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N-4-Substituted-benzyl-*N*′-*tert*-butylbenzyl thioureas as vanilloid receptor ligands: investigation on the role of methanesulfonamido group in antagonistic activity

Hyeung-geun Park,[a,*] Ji-yeon Choi,[a] Sea-hoon Choi,[a] Mi-kyung Park,[a] Jihye Lee,[a] Young-ger Suh,[a] Hawon Cho,[a] Uhtaek Oh,[a] Jiyoun Lee,[a] Sang-Uk Kang,[a] Jeewoo Lee,[a] Hee-Doo Kim,[b] Young-Ho Park,[c] Yeon Su Jeong,[c] Jin Kyu Choi[c] and Sang-sup Jew[a,*]

[a]*Research Institute of Pharmaceutical Sciences and College of Pharmacy, Seoul National University, Seoul 151-742, South Korea*
[b]*College of Pharmacy, Sookmyung Women's University, Seoul 140-742, South Korea*
[c]*AmorePacific R&D Center, Youngin-Si, Kyounggi-do 449-900, South Korea*

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Abstract—A series of *N*-4-substituted-benzyl-*N*′-*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 not only the two oxygens and amide hydrogen of sulfonamido group, but also the optimal size of methyl in methanesulfonamido group play an integral role for the antagonistic activity on vanilloid receptor.

Capsaicin (**1**), proton, and heat initiate the activation of the vanilloid receptor (VR1), which has been cloned from rat dorsal root ganglia and human.[1] It is a non-selective cation channel placed in the plasma membrane of peripheral sensory neurons.[2,3] The activation of VR1 by agonists such as capsaicin and resiniferatoxin (RTX) initially induce excitation of primary sensory neuron by influx of cations, especially Ca^{2+}, into neuronal cell.[4] The subsequent desensitization leads the agonists to have the effect of analgesia.[5] However, the excitatory side effects, such as initial irritancy, hypothermia, bronchoconstriction, and hypertension, is derived from its inherent mechanism and become obstacles to develop as systemic analgesics.[6] A complementary approach to overcome the unavoidable excitatory side effect by agonists is the competitive antagonism of VR1. Whereas several potent synthetic agonists were developed through the extensive structure–activity relationship studies,[7,8] only a few synthetic antagonists have been reported so far. Among the antagonists, while capsazepine[9] ($IC_{50}=0.65$ μM), *N*-alkyl glycine trimer[10] ($IC_{50}=2$ μM), pyrrolidine-thiourea[11] ($IC_{50}=3$ μM), and *N*,*N*′,*N*″-tri-substituted-thiourea[12] ($IC_{50}=0.32$ μM), showed only modest potency of antagonism in rat DRG, 5-iodo-RTX,[13] prepared by iodination of RTX, was reported to have potent antagonism with an IC_{50} of 3.9 nM for the inhibition of rat VR1 so far.

As part of our program to develop novel VR antagonists as potent analgesics, we recently reported a series of *N*-4-(methylsulfonylamino)benzyl thiourea analogues as highly potent VR1 antagonists with high affinity.[14,15] The previous results revealed that the agonistic activity of **4a** was changed to antagonism by the replacement of 3-methoxy-4-hydroxy group with 4-methansulfonamido group (**4b**) (Fig. 1). In this communication, we report the synthesis and functional assay on receptor of *N*-4-substituted-benzyl-*N*′-*tert*-butylbenzyl thioureas, and discuss the role of sulfonamido group for receptor antagonism based on their pharmacophoric analysis from systematic structure–activity relationship study.

Based on the previous results, we expected that the agonistic/antagonistic properties of VR1 ligands could be dramatically changed depending upon the functional group substituted on 4-position in vanilloid moiety. The importance of methansulfonyl group for antagonistic

* Corresponding authors. Fax: +82-2-872-9129; e-mail: hgpk@plaza.snu.ac.kr

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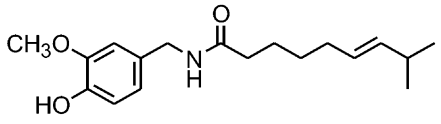
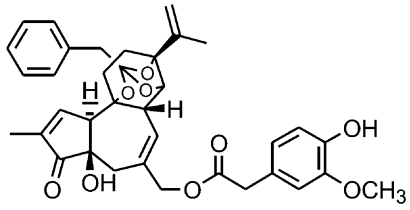
activity could be confirmed by 4-methansulfonate derivative (**5b**, $IC_{50} = 0.30$ μM in rat DRG) of SDZ249482 (**5a**) (Fig. 1). For the convenient preparation of target compounds with various functional groups on 4-position (Fig. 2), we employed 4-*tert*-butylbenzyl group of SDZ249482[7e] as a basic template, which has been proven as a potent agonist (Norvatis's group).

