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Halogenation of 4-hydroxy-3-methoxybenzyl thiourea TRPV1 agonists showed enhanced antagonism to capsaicin

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Abstract—Selected potent TRPV1 agonists (**1**–**6**) have been modified by 5- or 6-halogenation on the aromatic A-region to analyze their effects on potency and efficacy (agonism versus antagonism). The halogenation caused enhanced functional antagonism at TRPV1 compared to the corresponding prototype agonists. The analysis of SAR indicated that the antagonism was enhanced as the size of the halogen increased (I > Br > Cl) and when the 6-position was halogenated. Compounds **23c** and **31b** were found to be potent full antagonists with K_i (as functional antagonist) = 23.1 and 30.3 nM in rTRPV1/CHO system, respectively.

TRPV1 is a member of the transient receptor potential (TRP) superfamily;[1] the TRP family proteins form non-voltage activated cation channels and share a structural characteristic of six transmembrane segments.[2,3] TRPV1 functions as 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. It is activated by protons,[4] heat,[5] endogenous substances such as anandamide[6] and lipoxygenase products,[7] by vanilloids such as capsaicin (CAP)[8] and resiniferatoxin (RTX),[9] or indirectly by bradykinin.[10] Since TRPV1 is a non-selective 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 the primary sensory neurons (Fig. 1).

The receptor activation can be blocked either by desensitization subsequent to agonist exposure or by direct antagonism. Both strategies would have considerable therapeutic utility targeting inflammatory and neuropathic pain, cystitis, and bladder hyperreflexia. TRPV1 antagonists have attracted much attention so far 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 receptor. A therapeutic 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. A further advantage of antagonists is that their effects are readily reversible (Fig. 2).

Previously, we have demonstrated that so-called simplified RTX analogues, *N*-(3-pivaloyloxy-2-benzylpropyl)-*N*′-(4-hydroxy-3-methoxybenzyl)thioureas (**1** and **2**),[11] *N*-[2-pivaloyloxy-1-(phenethyl)ethyl]-*N*′-(4-hydroxy-3-methoxybenzyl)thioureas (**3** and **4**),[12] and *N*-[2-pivaloyloxy-1-(4-*t*-butylbenzyl)ethyl]-*N*′-(4-hydroxy-3-methoxybenzyl)thiourea (**5**),[12] possess potent TRPV1 agonism with high affinity, having a range of K_i (binding) = 6.35–56 nM and EC_{50} (agonism) = 1.97–21.5 nM in rat TRPV1 heterologously expressed in Chinese hamster ovary (CHO) cells and excellent analgesic activities. *N*-(4-*t*-Butylbenzyl)-*N*′-(4-hydroxy-3-methoxybenzyl) thiourea (**6**) also proved to be a highly potent agonist by the Novartis group[13] and by us.[14] Interestingly, we have found that isosteric replacement of the phenolic hydrox-

Keywords: TRPV1 agonist; TRPV1 antagonist; Halogenation; Resiniferatoxin; Capsaicinoid.
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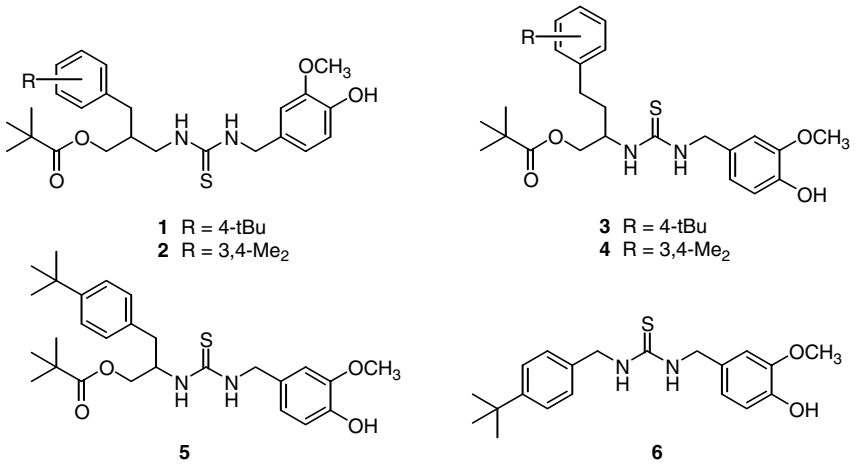


Figure 1.

