Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 6043–6053
Bioorganic & Medicinal Chemistry

α-Substituted *N*-(4-*tert*-butylbenzyl)-*N*′-[4-(methylsulfonylamino)-benzyl]thiourea analogues as potent and stereospecific TRPV1 antagonists

Jae-Uk Chung,[a] Su Yeon Kim,[a] Ju-Ok Lim,[a] Hyun-Kyung Choi,[a] Sang-Uk Kang,[a] Hae-Seok Yoon,[a] HyungChul Ryu,[a] Dong Wook Kang,[a] Jeewoo Lee,[a,*] Bomi Kang,[b] Sun Choi,[b] Attila Toth,[c] Larry V. Pearce,[c] Vladimir A. Pavlyukovets,[c] Daniel J. Lundberg[c] and Peter M. Blumberg[c]

[a]*Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea*
[b]*College of Pharmacy and National Core Research Center for Cell Signaling & Drug Discovery, Ewha Womans University, Seoul 120-750, Republic of Korea*
[c]*Laboratory of Cancer Biology and Genetics, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD 20892, USA*

Received 21 May 2007; accepted 20 June 2007
Available online 26 June 2007

Abstract—A series of α-substituted *N*-(4-*tert*-butylbenzyl)-*N*′-[4-(methylsulfonylamino)benzyl]thiourea analogues have been investigated as TRPV1 receptor antagonists. α-Methyl substituted analogues showed potent and stereospecific antagonism to the action of capsaicin on rat TRPV1 heterologously expressed in Chinese hamster ovary cells. In particular, compounds **14** and **18**, which possess the *R*-configuration, exhibited excellent potencies (respectively, K_i = 41 and 39.2 nM and $K_{i(ant)}$ = 4.5 and 37 nM).

1. Introduction

The transient receptor potential V1 (TRPV1) receptor[1,2] is a molecular tegrator 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 functions as a non-selective cation channel with high Ca^{2+} permeability and is activated by protons,[3] by heat,[4] by endogenous substances such as anandamide[5] and lipoxygenase products,[6] and by natural products such as capsaicin (CAP)[7] and resiniferatoxin (RTX).[8] The receptor 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.

TRPV1 antagonists have attracted much attention as promising drug candidates for inhibiting the transmission of nociceptive signaling from the periphery to the CNS and for blocking other pathological states associated with this receptor. They have thus emerged as novel and promising analgesic and anti-inflammatory agents, particularly for chronic pain and inflammatory hyperalgesia. Following the identification of capsazepine as the first competitive TRPV1 antagonist, a growing number of antagonists have been disclosed and some of them are in clinical development.[9]

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.[10,11]

A prototype antagonist (**1**), having a 4-(methylsulfonylamino)phenyl group in the A-region, showed high binding affinity and potent antagonism (K_i = 63 nM and $K_{i(ant)}$ = 54 nM in rTRPV1/CHO).[10] We further found that 3-substituents in the A-region affected the extent of agonism/antagonism. Thus, the 3-fluoro derivative **2** (K_i = 53.5 nM, $K_{i(ant)}$ = 9.2 nM in rTRPV1/CHO[10]; IC_{50} = 37 nM in rTRPV1/DRG[12]) was a potent antagonist not only of capsaicin stimulation of rTRPV1

Keywords: TRPV1 antagonists; Analgesic.
* Corresponding author. Tel.: +82 2 880 7846; fax: +82 2 888 0649; e-mail: jeewoo@snu.ac.kr

0968-0896/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2007.06.041

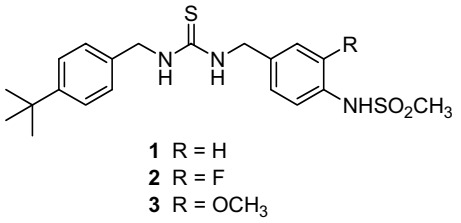


Figure 1.

but also of stimulation by temperature and pH. Conversely, the 3-methoxy derivative **3** showed a shift to partial agonism (K_i = 51 nM, 17% agonism and 84% antagonism in rTRPV1/CHO).[13]

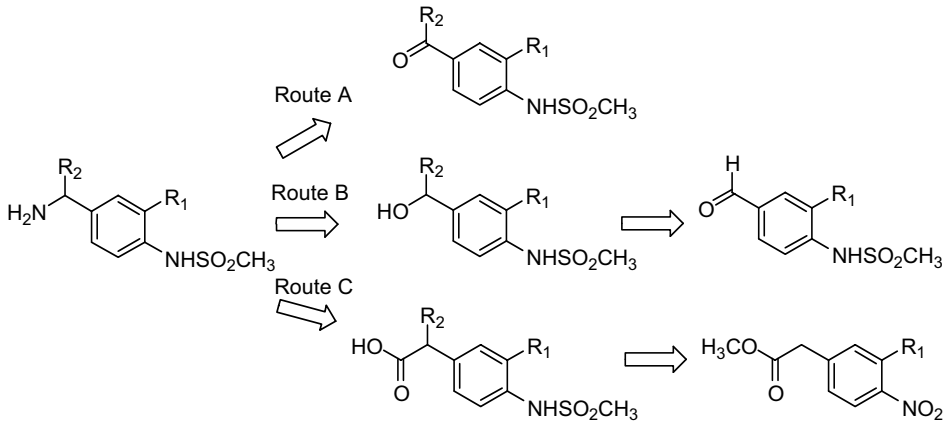
In order to optimize in vitro activities of 4-methylsulfonamide TRPV1 antagonists, we have investigated extensively their structure–activity relationships as a function of the structural regions designated as the A, B, and C-regions. From these analyses, antagonist **2** was found to possess the highest in vitro potency and is being developed as a preclinical candidate for analgesia.[12]

As part of our on-going effort, we describe in the current report the structure–activity relationships of the benzylic position in the A-region of the high affinity lead compounds **1**–**3**. The incorporation of substituents which should restrict the antagonists conformationally was able to stabilize a favorable 'bioactive conformation' and provides insights concerning additional pharmacophoric interactions with the receptor.