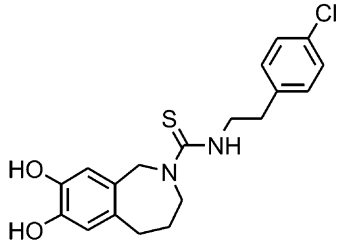
We prepared 10 4-substituted derivatives (**6a–j**) (Scheme 1). Compounds **6a–d** were easily prepared by the coupling of 4-*tert*-butylbenzyl isothiocyanate with the corresponding benzyl amines (**8a–d**), respectively. The *O*-mesylation of **6b** and the hydrogenation of **6d** gave **6j** and **6e**, respectively. Compounds **6f–h** were prepared from **6e** by the addition of methansulfonyl



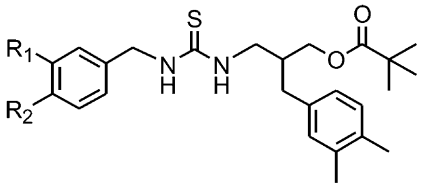
1, Capsaicin



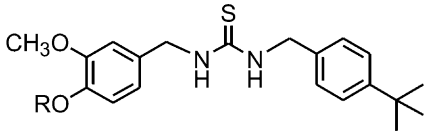
2, Resiniferatoxin



3, Capsazepine

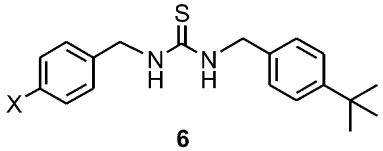


4 a $R_1 = OCH_3$ $R_2 = OH$
b $R_1 = H$ $R_2 = NHMs$



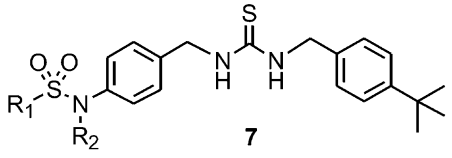
5a R = H SDZ249482
b R = Ms

Figure 1.



6

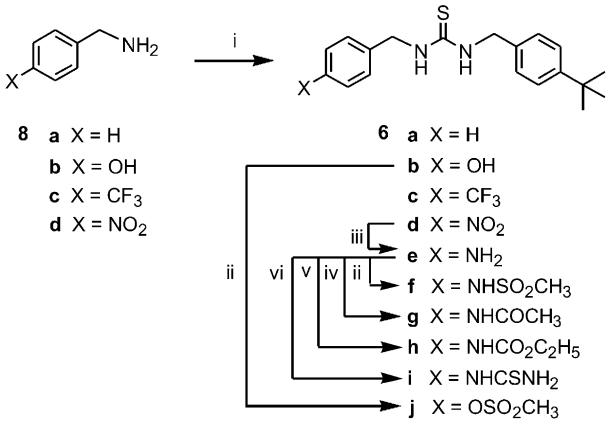
X = H
X = OH
X = OMs
X = CF_3
X = NO_2
X = NH_2
X = $NHSO_2CH_3$
X = $NHCOCH_3$
X = $NHCO_2C_2H_5$
X = $NHCSNH_2$



7

R_1 = Et	R_2 = H
R_1 = n-Pr	R_2 = H
R_1 = n-Bu	R_2 = H
R_1 = i-Pr	R_2 = H
R_1 = CF_3	R_2 = H
R_1 = CH_2CF_3	R_2 = H
R_1 = 2-thiopene	R_2 = H
R_1 = p-CH_3Ph	R_2 = H
R_1 = Bn	R_2 = H
R_1 = CH_3	R_2 = CH_3
R_1 = NH_2	R_2 = H
R_1 = $NHCH_3$	R_2 = H
R_1 = $N(CH_3)_2$	R_2 = H

Figure 2.