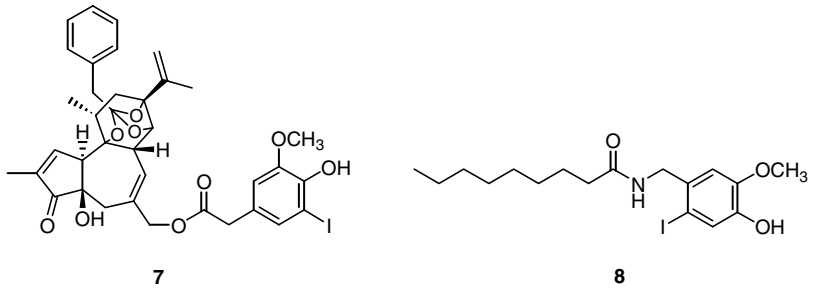
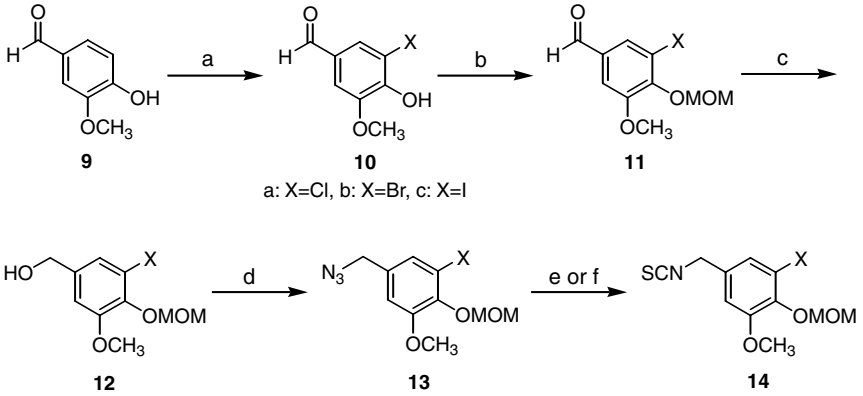


Figure 2.

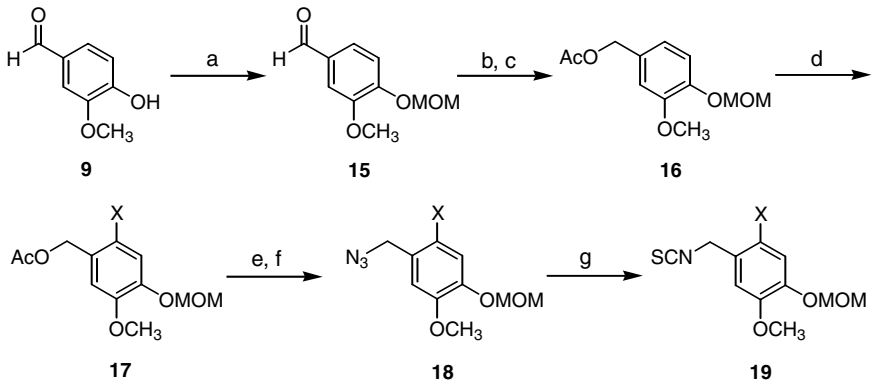
yl group in lead agonists with the methylsulfonamido group provided a series of antagonists effective against the action of capsaicin. Among them, the 3-fluoro-4-methylsulfonylamino analogue of the A-region in agonists **2** and **6** showed excellent antagonism with values of K_i (ant) = 7.8 and 9.16 nM, respectively.[14,15]

Recently it was reported that the halogenation of the aromatic A-ring of agonists also shifted the agonism of the ligands toward antagonism. Two leading examples are 5-iodoresiniferatoxin (**7**)[16] and 6-iodononivamide (**8**),[17] iodinated products of the agonists RTX and nonivamide, which showed potent antagonism with a K_i = 5.8 nM in the rTRPV1/HEK293 system and an IC_{50} = 10 nM in the hTRPV1/HEK293 system, respectively. The result prompted us to investigate how the halogenation on the aromatic A-region of our potent agonists modulates their functional activity.