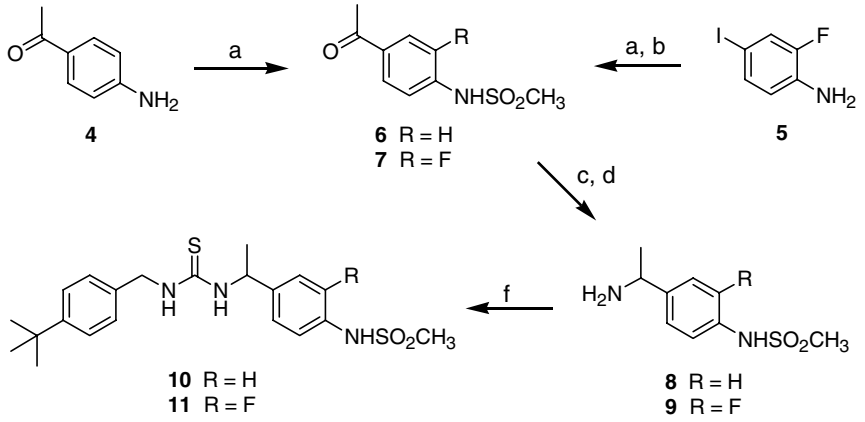
2. Chemistry

The target compounds were synthesized in general by the coupling of 4-*tert*-butylbenzyl isothiocyanate with the α-substituted benzyl amines of the A-region, which were obtained by the three different routes represented in Scheme 1 (Route A: from commercially available alkylketone, Route B: benzylic amination from the corresponding benzyl alcohol prepared from the aldehyde, Route C: Curtius rearrangement of α-substituted 2-phenylacetate) (Fig. 1).

The syntheses of α-methyl analogues with 3-hydrogen and 3-fluoro in the A-region are outlined in Scheme 2. Methylketones of (4-methylsulfonylamino)acetophenones (**6**, **7**), prepared from commercially available 4-aminoacetophenone or 2-fluoro-4-iodoaniline, respectively, were converted to the corresponding amines (**8**, **9**) via oximes, which were condensed with 4-*tert*-butylbenzyl isothiocyanate to afford the α-methyl analogues



Scheme 1.



Scheme 2. Reagents and conditions: (a) CH_3SO_2Cl, pyridine, 95%; (b) butyl vinyl ether, $Pd(OAc)_2$, DPPP, TlOAc, DMF, 78%; (c) NH_2OH, pyridine, 85–88%; (d) H_2, Pd-C, HCl, MeOH, 96–98%; (f) 4-*t*-BuBnNCS, CH_2Cl_2, 82–93%.

(**10**, **11**). The chiral isomers of **10** were readily obtained from the commercially available optical reagents (*R* or *S*)-α-methyl-4-nitrobenzylamine (**12**, **13**), as shown in Scheme 3. However, the syntheses of the optical isomers of **11** were carried out employing Ellman's asymmetric synthesis[14] starting from methylketone **7** as described in Scheme 4. Direct condensation of the commercially available optically active (*R*)-butanesulfinamide with ketone (**7**) provided the corresponding *tert*-butanesulfinyl ketimine, which was stereoselectively reduced in situ with $NaBH_4$ to afford the chiral *tert*-butanesulfinyl-protected amine (**16**). The acidic hydrolysis followed by isothiocyanate condensation gave (*R*)-α-methyl thiourea (**18**). The same protocol with (*S*)-butanesulfinamide gave the corresponding (*S*)-isomer (**19**). The optical purities of the final compounds, **18** and **19**, were determined by HPLC using chiral column, respectively (Fig. 2).

The syntheses of other α-alkyl and -aryl analogues are shown in Scheme 5. Starting from 2-fluoro-4-iodoaniline, the iodide of **20** was transformed to the aldehyde **22** in three steps through palladium-catalyzed vinyl coupling followed by oxidative cleavage. The aldehyde of **22** was reacted with Grignard reagents to produce alcohols, which were converted to the corresponding amines (**27**–**33**) in two steps and then condensed with isothiocyanate to afford α-substituted analogues (**31**–**34**).

The syntheses of α-dimethyl and α-cyclopropyl analogues were accomplished through a benzylic alkylation approach and are described in Scheme 6. Methyl 2-phenylacetates (**35**–**37**), prepared from the corresponding benzyl cyanides previously reported,[15] were alkylated with methyl iodide or dibromoethane to yield alkylated products. The methyl esters of **38**–**43** were hydrolyzed and then underwent Curtius rearrangement to give the corresponding amines, which were condensed with isothiocyanates to afford the final compounds (**56**–**61**).

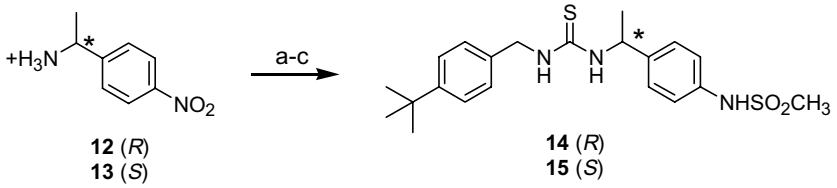
3. Results and discussion

3.1. Biological activity

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 by a functional $^{45}Ca^{2+}$ uptake assay using rat TRPV1 heterologously expressed in Chinese hamster ovary (CHO) cells, as previously described.[10,11] The results are summarized in Table 1, together with the potencies of capsazepine and the previously reported antagonists **1**–**3**.[16]