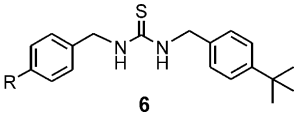


Scheme 1. Reagents and conditions: (i) 4-*t*-Bu-BnNCS, CH_2Cl_2, rt, 98%; (ii) MsCl, pyridine, CH_2Cl_2, rt, 66%; (iii) Pd/C, H_2, CH_3OH, rt, 90%; (iv) Ac_2O, Et_3N, CH_2Cl_2, rt, 86%; (v) $ClCO_2Et$, Et_3N, CH_2Cl_2, rt, 80%; (vi) DPT, CH_2Cl_2, rt, then *c*-NH_4OH, 65%.

chloride, acetyl chloride, and ethyl chloroformate under basic condition, respectively. The isothiocyanate formation from **6e** with dipyridylthiocarbonate (DPT), followed by the addition of *c*-NH_4OH provided **6i**.

The agonistic or antagonistic activities of the prepared derivatives[16] on receptor were evaluated by the $^{45}Ca^{2+}$-influx assay previously reported[17] by using the neonatal rat cultured spinal sensory neurons (Table 1). As shown in Table 1, 4-methanesulfonamido derivative, **6f** ($IC_{50}=0.11$ μM), showed the highest antagonistic activity among the prepared derivatives, but 4-methanesulfonate derivatives **6j** ($IC_{50}=9.3$ μM) showed relatively modest antagonistic activity. The result implies that N–H of sulfonamide group, hydrogen bonding donor, is more preferable to *O* of sulfonate group, hydrogen bonding acceptor, for antagonism. However, the other N–H types, such as amide **6g**, carbamate **6h**, and thiourea **6i**, showed little activity as agonist or antagonist, suggesting that the sulfonyl amide moiety is quite critical and sensitive for its antagonistic activity.

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



6

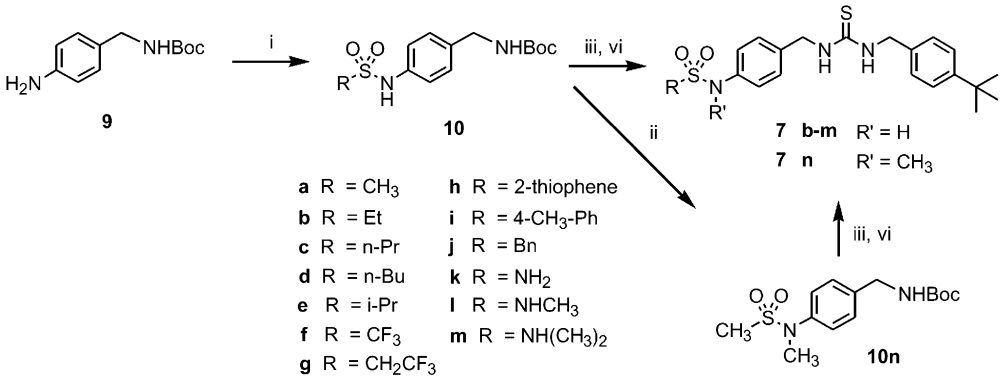
No.	R	$^{45}Ca^{2+}$-influx activity (μM)[a]	
		Agonist (EC_{50})	Antagonist (IC_{50})
1	Capsaicin	0.03	NE[b]
2	Capsazepine	NE	0.65
6a	H	NE	NE
6b	OH	>30[c]	NE
6c	CF_3	NE	12.4
6d	NO_2	NE	>30[c]
6e	NH_2	7.0	NE
6f	CH_3SO_2NH	NE	0.11
6g	CH_3CONH	NE	NE
6h	C_2H_5OCONH	NE	NE
6i	NH_2CSNH	>30[c]	>30[c]
6j	CH_3SO_3	NE	9.3

[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 three 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.
[c] Only partial activity was observed at 30 μM.

To investigate further pharmacophoric analysis of sulfonamido group for antagonistic activity, we prepared 10 sulfonamido derivatives (**7b–j**, **7n**) and three sulfamide derivatives (**7k–m**) (Scheme 2). Compound **7b–j** were prepared from **9** in three steps. The coupling of **9** with various sulfonyl chlorides or sulfamoyl chlorides under basic condition gave the corresponding sulfonamides or sulfamides (**10b–m**), respectively. The deprotection of the *N*-Boc group of **10b–m** with trifluoroacetic acid, followed by the coupling with 4-*tert*-butylbenzyl isothiocyanate provided **7b–m**. The *N*-methylation of **10a** and the following same procedure as above gave **7n**.