In the present study, we describe the syntheses, receptor activities, and the analysis of structure–activity relationships of 5- and 6-halogenated analogues of our lead agonists (**1**–**6**).



Scheme 1. Reagents and conditions: (a) NCS, NaH, THF, 78% for X = Cl; Br_2, CH_2Cl_2, 91% for X = Br; H_3BO_3, 1 N NaOH, KI, I_2, H_2O, 50% for X = I; (b) MOMCl, DBU, DMF, 75% for X = Cl; MOMCl, NEt_3, CH_2Cl_2, 92% for X = Br, 91% for X = I; (c) $NaBH_4$, LiCl, THF–EtOH, 93–99%; (d) DPPA, DBU, toluene, 90–99%; (e) CS_2, PPh_3, THF, 83% for X = Cl; (f) i—PPh_3, H_2O, THF, 71–96%; ii—TDI, NEt_3, DMF, 50% for X = Br, 46% for X = I.



Scheme 2. Reagents and conditions: (a) MOMCl, NEt_3, CH_2Cl_2, 98%; (b) $NaBH_4$, LiCl, THF–EtOH; (c) Ac_2O, pyridine, 99% in 2 steps; (d) oxone, NaCl, acetone–H_2O, 81% for X = Cl; oxone, NaBr, acetone–H_2O, 88% for X = Br; CF_3CO_2Ag, I_2, CH_2Cl_2, 87% for X = I; (e) LiOH, THF–H_2O; (f) DPPA, DBU, toluene, 63–77% in 2 steps; (g) CS_2, PPh_3, THF, 86–91%.

The target halogenated thiourea compounds (**20**–**31**) were synthesized in general by the coupling of the corresponding isothiocyanates with the C-region amines reported previously. 5- or 6-halogenated isothiocyanates of vanillin were prepared employing the two different regioselective halogenation strategies in which the presence of a free phenolic hydroxyl directed halogenation to the 5-position and the protection of the 4-hydroxyl switched the regiochemistry of halogenation from the 5-position to the 6-position.

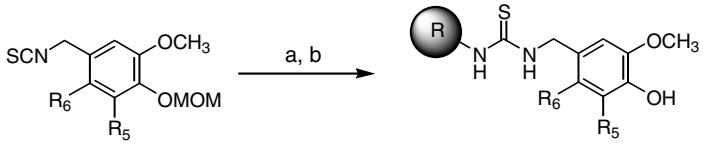
The syntheses of 5-halogenated isothiocyanates are outlined in Scheme 1. Starting from commercially available vanillin, the ortho carbon to the 4-hydroxyl was readily halogenated and then the hydroxyl group was protected with the methoxymethyl (MOM) group to provide **11**. The aldehyde **11** was reduced and then converted to the corresponding azide **13**, which was transformed to the corresponding isothiocyanate **14** using carbon disulfide and triphenylphosphine in a step.

The syntheses of 6-halogenated analogues are shown in Scheme 2. First, the 4-hydroxyl of vanillin was protected with the methoxymethyl (MOM) group and then the benzaldehyde was converted to the *O*-acetyl benzyl alcohol **16**. O-Protected **16** was halogenated regioselectively on 6-position by known methods[17] and then the acetoxymethyl group was changed to the corresponding isothiocyanates using a route similar to that described in Scheme 1.

