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N-4-Methansulfonamidobenzyl-*N*′-2-substituted-4-*tert*-butyl-benzyl thioureas as potent vanilloid receptor antagonistic ligands

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Abstract—A series of *N*-4-methansulfonamidobenzyl-*N*′-2-substituted-4-*tert*-butylbenzyl thioureas were prepared for the study of their agonistic/antagonistic activities to the vanilloid receptor in rat DRG neurons. Their structure–activity relationship reveals that there is a space for another hydrophobic binding interaction around 2-position in 4-*tert*-butylbenzyl region. Among the prepared derivatives, **6n** show the highest antagonistic activity against the vanilloid receptor (IC_{50} = 15 nM).

Vanilloid receptor (VR1), a nonselective cation channel placed in the plasma membrane of peripheral sensory neurons,[1,2] has been regarded as a new target for the treatment of pain.[3] The agonists initially induce the excitation of primary sensory neuron by influx of cations, especially Ca^{2+}, into neuronal cell,[4] but finally desensitize the peripheral sensory neurons, which leads analgesic effect.[3] However, the initial excitatory side effects of agonists, such as initial irritancy, hypothermia, bronchoconstriction, and hypertension, derived from its inherent mechanism, become obstacles to develop as systemic analgesics.[5] Recently, several research groups have focused on the investigation of antagonist to overcome the unavoidable excitatory side effect. Several synthetic and semi-synthetic antagonists have been reported so far, such as capsazepine[6] (IC_{50} = 0.65 μM), *N*-alkyl glycine trimer[7] (IC_{50} = 2 μM), pyrrolidine-thiourea[8] (IC_{50} = 3 μM), and *N*,*N*′,*N*″-trisubstituted-thiourea[9] (IC_{50} = 0.32 μM), 4-(2-pyridyl)piperazine-1-carboxamides (IC_{50} = 0.03 μM),[10] and 5-iodo-RTX (IC_{50} = 4 nM),[11] prepared by iodination of resiniferatoxin (RTX).

We recently reported a series of *N*-4-methylsulfonamidobenzyl thiourea analogues (**1**,[12] IC_{50}, = 70 nM; **2**,[13,14] IC_{50} = 110 nM; **3**,[13,15,16] IC_{50} = 37 nM) as highly potent VR1 antagonists with high affinity. These results revealed that the agonism could be dramatically changed to antagonism by the replacement of 3-methoxy-4-hydroxy group with 4-methansulfonamido group (Fig. 1).[12–14] In addition, the further introduction of fluoro group in 3-position enhanced the antagonistic activity.[15] As a subsequent research, we attempted to optimize the other part of the thioureas, *N*′-4-*tert*-butylbenzyl group, by the combination of the structure of **1** and **2**. In this communication, we report the synthesis and functional assay on receptor of *N*-4-methansulfonamidobenzyl-*N*′-2-substituted-4-*tert*-butylbenzyl thioureas, and discuss on the another-binding for receptor antagonism based on their pharmacophoric analysis from structure–activity relationship study.

Based on the structure of **1** and **2**, the 3,4-dimethylbenzyl group of *N*′-3-acetoxy-2-benzylpropyl group of **1** could be equivalent to the *N*′-4-*tert*-butylbenzyl group of **2** in hydrophobic binding with VR1. So we expected that the introduction of another potential binding moiety into **2**, such as the pivaloyl group in the case of **1**, might increase the antagonistic potency (Fig. 1). For the

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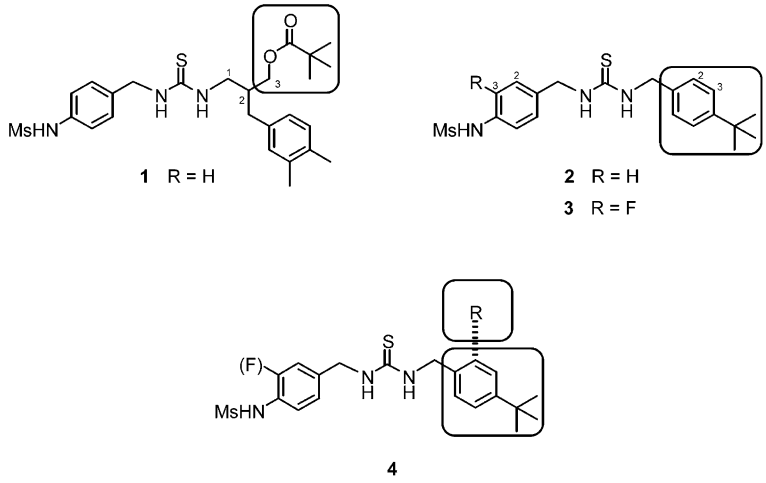


