by conversion to azides. The azides were reduced and finally coupled with isothiocyanate to afford the thioureas **5**–**8** and **10**–**11**. The 3-hydroxy (**9**) and 2-trifluoromethyl (**12**) analogues were prepared from 4-amino-3-hydroxybenzoic acid and 4-bromo-3-(trifluoromethyl)aniline, respectively, by straightforward conventional routes as described in Schemes 2 and 3. The 3-methylsulfonylamino (**13**) and 4-(methylsulfonylamino)methyl (**14**) analogues were obtained readily from the amines previously reported.[16] The syntheses of 3-methylsulfide, sulfoxide and sulfone analogues (**15**–**17**), shown in Scheme 4 started from 3-mercaptobenzoic acid, which was converted to benzyl azides with different oxidation states of sulfur. The *N*-4-(methylsulfonylamino)phenyl (**18**) and *N*-4-(methylsulfonylamino)phenethyl (**19**) analogues were prepared



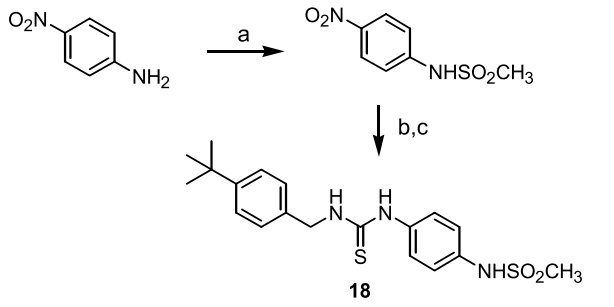
Scheme 1. Reagents: (a) for **5**, **6**: $(Boc)_2O$, NaH, DMF, 85–95%; for **7**: (i) $NaSCH_3$, THF–dH_2O; (ii) Fe, AcOH; (iii) $(Boc)_2O$, EtOH; (iv) m-CPBA, $CHCl_3$, 40% for 4 steps; for **8**: (i) $(Boc)_2O$, CH_2Cl_2; (ii) $(CH_3)_2CHBr$, K_2CO_3, DMF, 82% for 2 steps; for **10**, **11**: $(Boc)_2O$, THF, 90–95%; (b) NBS, AIBN, CCl_4, 40–60%; (c) NaN_3, DMF, 90–98%; (d) TFA, CH_2Cl_2; (e) MsCl, pyridine, 90–94% for 2 steps; (f) H_2, Pd–C, MeOH or PPh_3, H_2O, THF, 92–98%; (g) (4-*t*-Bu)$PhCH_2NCS$, NEt_3, DMF, 75–95%.



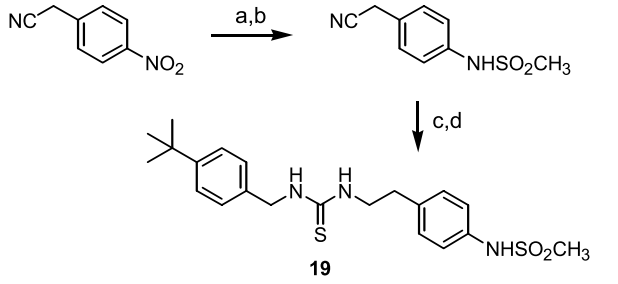
Scheme 2. Reagents: (a) H_2SO_4, MeOH, 76%; (b) MsCl, CH_2Cl_2, 85%; (c) $LiAlH_4$, THF, 88%; (d) NaN_3, BF_3–Et_2O, dioxane, 82%; (e) H_2, Pd–C, MeOH, 98%; (f) (4-*t*-Bu)$PhCH_2NCS$, NEt_3, DMF, 92%.



Scheme 3. Reagents: (a) MsCl, pyridine, 98%; (b) CuCN, NMP, 185 °C, 76%; (c) H_2, Pd—C, MeOH, 98%; (d) (4-*t*-Bu)$PhCH_2NCS$, NEt_3, CH_2Cl_2, 90%.

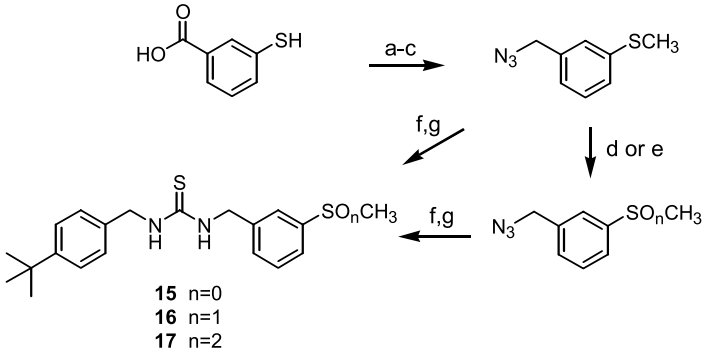


Scheme 5. Reagents: (a) MsCl, pyridine, 99%; (b) H_2, Pd–C, MeOH, 98%; (c) (4-*t*-Bu)$PhCH_2NCS$, CH_2Cl_2, 94%.