Finally, the coupling of the isothiocyantes (**14** and **19**) with the corresponding amines of the C-region[11,12] followed by acid hydrolysis of the protecting MOM group provided the final halogenated thioureas (**20**–**31**) in high yields as represented in Scheme 3.

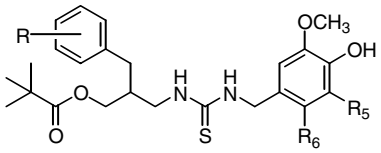
The binding affinities and agonistic/antagonistic activities of the synthesized TRPV1 ligands were assessed in vitro by a binding competition assay with [^{3}H]RTX and a functional $^{45}Ca^{2+}$ uptake assay using rat TRPV1 heterologously expressed in Chinese hamster ovary (CHO) cells, as previously described.[18] The results are summarized in Tables 1–4, together with the potencies of capsazepine and the agonists **1**–**6**. 6′-Iodononivamide (**8**),[17] previously reported as the most potent antagonist in a series of nonivamides, was also evaluated as a reference and displayed a full antagonism with K_i (binding) = 1320 nM and K_i (ant) = 127 nM, respectively.

Our previous finding indicated that compounds **1** and **2**, *N*-(3-pivaloyloxy-2-benzylpropyl)-*N*′-(4-hydroxy-3-methoxybenzyl)thioureas, were potent high affinity TRPV1 agonists with EC_{50} (agonism) = 2.83 and 1.97 nM and K_i (binding) = 6.35 and 17.4 nM, respectively. The receptor activities of halogenated analogues of **1** and **2** are outlined in Table 1. With agonist **1**, 5-halogenation on the A-region shifted the agonism to antagonism and the extent was more enhanced as the size of halogen increases. For example, whereas 5-chlorination produced a partial antagonist **20a** with 37% antagonism, 5-bromination gave 85% antagonism and 5-iodination afforded a full antagonist **20c** with K_i (ant) = 103 nM. Interestingly, 6-halogenation shifted the agonism to antagonism further compared to 5-halo-



Scheme 3. Reagents and conditions: (a) R–NH_2, 50–98%; (b) CF_3CO_2H, CH_2Cl_2 (1:2), 60–96%.

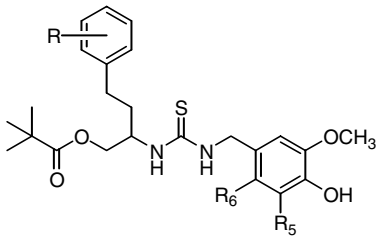
Table 1.



Compound	R	R_5	R_6	K_i (nM) binding affinity	EC_{50} (nM) agonism[a]	K_i (nM) antagonism[b]
CZP				1300	NE	520
8				1320 ± 120	NE	127 ± 29
1	4-*t*-Bu	H	H	6.35 ± 0.48	2.83 ± 0.55	NE
20a	4-*t*-Bu	Cl		21.3 ± 2.7	(49%)	(37%)
20b	4-*t*-Bu	Br		18.8 ± 6.3	(9%)	(85%)
20c	4-*t*-Bu	I		45 ± 15	NE	103 ± 14
21a	4-*t*-Bu		Cl	12.2 ± 2.6	(24%)	(71%)
21b	4-*t*-Bu		Br	12.8 ± 2.1	NE	55 ± 18
21c	4-*t*-Bu		I	13.3 ± 3.6	NE	71 ± 34
2	3,4-Me_2	H	H	17.4 ± 4.1	1.97 ± 0.56	NE
22a	3,4-Me_2	Cl		30.9 ± 2.2	(93%)	NE
22b	3,4-Me_2	Br		33.1 ± 4.4	(29%)	(70%)
22c	3,4-Me_2	I		232 ± 63	NE	121 ± 30
23a	3,4-Me_2		Cl	24.2 ± 7.1	(63%)	(41%)
23b	3,4-Me_2		Br	19.9 ± 5.1	(13%)	(91%)
23c	3,4-Me_2		I	18.9 ± 2.6	NE	23.1 ± 8.0

Values represent means ± SEM from three or more experiments.
[a] The values in parentheses indicate the percentage of maximal calcium uptake compared with that induced by 300 nM capsaicin.
[b] The values in parentheses indicate the extent of partial antagonism.