Figure 1.

convenient preparation of target compounds (Fig. 2) with various functional groups at 2-position, we employed **2** as a basic template, which has been proven as a potent antagonist.[14]

We prepared fourteen 2-substituted derivatives (**5a–l**) (Scheme 1). Compounds **5a–k** were prepared in 4 steps from **7**. The introduction of cyano group into **7** by using trichloroacetonitrile,[15] followed by *O*-alkylation in basic condition gave **9**. The reduction of cyano group of **9** with $LiAlH_4$ and the subsequent coupling with 4-*tert*-butylbenzylisothiocyanate provided **5a–k**. Compound **5l** was prepared in 3 steps from **8**. The reduction of **8**, followed by the coupling with 4-*tert*-butylbenzylisothiocyanate gave **12**, which was treated with pivaloyl anhydride to afford **5l**. Compounds **5m** and **5n** were obtained in 5 steps from **8** (Scheme 2). The *O*-triflate formation of **8**, followed by the insertion of CO in the presence of methanol and the catalytic amount of $Pd(OAc)_2$ gave the methyl ester **14**. The reduction of **14** with $LiAlH_4$ and the followed coupling with 4-*tert*-butylbenzylisothiocyanate provided **16**. Finally, the acylation with acetic anhydride and pivaloyl anhydride afforded **5m** and **5n**, respectively.

The agonistic or antagonistic activities of the prepared derivatives[17] on receptor were evaluated by the $^{45}Ca^{2+}$-influx assay previously reported[18] by using the neonatal rat cultured spinal sensory neurons (Table 1). As shown in Table 1, the antagonistic activities were quite dependent upon the length of carbon chain. The optimal length of alkyl carbon chain was 4 (**5d**, $IC_{50}=60$ nM) or 5 (**5e**, $IC_{50}=70$ nM). The shorter or the longer carbon chain decreases the antagonistic activity. Similar tendency was observed in the methoxy ether derivatives, **5j** ($IC_{50}=500$ nM) and **5k** ($IC_{50}=60$ nM). Among the branched alkyl groups, the neopentyloxy derivative (**5h**, $IC_{50}=60$ nM) showed the higher antagonistic activity than that of isopropyloxy derivative (**5g**, $IC_{50}=350$ nM). The benzyloxy derivative (**5i**, $IC_{50}=770$ nM) showed far less antagonistic activity than that of the similar sized aliphatic ones (**5d**,**e**). *O*-Pivaloyl ester derivative **5l** ($IC_{50}=220$ nM) exhibited relatively low antagonistic activity, although the pivaloyl group is

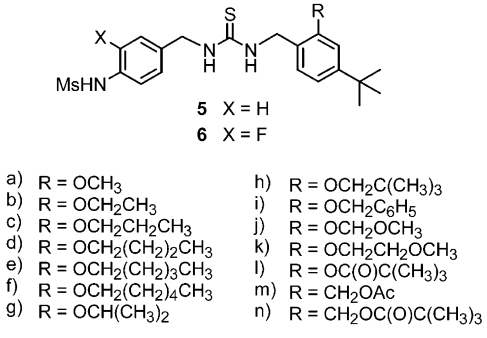
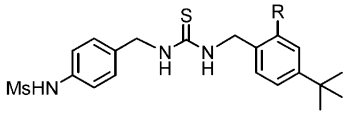


Figure 2.