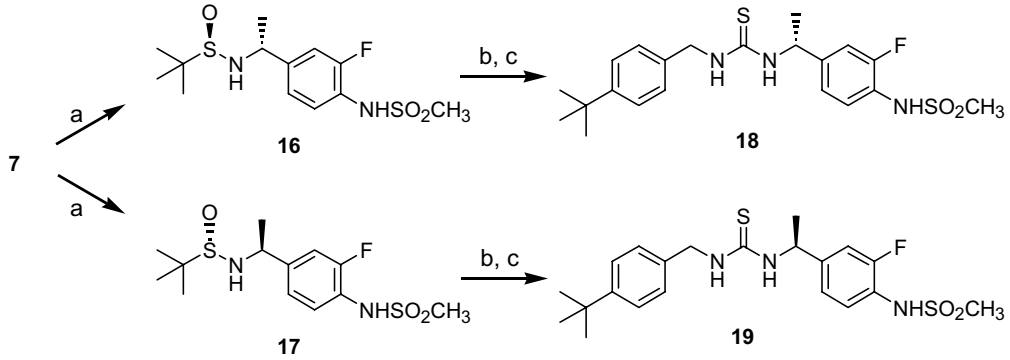
Initially, a methyl group was incorporated at the benzylic position of the A-region in the lead compounds (**1**–**3**) to provide **10**, **11**, and **59**. The results indicated that the binding affinities of α-methyl analogues were comparable to those of the parent lead compounds. In contrast, their potencies as antagonists depended on the specific 3-substituents in the A-region. Whereas α-methylation of **1** (R_1 = H) to give **10** led to a 4-fold enhancement of antagonist potency, that of **2** (R_1 = F) and **3** (R_1 = OMe) resulted in no change or an 8-fold reduction, respectively, in antagonist potency.

The interesting results with the α-methyl analogues prompted us to explore their two optical isomers. The activities of the compounds showed marked stereodependence. The enantiomeric (*S*)-isomers (**15** and **19**) of

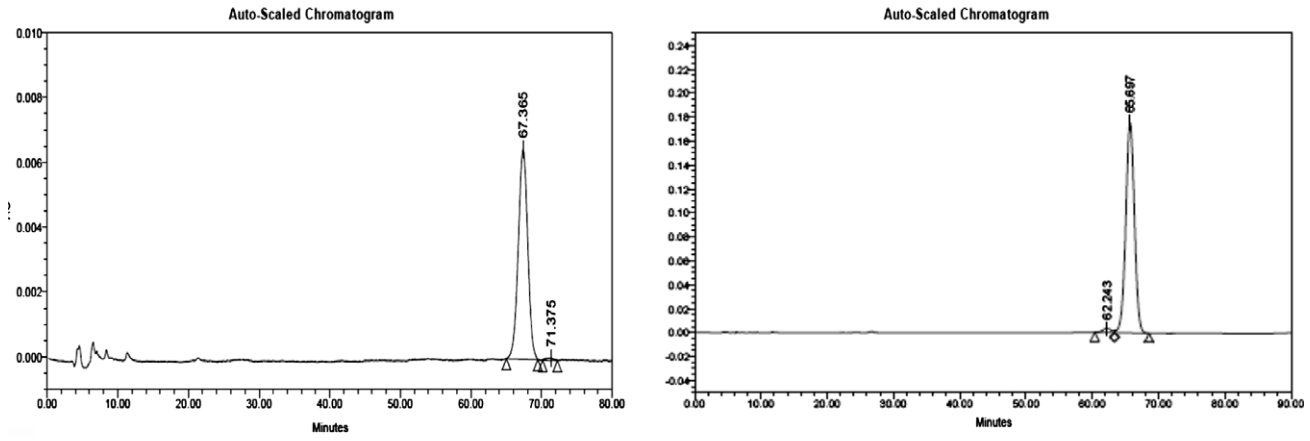


12 (*R*)
13 (*S*)
14 (*R*)
15 (*S*)

Scheme 3. Reagents and conditions: (a) 4-*t*-BuBnNCS, NEt_3, CH_2Cl_2, 82–84%; (b) H_2, Pd-C, MeOH, 96–98%; (c) CH_3SO_2Cl, pyridine, 92–94%.

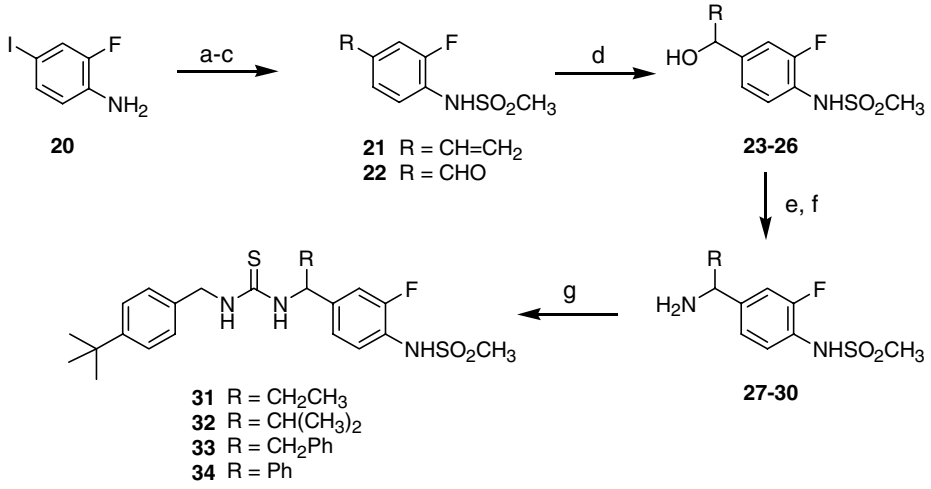


Scheme 4. Reagents and conditions: (a) i—(*R*)-*t*-BuS(O)NH_2 for **16**, (*S*)-*t*-BuS(O)NH_2 for **17**, $Ti(OEt)_4$, THF; ii—$NaBH_4$, 36% for *R*, 31% for *S*; (b) HCl–MeOH, dioxane, 80–88%; (c) 4-*t*-BuBnNCS, NEt_3, DMF, 84–86%.



(a) Compound **18** (99% ee) (b) Compound **19** (98% ee)

Figure 2. Conditions; column: Chiralcel OJ-RH (4.6 × 150 mm, Daicel), eluent: acetonitrile/water = 40/60 (v/v), flow rate: 0.3 mL/min, column temperature: 25 °C, injection volume: 1 μL, detection wavelength: 224 nm, sample preparation: 100 μg/mL eluent.