Table 2.



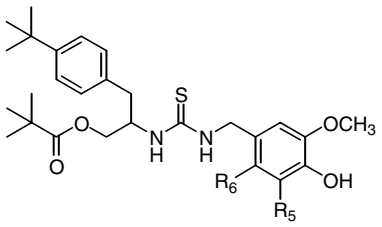
Compound	R	R_5	R_6	K_i (nM) binding affinity	EC_{50} (nM) agonism[a]	K_i (nM) antagonism[b]
3	4-*t*-Bu	H	H	36.5 ± 2.6	21.5 ± 4.1	NE
24a	4-*t*-Bu	Cl		122 ± 21	(44%)	(47%)
24b	4-*t*-Bu	Br		143 ± 48	(19%)	(73%)
24c	4-*t*-Bu	I		214 ± 35	NE	525 ± 130
25a	4-*t*-Bu		Cl	79 ± 15	NE	72.2 ± 7.0
25b	4-*t*-Bu		Br	75 ± 13	NE	76.1 ± 7.2
25c	4-*t*-Bu		I	79 ± 7	NE	69 ± 13
4	3,4-Me_2	H	H	56 ± 23	14.7 ± 2.1	NE
26a	3,4-Me_2	Cl		86 ± 12	(88%)	(16%)
26b	3,4-Me_2	Br		102 ± 11	(50%)	(59%)
26c	3,4-Me_2	I		127 ± 26	NE	115 ± 31
27a	3,4-Me_2		Cl	103 ± 18	(60%)	(45%)
27b	3,4-Me_2		Br	109 ± 7	(13%)	(75%)
27c	3,4-Me_2		I	140 ± 20	NE	176 ± 21

Values represent means ± SEM from three or more experiments.
[a] The values in parentheses indicate the percentage of maximal calcium uptake compared with that induced by 300 nM capsaicin.
[b] The values in parentheses indicate the extent of partial antagonism.

genation. Indeed, 6-chlorination of **1** was able to exert 71% antagonism, and 6-bromination and 6-iodination provided full antagonists **21b** and **21c** with K_i (ant) = 55 and 71 nM, respectively.

A similar SAR pattern was observed in the halogenated analogues of agonist **2**, Both 5- and 6-halogenation shifted the agonism to antagonism with the order of I > Br > Cl and 6-halogenation produced more en-

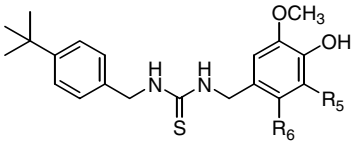
Table 3.



Compound	R_5	R_6	K_i (nM) binding affinity	EC_{50} (nM) agonism[a]	K_i (nM) antagonism[b]
5	H	H	34.6 ± 9.6	5.7 ± 2.1	NE
28a	Cl		28.8 ± 5.3	60 ± 18	NE
28b	Br		38.2 ± 6.8	(57%)	(29%)
28c	I		39.4 ± 6.1	NE	178 ± 72
29a		Cl	41.5 ± 1.4	(94%)	(11%)
29b		Br	74.9 ± 3.7	(41%)	(51%)
29c		I	96 ± 10	NE	310 ± 100

Values represent means ± SEM from three or more experiments.
[a] The values in parentheses indicate the percentage of maximal calcium uptake compared with that induced by 300 nM capsaicin.
[b] The values in parentheses indicate the extent of partial antagonism.

Table 4.