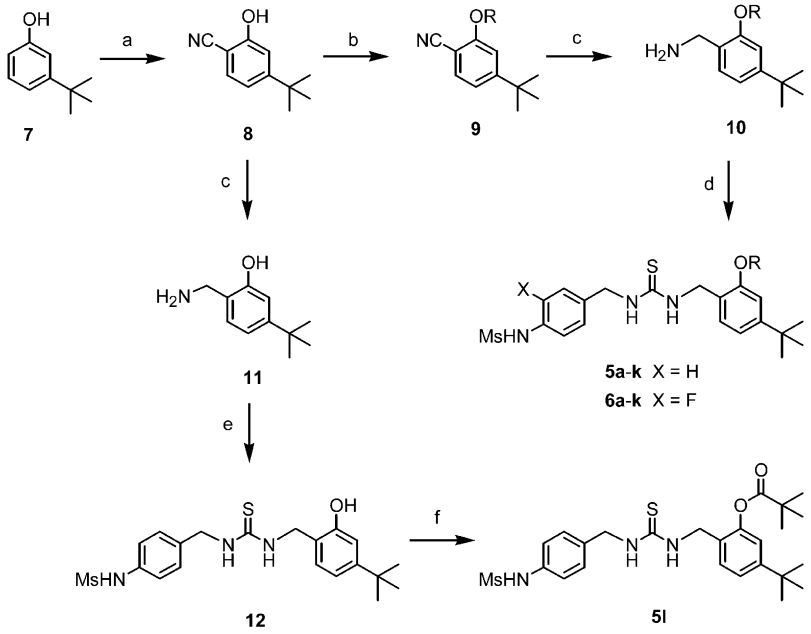
Table 1. In vitro biological activity of the derivatives by $^{45}Ca^{2+}$-influx assay in rat DRG neurons



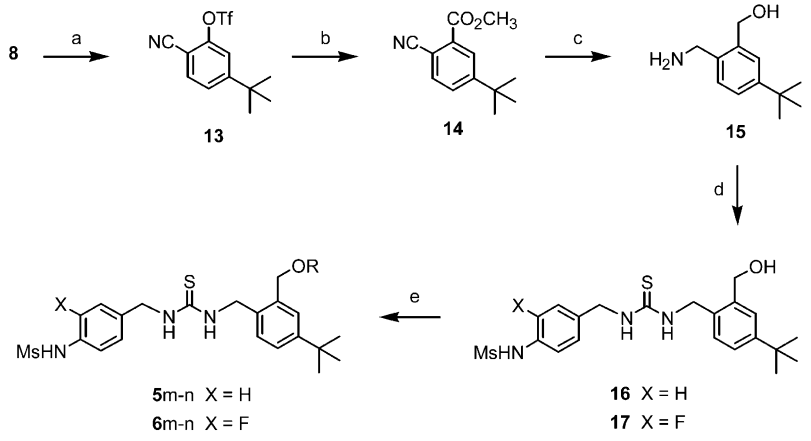
No	R_1	$^{45}Ca^{2+}$-influx activity (μM)[a]	
		Agonist (IC_{50})	Antagonist (IC_{50})
2	H	NE[b]	0.11
5a	OCH_3	NE	2.40
5b	OCH_2CH_3	NE	1.10
5c	$OCH_2CH_2CH_3$	NE	0.25
5d	$OCH_2(CH_2)_2CH_3$	NE	0.06
5e	$OCH_2(CH_2)_3CH_3$	NE	0.07
5f	$OCH_2(CH_2)_4CH_3$	NE	0.15
5g	$OCH(CH_3)_2$	NE	0.35
5h	$OCH_2C(CH_3)_3$	NE	0.06
5i	$OCH_2C_6H_5$	NE	0.77
5j	OCH_2OCH_3	NE	0.50
5k	$OCH_2CH_2OCH_3$	NE	0.06
5l	$OC(O)C(CH_3)_3$	NE	0.22
5m	$CH_2OC(O)CH_3$	NE	0.58
5n	$CH_2OC(O)C(CH_3)_3$	NE	0.07

[a] EC_{50} (the concentration of derivative necessary to produce 50% of the maximal response) and IC_{50} values (the concentration of derivative necessary to reduce the response to 0.5 μM capsaicin by 50%) were estimated with at least 3 replicates at each concentration. Each compound was tested in two independent experiments. Antagonist data were fitted with a sigmoidal function.

[b] NE: not effective at 30 μM.



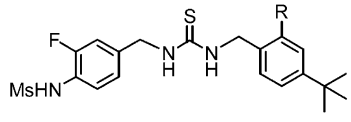
Scheme 1. Reaction and conditions: (a) (i) Cl_3CCN, $ClCH_2CH_2Cl$, reflux, 17 h; (ii) KOH, *tert*-BuOH, reflux, 20 min, 65%; (b) RX, K_2CO_3, DMF, reflux, 2 h, 95–99%; (c) $LiAlH_4$, ether, reflux, 1 h; (d) 4-MsNHPhCH_2NCS or 3-F-4-MsNHPhCH_2NCS, Et_3N, CH_2Cl_2, rt, 2 h, 60–70% from **9**; (e) 4-MsNHPhCH_2NCS, Et_3N, CH_2Cl_2, rt, 2 h, 55% from **8**; (f) Et_3N, CH_2Cl_2, Piv_2O, rt, 12 h, 52%.