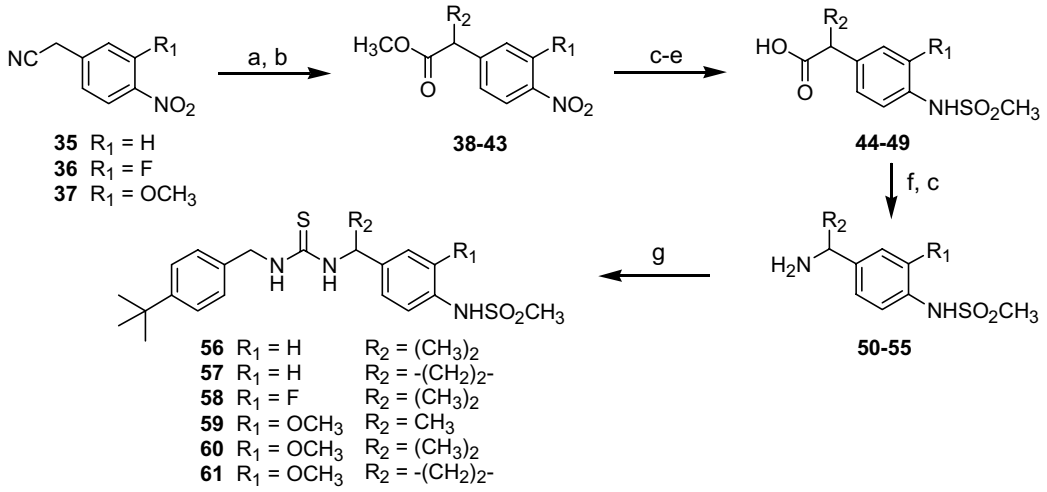
Scheme 5. Reagents and conditions: (a) $CH_2{=}CHSnBu_3$, $Pd(PPh_3)_4$, toluene, 93%; (b) CH_3SO_2Cl, pyridine, CH_2Cl_2, 91%; (c) OsO_4, $NaIO_4$, acetone–H_2O, 64%; (d) RMgCl, THF, 92–99%; (e) DPPA, DBU, toluene, 84–94%; (f) H_2, Pd-C, MeOH, 94–98%; (g) 4-*t*-BuBnNCS, DMF, 84–92%.

10 and **11** showed very weak potencies compared to the racemates. Conversely, the enantiomeric (*R*)-isomers (**14** and **18**) displayed enhanced potencies. In particular, compound **14** was found to be a potent antagonist with a value of $K_{i(ant)}$ = 4.5 nM, which was 115-fold more potent than capsazepine.

Previously, Ryu et al. reported VR1 functional activities of compounds **10**, **14**, and **15** using cultured spinal sensory neurons from neonatal rats.[17] That report described that the (*R*)-isomer (**14**) was an active and potent antagonist, consistent with our present results. However, in that case they reported that the (*S*)-isomer (**15**) also showed modest antagonism, whereas we found that **15** was a very weak agonist devoid of antagonism.

As a next step, we explored additional steric substituents, such as ethyl, isopropyl, phenyl, and benzyl groups, on the lead compound **2** to afford **31**–**34**. Unfortunately, incorporation of these bulkier groups reduced both binding affinity and antagonist potency. The antagonist potencies diminished as the size of the substituents increased, Et (**31**) > *i*-Pr (**32**) > Bn (**33**) > Ph (**34**). The result indicates that the improvement in potency upon the incorporation of an α-methyl group (in the *R*-configuration) results from its specific effect on the receptor binding interaction, not from an unspecific effect on the physico-chemical properties.

The potent antagonism conferred by the methyl group might derived from the specific interaction of the methyl group per se with the receptor or, alternatively, from conformational restriction imposed by the methyl group on the whole molecule. To distinguish these alternatives, dimethyl (**56**, **58**, and **60**) and cyclopropyl analogues (**57**, **61**) of the lead compounds (**1**–**3**) were investigated. All dimethyl analogues were almost devoid of receptor activity or very weak antagonists. On the other hand, the relatively smaller cyclopropyl analogues were better tolerated compared to the dimethyl analogues. Incorporation of a cyclopropyl group into lead compound **1** to afford **57** reduced binding affinity only 2.7-fold compared to



Scheme 6. Reagents and conditions: (a) HCl, MeOH, 80–90%; (b) MeI or $BrCH_2CH_2Br$, NaH, DMF, 75–90%; (c) H_2, Pd-C, MeOH, 92–98%; (d) MsCl, pyridine, 94–98%; (e) LiOH, THF–H_2O, 80–94%; (f) i—DPPA, NEt_3, toluene, reflux, ii—BnOH, reflux, 70–85%; (g) 4-*t*-BuBnNCS, CH_2Cl_2, 80–90%.

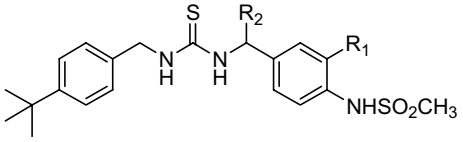
1 and its potency as an antagonist was comparable to that of **1**. The cyclopropyl analogue **61** of lead compound **3** was less potent by 5- to 10-fold compared to the parent **3** but was more potent than the corresponding dimethyl analogue **60**. Our data thus suggest that the α-position is tightly constrained sterically and that the dimethyl substitution is too large to be tolerated.

3.2. Molecular modeling

We performed conformational analysis of **1**, **14**, and **15** using Grid Search in the Tripos SYBYL molecular modeling program to provide insights into the potential bioactive conformation of the antagonists and the possible basis for the stereoselective receptor activities of the *R*- and *S*-isomers. The resulting lowest energy conformers in the gas phase were solvated with water, and then the solvated molecules were energy minimized to find the lowest energy conformations in aqueous solution.

As shown in Figure 3, the resulting conformers of the *R*- and *S*-isomers (**14** and **15**) show distinctive conformational differences and the conformation of the nonchiral compound **1** (white, K_i = 63 nM) is relatively close to that of the active *R*-isomer, compound **14** (magenta, K_i = 41 nM), explaining why the potencies of the

Table 1.