Compound	R_5	R_6	K_i (nM) binding affinity	EC_{50} (nM) agonism[a]	K_i (nM) antagonism[b]
6	H	H	58.6 ± 9.0	2.5 ± 1.1	NE
30a	Cl		76.0 ± 13	(5%)	(92%)
30b	Br		51.6 ± 6.7	NE	49 ± 12
30c	I		342 ± 97	NE	176 ± 27
31a		Cl	36.9 ± 3.5	(33%)	(81%)
31b		Br	42.1 ± 3.5	NE	30.3 ± 6.3
31c		I	130 ± 16	NE	156 ± 58

Values represent means ± SEM from three or more experiments.
[a] The values in parentheses indicate the percentage of maximal calcium uptake compared with that induced by 300 nM capsaicin.
[b] The values in parentheses indicate the extent of partial antagonism.

hanced antagonism. On the result, 6-iodination of agonist **2** led to very potent antagonist **23c** with K_i (ant) = 23.1 nM, which was 5.5-fold and 20-fold more potent than 6-iodononivamide (**8**) and capsazepine, respectively. All halogenated analogues (**20**–**23**) displayed comparable or slightly less binding affinities compared to the parent compound (**1** and **2**) except compound **22c**.

The SAR of halogenated analogues of *N*-[2-pivaloyloxy-1-(phenethyl)ethyl]-*N*′-(4-hydroxy-3-methoxybenzyl) thiourea (**3** and **4**) is described in Table 2. Similar to the results described in Table 1, the halogenation of **3** and **4** also converted the agonists to partial antagonists or full antagonists, and the antagonism was enhanced with the order of I > Br > Cl and 6-halogenation > 5-halogenation. In particular, all 6-halogenated analogues of agonist **3** (**25a**–**c**) were found to be full antagonists with a range of K_i (ant) = 69–76 nM.

A similar SAR pattern was observed in the halogenated analogues of *N*-[2-pivaloyloxy-1-(4-*tert*-butylbenzyl)ethyl]-*N*′-(4-hydroxy-3-methoxybenzyl)thiourea (**5**) and *N*-(4-*tert*-butylbenzyl)-*N*′-(4-hydroxy-3-methoxybenzyl)thiourea (**6**) as shown in Tables 3 and 4, respectively. Among them, *N*-(4-*tert*-butylbenzyl)-*N*′-(6-bromo-4-hydroxy-3-methoxybenzyl)thiourea (**31b**) displayed very potent antagonism with K_i = 30.3 nM.

Previously it was established that halogenation of RTX or capsaicinoid reverted the activity of the agonists to generate antagonists. However, halogenated RTX and capsainoid analogues showed different structure–activity relationships for the reversal of activity. In the RTX series, whereas 5-iodination produced a maximal full

antagonism,[16] 6-iodination led to partial agonism with 50% efficacy in the hTRPV1/HEK system.[19] On the other hand, in the capsaicinoid series, although both 5- and 6-iodo derivatives behaved as powerful antagonists under the same assay conditions, the 6-iodo derivative was more potent than the corresponding 5-iodo analogue. The analysis indicated that our lead agonists (**1**–**6**) investigated in this study are more like capsaicinoid-type ligands rather than RTX-type ligands although the ligands were initially designed as simplified RTX analogues.

In summary, we have modified the aromatic A-region of potent *N*-(4-hydroxy-3-methoxybenzyl)thiourea agonists (**1**–**6**) by 5- and 6-halogenation in a systematic way to understand the role of halogens for the reversal of activity and to analyze their structure–activity relationships. In general, the halogenation shifted the activity of the ligands from agonism to antagonism and the extent depended on both the size of the halogen and the halogenated position. Iodination conferred the most enhancement of antagonism, with lesser effects upon bromination and chlorination in that order. Antagonism by 6-halogenation was higher than that of the corresponding 5-halogenation. Among the halogenated ligands synthesized, compounds **23c** and **31b** were found to be very potent full antagonists with K_i (ant) = 23.1 and 30.3 nM; they were thus more potent than 6′-iodononivamide by 5.5-fold and 4-fold, respectively. The analysis indicated that the SAR of agonists (**1**–**6**) by halogenation is similar to that of the capsaicinoids previously reported.

Acknowledgments

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