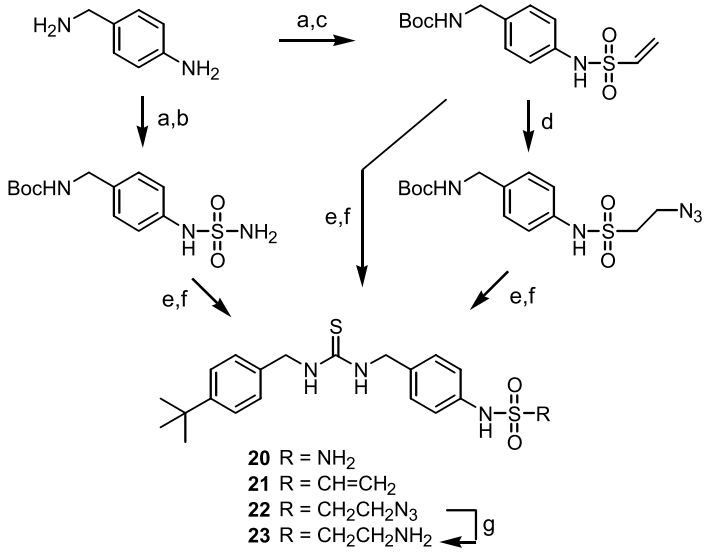


Scheme 6. Reagents: (a) H_2, Pd–C, MeOH, 99%; (b) MsCl, pyridine, 92%; (c) H_2, Pd–C, conc. HCl, MeOH, 40%; (d) (4-*t*-Bu)$PhCH_2NCS$, CH_2Cl_2, 88%.

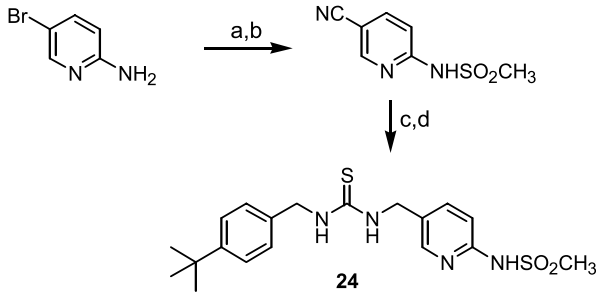
starting from 4-nitroaniline and 4-nitrophenylaceto nitrile, respectively, in a conventional manner, as described in Schemes 5 and 6. The syntheses of sulfonylamino analogues **20**–**23** are depicted in Scheme 7. While the sulfamide analogue (**20**) was prepared through N-sulfonylation of *N*-Boc-4-aminobenzyl amine with sulfamyl chloride, compounds **21**–**23** were obtained through key steps including N-sulfonylation with 2-chloroethylsulfonyl chloride to provide (vinylsulfonyl)amino and subsequent azidation with trimethylsilyl azide. The syntheses of heterocyclic analogues are shown in Schemes 8 and 9. Whereas the 2-methylsulfonylamino pyridine analogue (**24**) was synthesized from 2-amino-5-bromopyridine using a route similar to that described in Scheme 3, the 5-methylsulfonylamino pyridine (**25**) and pyrazine (**26**) analogues were prepared from 6-methyl nicotinic acid and 5-methyl-2-pyrazine carboxylic acid, respectively, employing Curtius rearrangement



Scheme 4. Reagents: (a) CH_3I, K_2CO_3, acetone, 98%; (b) $LiAlH_4$, THF, 96%; (c) DPPA, DEAD, PPh_3, THF, 68%; (d) H_2O_2, AcOH, 85% for $n = 1$; (e) m-CPBA, $CHCl_3$, 65% for $n = 2$; (f) H_2, Pd–C, MeOH, 96%; (g) (4-*t*-Bu)$PhCH_2NCS$, CH_2Cl_2, 92%.