	R_1	R_2	K_i (nM) Binding affinity	EC_{50} (nM) Agonism	K_i (nM) Antagonism
CPZ			1300 (±150)	NE	520 (±12)
1	H	H	63 (±10)	NE	54 (±8.7)
10	H	CH_3	59.3 (±5.2)	NE	14.7 (±2)
14	H	CH_3-(*R*)	41 (±11)	NE	4.5 (±1.9)
15	H	CH_3-(*S*)	3950 (±490)	(8%)	NE
56	H	$(CH_3)_2$	NE	NE	1800 (±600)
57	H	$-(CH_2)_2-$	171 (±36)	NE	60 (±19)
2	F	H	53.5 (±6.5)	NE	9.2 (±1.6)
11	F	CH_3	54 (±11)	NE	9.2 (±2.9)
18	F	CH_3-(*R*)	39.2 (±8.3)	NE	37 (±10)
19	F	CH_3-(*S*)	2140 (±270)	NE	2670 (±470)
31	F	CH_2CH_3	230 (±28)	NE	54 (±24)
32	F	$CH(CH_3)_2$	339 (±87)	NE	223 (±21)
33	F	CH_2Ph	1740 (±420)	NE	700 (±210)
34	F	Ph	100 (±19)	NE	860 (±260)
58	F	$(CH_3)_2$	7700 (±1500)	NE	980 (±200)
3	OCH_3	H	51 (±17)	(18%)	(84%)
59	OCH_3	CH_3	67 (±12)	NE	28.6 (±4.8)
60	OCH_3	$(CH_3)_2$	2890 (±460)	NE	663 (±60)
61	OCH_3	$-(CH_2)_2-$	370 (±180)	NE	243 (±53)

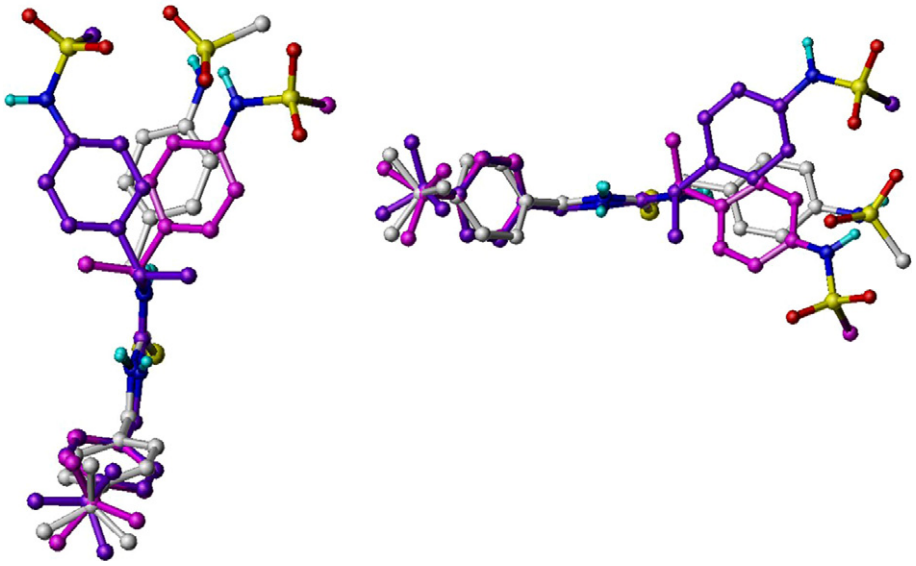


Figure 3. Overlay of the lowest energy conformers of **1**, **14**, and **15** (in two different views). These conformers were generated by grid conformational search, solvation with water, and then energy minimization as described in the experimental section. Carbon atoms are shown in white, magenta, and purple for **1**, **14**, and **15**, respectively. The non-polar hydrogen atoms are not displayed for clarity.

non-methyl analogues **1** (or **2**) are closer to those of the active *R*-isomers **14** (or **18**) than of the inactive *S*-isomers **15**(or **19**). Additionally, a further contribution to the loss of activity of the *S*-isomers might be because key pharmacophores in their conformations are disposed inappropriately when binding to the receptor compared with the active *R*-isomers and the corresponding non-chiral compounds (Fig. 3).

4. Conclusion

We have modified the benzylic position in the A-region of the prototype TRPV1 antagonists (**1**–**3**) to analyze their structure–activity relationships. Incorporation of a methyl group with an (*R*)-configuration enhanced specific binding to the receptor. Compound **14** showed the highest receptor potency with values of K_i = 41 nM and $K_{i(ant)}$ = 4.5 nM to capsaicin. However, larger substitutions or an (*S*)-configuration resulted in modest to dramatic decreases in binding affinities and antagonistic potencies. Molecular modeling analysis indicated that the conformation of the non-chiral compound (**1**) was closer to that of active methyl *R*-isomer (**14**) rather than the inactive *S*-isomer (**15**), consistent with their receptor activities.

5. Experimental

5.1. General

All Chemical reagents were commercially available. Melting points were determined on a melting point Buchi B-540 apparatus and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230–400 mesh, Merck. Proton NMR spectra were recorded on a JEOL JNM-LA 300 at 300 MHz. Chemical shifts are reported in ppm units with Me_4Si as a reference standard. Mass spectra were recorded on a VG Trio-2 GC-MS. Combustion analyses were performed on an EA 1110 Automatic Elemental Analyzer, CE Instruments.

5.1.1. 4′-(Methylsulfonylamino)acetophenone (6). A cooled solution of 4′-aminoacetophenone (**4**) (1.35 g, 10 mmol) in pyridine (10 mL) at 0 °C was treated with methanesulfonyl chloride (1.718 g, 15 mmol) and stirred at room temperature for 2 h. The reaction mixture was diluted with H_2O and extracted with EtOAc several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexanes to afford **6** as white solid (2.03 g, 95%). mp = 161 °C; ^1H NMR ($CDCl_3$) δ 7.97 (dd, 2H, J = 2, 6.8 Hz,), 7.26 (dd, 2H, J = 2, 6.8 Hz), 6.87 (br s, 1H, $NHSO_2$), 3.10 (s, 3H, SO_2CH_3), 2.59 (s, 3H, $COCH_3$).