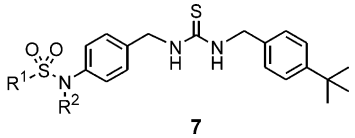
The $^{45}Ca^{2+}$-influx activity of the prepared derivatives[16] was shown in Table 2. Generally, the bulkier R^1 showed the less antagonistic activity (**7b–j**). This finding is in accord with the previous results obtained from a series of 3-acyloxy-2-benzylpropyl analogues,[15] and suggests that there is very limited binding space between sulfonamido group and VR1 receptor. Also the fluoro derivatives **7f** and **7g** of **6f** and **7b**, respectively, exhibited dramatic decrement in antagonistic activity, suggesting that polar substituents are not favorable for antagonistic binding. The *N*-methylated derivative of sulfonamido group (**7n**) led to 20 times less antagonistic activity than that of **6f**, implying that the N–H, acting as hydrogen bonding donor, may be important for the antagonistic binding with the receptor. In a series of sulfamides (**7k–m**), we expected that the potential hydrogen bonding via the additional N–H in sulfamides might enhance the antagonistic activity. However their activities were dropped by 3–5 times lower than that of the corresponding alkyl derivatives (**6f**, **7b**, **7e**), respectively. There is another potential factor for the dramatic variation in the antagonistic activity of **6f**, **7f**, and **7k**, which have similar sized substituents (CH_3, CF_3, NH_2, respectively). The optimal pK_a of the N–H in sulfonamido groups, depending on the electronic effect of the substituents, might be important for the binding with receptor. These cumulative findings might suggest that the two oxygen (as hydrogen bonding acceptors) and N–H (as a hydrogen bonding donor) of sufonamido group play an integral role in the antagonistic binding with VR1 as drawn in Figure 3 and the spatial environment between sulfonamido group and binding site is very limited.

In conclusion, 28 *N*-4-substituted-benzyl-*N*-*tert*-butylbenzyl thiourea derivatives were prepared for the systematic studies on the pharmacophoric information required for the antagonistic activity on VR1. Among them, the best antagonistic activity was observed with the methanesulfonamide derivative (**6f**). Any modification, such as *N*-methylation and the replacement of



Scheme 2. Reagents and conditions: (i) RSO_2Cl, pyridine, CH_2Cl_2, rt, 70–95%; (ii) NaH, CH_3I, THF, 0 °C, 85%; (iii) TFA, CH_2Cl_2, rt; (iv) 4-*t*-Bu-BnNCS, CH_2Cl_2, rt, 85–95% from **10**.

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



7

No.	R^1	R^2	$^{45}Ca^{2+}$-influx activity (μM)[a]	
			Agonist (EC_{50})	Antagonist (IC_{50})
1	Capsaicin		0.03	NE[b]
2	Capsazepine		NE	0.65
6f	CH_3	H	NE	0.11
7b	Et	H	NE	1.3
7c	*n*-Pr	H	NE	4.5
7d	*n*-Bu	H	NE	4.1
7e	*i*-Pr	H	NE	3.8
7f	CF_3	H	NE	12.4
7g	CF_3CH_2	H	NE	11.7
7h	2-Thiophene	H	NE	>30[c]
7i	4-CH_3Ph	H	NE	NE
7j	Bn	H	NE	1.5
7k	NH_2	H	NE	1.0
7l	CH_3NH	H	NE	2.5
7m	$(CH_3)_2N$	H	NE	14.6
7n	CH_3	CH_3	NE	2.5

[a] EC_{50} and IC_{50} values were estimated by the same method described in Table 1.
[b] NE, not effective at 30 μM.
[c] Only partial activity was observed at 30 μM.

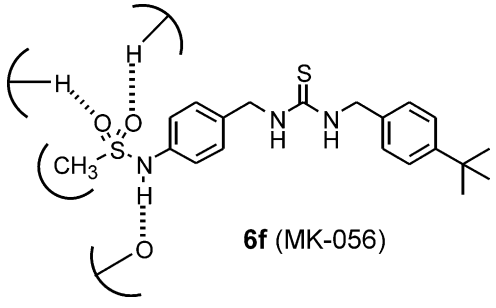


Figure 3.

methyl group with larger alkyl groups or amine groups, in methanesulfonamide group of **6f** resulted in dramatic decrease of antagonistic activity. We believe this pharmacophoric information on the antagonistic activity would be very useful to design more potent antagonistic scaffolds for the development of potential analgesics. Further investigation for new scaffolds based on these results is now under way.

Acknowledgements

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16. All compounds gave satisfactory spectroscopic data consistent with the proposed structures.
17. 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 ref 5b.