Scheme 7. Reagents: (a) $(Boc)_2O$, THF, 90%; (b) $ClSO_2NH_2$, Et_3N, CH_2Cl_2, 60%; (c) $ClSO_2CH_2CH_2Cl$, Et_3N, CH_2Cl_2, 97%; (d) $TMSN_3$, MeOH, DMF, sealed tube, 100 °C, 75%; (e) CF_3CO_2H, CH_2Cl_2; (f) (4-*t*-Bu)$PhCH_2NCS$, NEt_3, CH_2Cl_2, 80–90% for 2 steps; (g) PPh_3, H_2O, THF, 80%.



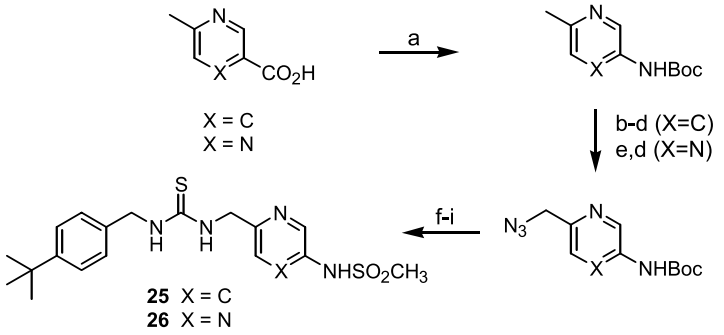
Scheme 8. Reagents: (a) $(CH_3SO_2)O$, CH_2Cl_2, 55%; (b) CuCN, NMP, 180 °C, 60%; (c) H_2, Pd–C, conc. HCl, MeOH; (d) (4-*t*-Bu)$PhCH_2NCS$, NEt_3, CH_2Cl_2, 50% for 2 steps.

and the benzylic azidation method described in Scheme 1.

3. Results and discussion

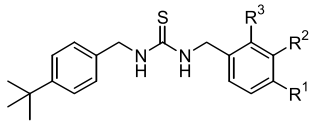
The binding affinities and agonistic/antagonistic potencies of the synthesized TRPV1 ligands were assessed in vitro by a binding competition assay with [^{3}H]RTX and a functional $^{45}Ca^{2+}$ uptake assay using rat TRPV1 heterologously expressed in Chinese hamster ovary (CHO) cells, as previously described.[15,16] The results are summarized in Tables 1 and 2, together with the potencies of capsazepine and the antagonists **2**–**4**.

Our previous findings on the SAR of *N*-(4-*t*-butylbenzyl)-*N*′-4-[(methylsulfonylamino)benzyl]thiourea antagonists indicated that the incorporation of 3-fluoro on the A-region further enhanced antagonism, whereas a 3-methoxy group favored agonism. Therefore, we decided to explore the effect on potency as well as agonism/antagonism of substituents at various positions in the A-region. The incorporation of electron-withdrawing groups, including the lipophilic 3-chloro (**5**, $+\sigma$, $+\pi$), 3-nitro (**6**, $+\sigma$, $+\pi$) and hydrophilic 3-methylsulfone (**7**, $+\sigma$, $-\pi$), led to much reduced potencies in binding affinity (>20-fold lower) and antagonism (>7-fold lower) compared to **2**. Indeed, the 3-methylsulfone analogue (**7**) was devoid of any receptor activity. The analogues with electron-donating groups, such as the lipophilic 3-isopropoxy (**8**) and hydrophilic 3-hydroxy (**9**), showed



Scheme 9. Reagents: (a) DPPA, Et_3N, *t*-BuOH, toluene, 85%; (b) m-CPBA, $CHCl_3$, 45%; (c) MsCl, Et_3N, CH_2Cl_2, 78%; (d) NaN_3, DMF, 80% in X = C, 85% in X = N; (e) NBS, AlBN, CCl_4, 80%; (f) CF_3CO_2H, CH_2Cl_2, 82%; (g) MsCl, pyridine, 60%; (h) H_2, Lindler cat, MeOH; (i) (4-*t*-Bu)$PhCH_2NCS$, NEt_3, DMF, 80% for 2 steps.