5.1.2. 3′-Fluoro-4′-(methylsulfonylamino)acetophenone (7). A cooled solution of amine (2.37 g, 10 mmol) in pyridine (10 mL) at 0 °C was treated with methanesulfonyl chloride (1.718, 15 mmol) and stirred at room temperature for 2 h. The reaction mixture was diluted with H_2O and extracted with EtOAc several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexanes to give the mesylated compound (2.99 g, 95%). A solution of mesylated product (1.576 g, 5 mmol) in DMF (10 mL) was added palladium(II)acetate (0.034 g, 0.15 mmol), 1,3-bisdiphenyl phosphinopropane (0.124 g, 0.3 mmol), thallium(I)acetate (1.450 g, 5.5 mmol) and butylvinylether (1.3 mL, 10 mmol), and heated at 95 °C for 19 h. The reaction mixture was cooled to room temperature, diluted with THF, treated with 10% HCl (10 mL), and stirred at room temperature for 0.5 h. The reaction

mixture was diluted with EtOAc and washed with NH_4Cl(aq) three times. The combined organic layers were washed with $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexanes to afford **7** as yellow solid (0.9 g, 78%); mp = 141 °C; ^{1}H NMR ($CDCl_3$) δ 7.65–7.80 (m, 3H, Ar), 6.89 (br s, 1H, $NHSO_2$), 3.12 (s, 3H, SO_2CH_3), 2.59 (s, 3H, $COCH_3$).

5.1.3. General procedure for oxime formation and hydrogenation. A mixture of ketone (5 mmol) and hydroxylamine hydrochloride (0.695 g, 10 mmol) in pyridine (5 mL) was heated at 70 °C for 3 h. The reaction mixture was cooled to room temperature, diluted with H_2O, and extracted with EtOAc several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexanes (1:1) as eluant. A suspension of oxime (5 mmol) and 10% palladium on carbon (150 mg) in MeOH (25 mL) was treated with concentrated hydrochloric acid (10 drops) and was hydrogenated under a balloon of hydrogen for 6 h. The reaction mixture was neutralized with solid $NaHCO_3$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH as eluant.

5.1.4. 1-[4-(Methylsulfonylamino)phenyl]ethyl amine (8). 82% yield; white solid; mp = 211 °C; ^{1}H NMR ($CDCl_3$) δ 7.35 (d, 2H, J = 8.6 Hz), 7.18 (d, 2H, J = 8.6 Hz), 4.13 (q, 1H, J = 7 Hz, CHMe), 3.00 (s, 3H, SO_2CH_3), 1.37 (d, 3H, J = 7 Hz, CH_3).

5.1.5. 1-[3-Fluoro-4-(methylsulfonylamino)phenyl]ethyl amine (9). 85% yield; white solid; mp = 162–165 °C; ^{1}H NMR (CD_3OD) δ 7.45 (t, 2H, J = 8.2 Hz, H-5), 7.24 (dd, 1H, J = 11.5, 2 Hz, H-2), 7.18 (dd, 1H, J = 8.3, 2 Hz, H-6), 4.15 (q, 1H, J = 7 Hz, CHMe), 2.97 (s, 3H, SO_2CH_3), 1.43 (d, 3H, J = 7 Hz, CH_3).

5.1.6. General procedure for coupling to thioureas (10, 11). A mixture of amine (1 mmol) and isothiocyanate (1 mmol) in DMF (2 mL) was stirred at room temperature for 2 h. The reaction mixture was diluted with H_2O and extracted with EtOAc several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:1) as eluant.

5.1.7. *N*-(4-*tert*-Butylbenzyl)-*N*′-{1-[4-(methylsulfonylamino)phenyl]ethyl}thiourea (10). 88% yield; white solid; mp = 101 °C; ^{1}H NMR ($CDCl_3$) δ 7.33 (d, 2H, J = 8 Hz,), 7.23 (d, 2H, J = 8.5 Hz), 7.16 (d, 2H, J = 8.5 Hz), 7.07 (d, 2H, J = 8 Hz), 6.66 (s, 1H, $NHSO_2$), 6.14 (br s, 1H, NH), 5.86 (br s, 1H, NH), 5.02 (br s, 1H, CHMe), 4.58 (d, 2H, J = 4.6 Hz, CH_2NH), 3.00 (s, 3H, SO_2CH_3), 1.47 (d, 3H, J = 6.6 Hz, CH_3), 1.30 (s, 9H, $C(CH_3)_3$); MS (FAB) m/z 420 (MH^+); Anal. calcd for $C_{21}H_{29}N_3O_2S_2$: C, 60.11; H, 6.97; N, 10.01; S, 15.28. Found: C, 60.35; H, 6.99; N, 9.98; S, 15.25.

5.1.8. *N*-(4-*tert*-Butylbenzyl)-N′-{1-[3-fluoro-4-(methylsulfonylamino)phenyl]ethyl}thiourea (11). 93% yield; white solid; mp = 175 °C; ^{1}H NMR ($CDCl_3$) δ 7.50 (t, 1H, J = 8.04 Hz, H-5), 7.36 (d, 2H, Ar), 7.14 (d, 2H, Ar), 7.0–7.05 (m, 2H, Ar), 6.48 (s, 1H, $NHSO_2$), 5.95 (br s, 2H, NH), 5.17 (br s, 1H, NHC*H*Me), 4.56 (d, 2H, J = 5.1 Hz, CH_2NH), 3.02 (s, 3H, SO_2CH_3), 1.46 (d, 3H, J = 6.8 Hz, $CHCH_3$), 1.31 (s, 9H, $C(CH_3)_3$); MS (FAB) m/z 438 (MH^+); Anal. calcd for $C_{21}H_{28}FN_3O_2S_2$: C, 57.64; H, 6.45; N, 9.60; S, 14.66. Found: C, 57.86; H, 6.47; N, 9.58; S, 14.63.