Scheme 2. Reaction and conditions: (a) Tf_2O, Et_3N, CH_2Cl_2, 0 °C, 1 h, 93%; (b) $Pd(OAc)_2$, dppf, Et_3N, MeOH, CO, DMSO, 50 °C, 3 h, 83%; (c) $LiAlH_4$, ether, reflux, 1 h; (d) 4-MsNHPhCH_2NCS or 3-F-4-MsNHPhCH_2NCS, Et_3N, CH_2Cl_2, rt, 2 h, 50%; (e) Et_3N, CH_2Cl_2, Ac_2O or Piv_2O, rt, 12 h, 50–60%.

spatially located in similar position with that of **1**. The low activity might be come from the low stability of the phenolic ester in **5l**. In case of 2-acyloxymethyl derivatives (**5m**, $IC_{50} = 580$ nM; **5n**, $IC_{50} = 70$ nM), the pivaloyl ester derivative **5n**, showed 8 times higher antagonistic activity than that of the acetyl ester derivative **5m**, which is in accord with the case in alkyl derivatives (**5c**,**h**). Especially, **5n**, one carbon extended derivative of **5l**, showed 3 times higher antagonist activity than **5l** itself. These accumulated findings may imply that there is a space for another hydrophobic binding, but it is not bigger than the size of C_4– C_5 alkyl chain. To increase the potency by the introduction of fluoro group at 3-position, 3-fluoro derivatives of **5d**, **5h**, and **5n** were prepared by using 3-fluoro-4-methansulfonamidobenzylisothiocynate, instead of 4-methansulfonamidobenzylisothiocynate in Schemes 1 and 2. As shown in Table 2, **6d** and **6n** showed 3 times higher

Table 2. In vitro biological activity of the derivatives by $^{45}Ca^{2+}$-influx assay in rat DRG neurons



No	R	$^{45}Ca^{2+}$-influx activity (nM)[a]	
		Agonist (IC_{50})	Antagonist (IC_{50})
3	H	NE[b]	37
6d	$OCH_2(CH_2)_2CH_3$	NE	20
6h	$OCH_2C(CH_3)_3$	NE	66
6n	$CH_2OC(O)C(CH_3)_3$	NE	15

[a] EC_{50} and IC_{50} values were estimated by the same method described in Table 1.

[b] NE: not effective at 30 μM; Only partial activity was observed at 30 μM.

antagonistic activity than that of **5d** and **5n**, respectively, but the comparable potency was observed in case of the neopentyloxy derivative (**6h**).

In conclusion, seventeen *N*′-2-substituted-4-*tert*-butylbenzyl thioureas were prepared for the optimization of *N*′-4-*tert*-butylbenzyl group of **2**. Among them, the best antagonistic activity was observed with the *N*-3-fluoro-4-methansulfonamidobenzyl-*N*′-2-pivaloyloxymethyl-4-*tert*-butylbenzyl thiourea (**6n**). We believe this pharmacophoric information would be very useful to design more potent antagonistic scaffolds for the development of potential analgesics.

Acknowledgements

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17. All compounds gave satisfactory spectroscopic data consistent with the proposed structures. Selected spectral data for **6n**; mp 65 °C. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.37 (m, 4H), 6.97 (t, J = 7.8 Hz, 2H), 6.56 (br, 1H), 5.10 (s, 2H), 4.71 (s, 4H), 2.99 (s, 3H), 1.28 (s, 9H), 1.11 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz), δ 181.71, 178.98, 155.51, 153.07, 151.23, 137.45, 133.83, 131.71, 128.15, 125.76, 125.63, 124.08, 123.66, 123.37, 123.24, 114.78, 114.58, 63.37, 47.16, 44.98, 39.69, 38.67, 34.46, 31.13, 26.94. IR (KBr) 3353, 2964, 1717, 1549, 1512 cm^{-1}. MS (FAB) *m*/*e*, 538 [M + H$^+$]. Anal. calcd for $C_{26}H_{36}FN_3O_4S_2$: C, 58.08; H, 6.75; N, 7.31. Found: C, 57.37; H, 6.99; N, 7.02.
18. The uptake and accumulation of $^{45}Ca^{2+}$ by the 4-substituted-benzyl-*tert*-benzyl thiourea derivatives was studied in neonatal rat cultured spinal sensory neurons by the method described in detailed in the ref 3b.