Table 1. Potencies of TRPV1 ligands for binding to rat VR1 and for inducing calcium influx in VR1/CHO cells



	R^1	R^2	R^3	K_i (nM)[c] binding affinity	EC_{50} (nM)[c] agonism	K_i (nM)[c] antagonism
CPZ				1300	NE	520
2	$NHSO_2CH_3$	H	H	63 (±10)	NE	54 (±8.7)
3	$NHSO_2CH_3$	F	H	53.5 (±6.5)	NE	9.16 (±1.6)
4	$NHSO_2CH_3$	OCH_3	H	50.4 (±16.5)	WE[a]	3.4 (±0.5)[b]
5	$NHSO_2CH_3$	Cl	H	1160 (±110)	NE	400 (±220)
6	$NHSO_2CH_3$	NO_2	H	1300 (±120)	NE	610 (±160)
7	$NHSO_2CH_3$	SO_2CH_3	H	NE	NE	NE
8	$NHSO_2CH_3$	$OCH(CH_3)_2$	H	8072 (±1900)	NE	3600 (±1100)
9	$NHSO_2CH_3$	OH	H	750 (±72)	WE[a]	WE[b]
10	$NHSO_2CH_3$	H	F	215 (±19)	NE	27.3 (±8.6)
11	$NHSO_2CH_3$	H	Cl	144 (±31)	NE	45.5 (±2)
12	$NHSO_2CH_3$	H	CF_3	440 (±56)	NE	119 (±21)
13	H	$NHSO_2CH_3$	H	6000 (±1200)	4100 (±1500)	NE
14	$CH_2NHSO_2CH_3$	H	H	6120 (±260)	1720 (±330)	NE
15	H	SCH_3	H	580 (±26.4)	WE[a]	NE
16	H	$SOCH_3$	H	3600 (±1100)	1890 (±190)	NE
17	H	SO_2CH_3	H	1000 (±23)	1010 (±46)	NE

[a] Only fractional calcium uptake compared with that induced by 300 nM capsaicin (**4**, 17%; **9**, 62%; **15**, 23%).
[b] Only fractional antagonism (**4**, 84%; **9**, 39%).
[c] Values represent means ± SEM from three or more experiments (K_i for **8** represents means ± range of two experiments). NE, no effect. WE, weak effect (quantitation of fractional agonism/antagonism is from 1–3 experiments; compounds were tested at a maximal concentration of 10–30 μM).

very low potencies both for binding and antagonism. These findings indicated that steric factors at the 3-position rather than electronic/lipophilic ones were most important for receptor activity, since receptor potencies diminished in proportion to the size of the 3-substituents. The small 3-substituents, such as hydrogen, fluoro and methoxy groups in **2**–**4**, were thus optimal for conferring potent binding activity in this study.

The effect of substituents at the 2-position was also investigated. The 2-fluoro analogue of **2** (**10**) exhibited 3.5-fold weaker potency in binding affinity but 2-fold greater potency for antagonism compared to **2**. The other electron-withdrawing groups, such as 2-chloro (**11**) and 2-trifluoromethyl (**12**), led to moderately reduced binding affinity (2- and 7-fold) but retention of potencies for antagonism. The modification of substituents in the A-region revealed that the 3-fluoro group appeared to be optimal for binding and antagonism to the receptor.

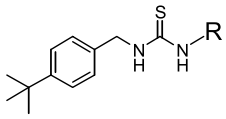
In order to discern the positional significance of the 4-methylsulfonylamino group in prototype **2** for antagonism, the corresponding 3-methylsulfonylamino (**13**) and one-carbon elongated, 4-(methylsulfonylamino)methyl, analogues (**14**) were examined. As expected, both exhibited a dramatic loss in binding affinity, ca. 100-fold compared to **2**, as well as a shift to full agonism. This result is consistent with a series of *N*-(3-acyl-2-benzylpropyl)thiourea analogues reported previously.[16]

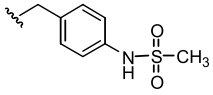
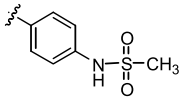
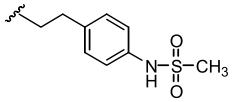
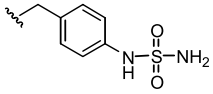
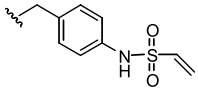
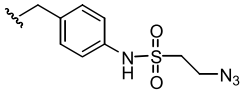
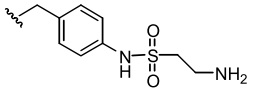
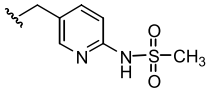
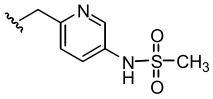
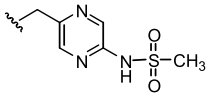
Recently, SB-366791 identified through high-throughout screening was a potent and selective hTRPV1 antagonist; structurally, it contains a 3-methoxyphenyl group in the A-region.[22] Thus, 3-methylsulfide, sulfoxide and sulfone analogues (**15**–**17**) were investigated as its congeners. Interestingly, these congeners showed agonism rather than antagonism even though **15** was found to be a partial agonist. Moreover, their binding affinities were low.