5.1.9. *N*-(4-*tert*-Butylbenzyl)-*N*′-{(1*R*)-1-[4-(methylsulfonylamino)phenyl]ethyl}thiourea (14). To a stirred solution of (*R*)-α-methyl-4-nitrobenzyl amine hydrochloride (**12**) (203 mg, 1 mmol) in anhydrous CH_2Cl_2 (10 mL) was added triethylamine (0.28 mL, 2 mmol) at room temperature. When the reaction mixture became clear, isothiocyanate (1 mmol) was added and stirred overnignt at room temperature. The mixture was evaporated by rotary evaporator and the residue was purified by flash column chromatography on silica gel with EtOAc/hexanes as eluant. A suspension of thiourea (5 mmol) and 10% palladium on carbon (150 mg) in MeOH (25 mL) was treated with concentrated hydrochloric acid (10 drops) and was hydrogenated under a balloon of hydrogen for 6 h. The reaction mixture was neutralized with solid $NaHCO_3$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH as eluant. A cooled solution of amine (0.25 mmol) in pyridine (2 mL) at 0 °C was treated with methanesulfonyl chloride (0.3 mmol) and stirred for 10 min at 0 °C. After aqueous work-up, the residue was purified by flash column chromatography on silica gel with EtOAc/hexanes as eluant to afford **14** as white solid in 75% yield; mp = 101 °C. The spectra of **14** were identical to those of **10**; [α] = −13.34 ($CHCl_3$, c = 1.08); Anal. calcd for $C_{21}H_{29}N_3O_2S_2$: C, 60.11; H, 6.97; N, 10.01; S, 15.28. Found: C, 60.37; H, 6.98; N, 9.97; S, 15.24.

5.1.10. *N*-(4-*tert*-Butylbenzyl)-*N*′-{(1*S*)-1-[4-(methylsulfonylamino)phenyl]ethyl}thiourea (15). This compound was prepared from **13** following the procedure for the synthesis of **14** in 75% yield; white solid, mp = 101 °C. The spectra of **15** were identical to those of **14**; [α] = +10.60 ($CHCl_3$, c = 1.08); Anal. calcd for $C_{21}H_{29}N_3O_2S_2$: C, 60.11; H, 6.97; N, 10.01; S, 15.28. Found: C, 60.38; H, 7.00; N, 9.98; S, 15.25.

5.1.11. (*R*)-Sulfinamide (16) and (*S*)-sulfinamide (17). To a 0.5 M solution of $Ti(OEt)_4$ (0.3 mL, 1.44 mmol) and **7** (0.2 g, 0.87 mmol) in THF (5 mL) under an N_2 atmosphere was added (*R*)-(+)-2-methyl-2-propanesulfinamide (0.087 g, 0.72 mmol) and the mixture was heated (70 °C). Upon completion, as determined by TLC, the mixture was cooled to room temperature and then to −40 °C before it was cannulated dropwise into a −40 °C solution of $NaBH_4$ (0.109 g. 2.88 mmol). The

mixture was stirred at −40 °C for 12 h, and then MeOH was added dropwise until gas was no longer evolved. The resulting suspension was filtered through a plug of Celite and the filter cake was washed with EtOAc. The filtrate was washed with brine, and the brine layer was extracted with EtOAc. The combined organic portions were dried (Na_2SO_4), filtered, and concentrated. After silica gel column chromatography (*n*-hexane/EtOAc), the (*R*)-sulfonamide (0.105 g, 0.31 mmol, 36%) was isolated; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (t, 1H, J = 8.4 Hz), 7.19 (m, 1H), 7.15 (m, 1H), 6.97 (br s, 1H), 4.53 (m, 1H), 3.50 (d, 1H, J = 3.8 Hz), 3.04 (s, 3H), 1.75 (br s, 1H), 1.51 (d, 3H, J = 6.5 Hz), 1.25 (s, 9H).

(*S*)-Sulfinamide (**17**) was prepared from **7** using (*S*)-(−)-2-methyl-2-propanesulfinamide in 31% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (m, 1H), 7.26 (br s, 1H), 7.17–7.08 (m, 2H), 4.48 (m, 1H), 3.54 (d, 1H, J = 3.8 Hz), 2.99 (s, 3H), 1.47 (d, 3H, J = 6.5 Hz), 1.21 (s, 9H).

5.1.12. (*R*)-*N*-(4-*tert*-Butylbenzyl)-*N*′-{1-[3-fluoro-4-(methylsulfonylamino)phenyl]ethyl}thiourea (18). To a (*R*)-sulfinamide (**16**) (0.105 g, 0.31 mmol) were added 1:1 (v/v) MeOH and HCl dioxane solution (4.0 M, 0.22 mL). The mixture was stirred at room temperature for 30 min and was then concentrated to near dryness. Diethyl ether was added to precipitate the amine hydrochloride. The precipitate was then filtered off and washed with diethyl ether to provide analytically pure (*R*)-1-[3-fluoro-4-(methylsulfonylamino)phenyl]ethyl amine hydrochloride (0.059 g, 0.22 mmol, 70%, 96 ee%); ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.71 (br s, 1H), 8.60 (br s, 3H), 7.52 (dd, 1H, J = 1.9, 11.8 Hz), 7.42 (t, 1H, J = 8.4 Hz), 7.33 (dd, 1H, J = 1.8, 8.4 Hz), 4.39 (m, 1H), 3.62 (m, 1H), 3.05 (s, 3H), 1.49 (d, 3H, J = 6.5 Hz).