To find the optimal pharmacophoric distance between the A- and B-regions, we varied the distance with a one-carbon shortened 4-(methylsulfonylamino)phenyl (**18**) and a one-carbon elongated 4-(methylsulfonylamino)phenethyl (**19**) analogues. These also displayed a dramatic decrease in binding affinity and antagonism with limited partial agonism.

From our previous SAR investigation on the 4-methylsulfonylamino group, we demonstrated that substitution of methyl with other alkyl groups diminished the potencies of binding and antagonism as the sizes increased.[16,23] In capsaicinoid agonists, a 2-aminoethyl group has been employed on the phenolic hydroxyl group of the A-region. The suggested rationale is that this substitution enhances metabolic stability in vivo, and the analogues indeed were orally effective with good analgesic potency.[17] Thus, we replaced the methyl group of 4-methylsulfonamido with other groups such as amine, vinyl, 2-azidoethyl and 2-aminoethyl to provide **20**–**23**. Unlike the methylsulfonamide derivative, the sulfamide (**20**) and vinylsulfonamide (**21**) congeners functionally proved to be a partial and a full agonist, respectively, with 10- and 3.5-fold reduced binding affinity compared to **2**. It is noteworthy that, although substitution of the 4-phenolic hydroxyl of the A-region in agonists with the 4-methylsulfonamido group shifted the ligands from agonism toward antagonism, the

Table 2. Potencies of TRPV1 ligands for binding to rat VR1 and for inducing calcium influx in VR1/CHO cells



	R	K_i (nM)[c] binding affinity	EC_{50} (nM)[c] agonism	K_i (nM)[c] antagonism
2		63 (±10)	NE	54 (±8.7)
18		9560 (±810)	WE[a]	6800 (±2800)
19		3900 (±1100)	WE[a]	699 (±81)
20		650 (±140)	WE[a]	WE[b]
21		231 (±90)	250 (±35.5)	NE
22		145 (±5.4)	NE	231 (±33)
23		4640 (±210)	NE	WE[b]
24		4100 (±1300)	NE	177 (±25)
25		11900 (±3900)	NE	4100 (±900)
26		NE	NE	NE

[a] Only fractional calcium uptake compared with that induced by 300 nM capsaicin (**18**, 7%; **19**, 18%; **20**, 50%).
[b] Only fractional antagonism (**20**, 35%; **23**, 50%).
[c] Values represent means ± SEM from three or more experiments. NE, no effect. WE, weak effect (quantitation of fractional agonism/antagonism is from 1 to 3 experiments; compounds were tested at a maximal concentration of 10-30 μM).

antagonism can be restored to agonism by even small modifications such as transposition to the 3-position (**13**), one-carbon elongation (**14**) and substitution of methyl with vinyl (**21**). The 2-azidoethyl congener (**22**) was an antagonist with moderately reduced binding affinity (2-fold) and antagonism (4-fold), whereas the 2-aminoethyl congener (**23**) was a partial antagonist with much reduced binding affinity (75-fold) compared to **2**. The result confirmed that other sulfonamides did not improve the potency of methylsulfonamide for receptor antagonism.

As a last series of modifications, we replaced the phenyl ring of **2** with polar isosteres, such as pyridine and pyrazine, for which one might expect improved pharmacokinetics. A series of 4-(2-pyridyl)piperazines, such as BCTC,[24] have been reported to show potent TRPV1 antagonism. Unfortunately, the pyridine analogues (**24** and **25**) exhibited much decreased receptor potencies compared to **2** and the pyrazine analogue (**26**) was devoid of activity.

In summary, we have modified the A-region of *N*-(4-*t*-butylbenzyl)-*N*′-[4-(methylsulfonylamino)benzyl]thiourea (**2**), a prototype TRPV1 antagonist with potent antagonism and high affinity, to analyze its structure–activity relationships. The approaches included incorporation of diverse substituents at the 2- and 3-positions, positional modification of 4-methylsulfonamide, 3-methoxyphenyl surrogates, variation of the distance between A- and B-regions, methyl modification of 4-methylsulfonamide and phenyl substitution with isosteric rings.

The 2-halogen analogues, namely **10** and **11**, showed enhanced antagonism. However, the other modifications generally conferred modest to the dramatic decreases in binding affinities and antagonistic potencies or shifted ligands from antagonism to agonism.

In the following paper, the extensive structure–activity relationships at the B-region will be described.

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