To a stirred solution of 4-[4-(1-amino-ethyl)-2-fluorophenyl]-methanesulfonamide hydrochloride (20 mg, 0.075 mmol) in DMF (1 mL), Et_3N (13 μL, 0.09 mmol), and 1-*tert*-butyl-4-isothiocyanatomethyl benzene (15 mg, 0.075 mmol) were added in the written order. The reaction mixture was stirred for 3 h at room temperature. And then the reaction solution was extracted by EtOAc and the organic phase was washed with H_2O, dried (Na_2SO_4), filtered, and concentrated. After silica gel column chromatography (*n*-hexane/EtOAc), *N*-{4-{1-[3-(4-*tert*-butyl-benzyl)-thioreido]-ethyl}-2-fluorophenyl}-methanesulfonamide (26 mg, 0.06 mmol, 85%) was isolated; white solid, mp = 175 °C; [α] −19.24 (*c* 0.7, $CHCl_3$); 98.41 ee%

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42 (t, 1H, J = 9.0 Hz), 7.35 (m, 1H), 7.33 (m, 1H), 7.12 (m, 2H), 7.00 (m, 2H), 6.90 (br s, 1H), 6.45–6.10 (br s, 2H), 5.18 (br s, 1H), 4.54 (m, 2H), 2.98 (s, 3H), 1.43 (d, 3H, J = 3.0Hz), 1.29 (s, 9H); MS (FAB) *m*/*z* 438 (MH^+); Anal. calcd for $C_{21}H_{28}FN_3O_2S_2$: C, 57.64; H, 6.45; N, 9.60; S, 14.66. Found: C, 57.88; H, 6.48; N, 9.57; S, 14.63.

5.1.13. (*S*)-*N*-(4-*tert*-Butylbenzyl)-*N*′-{1-[3-fluoro-4-(methylsulfonylamino)phenyl]ethyl}thiourea (19). The compound was prepared from (*S*)-sulfinamide (**17**) by following the procedure for the synthesis of **18**; white solid, mp = 175 °C; [α] = 16.04 (*c* 0.7, $CHCl_3$), 97.76 ee%. The spectra are identical to those of **18**; Anal. calcd for $C_{21}H_{28}FN_3O_2S_2$: C, 57.64; H, 6.45; N, 9.60; S, 14.66. Found: C, 57.85; H, 6.47; N, 9.57; S, 14.62.

5.1.14. *N*-(2-Fluoro-4-vinylphenyl)methanesulfonamide (21). A solution of 2-fluoro-4-iodoaniline **20** (2.37 g, 10 mmol) in toluene (50 mL) was treated with tetrakis(triphenylphosphine)palladium (0.578 g, 0.5 mmol), tributylvinyltin (3.5 mL, 12 mmol), and a catalytic amount of 2,6-di-*tert*-butyl-4-methylphenol. After being heated at 100 °C for 1 h, the reaction mixture was filtered through Celite and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexanes (1:5) as eluant to afford 2-fluoro-4-vinylaniline (1.275 g, 93%) as a yellow oil. A cooled solution of 2-fluoro-4-vinylaniline (0.96 g, 7 mmol) in pyridine (10 mL) at 0 °C was treated with methanesulfonyl chloride (0.644 mL, 8.4 mmol) and stirred at room temperature for 30 min. The reaction mixture was diluted with water, and extracted with EtOAc several times. The combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexanes (1:3) as eluant to afford **21** (1.372 g, 91%) as a white solid; mp = 82 °C; ^{1}H NMR ($CDCl_3$) δ 7.53 (t, 1H, J = 8 Hz, H-6), 7.15–7.25 (m, 2H, H-3,5), 6.64 (dd, 1H, J = 10.7, 17.5 Hz, C*H*=CH_2), 6.50 (br s, 1H, $NHSO_2$), 5.72 (d, 1H, J = 17.5 Hz, CH=CH_2), 5.32 (dd, 1H, J = 10.7 Hz, CH=CH_2), 3.03 (s, 3H, SO_2CH_3).

5.1.15. *N*-(2-Fluoro-4-formylphenyl)methanesulfonamide (22). A solution of **21** (1.076 g, 5 mmol) in acetone and water (1:1, 20 mL) was treated with a catalytic amount of osmium tetroxide (4 wt% solution in hydroxyperoxide) and sodium periodate (2.139 g, 10 mmol). After being stirred at room temperature for 1 h, the mixture was concentrated into a small volume in vacuo. The residue was treated with aqueous sodium thiosulfate solution and then extracted with EtOAc several times. The combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexanes (1:2) as eluant to afford **22** (0.521 g, 48%) as a white solid; mp = 151 °C; ^{1}H NMR ($CDCl_3$) δ 9.92 (d, 1H, J = 2.2 Hz, CHO), 7.78 (t, 1H, J = 8.6 Hz, H-6), 7.65–7.74 (m, 2 H, H-3,5), 6.92 (br s, 1H, $NHSO_2$), 3.15 (s, 3H, SO_2CH_3).

5.1.16. General procedure for Grignard reaction. A cooled solution of **22** (0.424 g, 2 mmol) in THF (20 mL) at 0 °C was treated with Grignard reagent (4 mmol) and stirred at 0 °C for 30 min. The reaction mixture was quenched with saturated ammonium chloride solution, diluted with water and extracted with EtOAc several times. The combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexanes (1:1) as eluant.

5.1.17. *N*-[2-Fluoro-4-(1-hydroxypropyl)phenyl]methanesulfonamide (23). 92% yield; colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.53 (t, 1H, J = 8.22 Hz, H-6), 7.19 (dd, 1H, J = 1.8, 11.2 Hz, H-3), 7.12 (dd, 1H, J = 1.8 Hz, 8 Hz, H-5), 6.45 (br s, 1H, $NHSO_2$), 4.61 (m, 1H, CHOH), 3.02 (s, 3H, SO_2CH_3), 1.87 (m, 1H, OH), 1.7–1.8 (m, 2H, CH_2), 0.93 (t, 3H, J = 7.3 Hz, CH_3).

5.1.18. *N*-[2-Fluoro-4-(1-hydroxy-2-methylpropyl)phenyl]-methanesulfonamide (24). 90% yield; colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.50 (t, 1H, J = 8.28 Hz, H-6), 7.15 (dd, 1H, J = 1.95, 11.2 Hz, H-3), 7.07 (dd, 1 H, J = 1.8 Hz, 8 Hz, H-5), 6.62 (br s, 1H, $NHSO_2$), 4.38 (d, 1H, J = 6.36 Hz, CHOH), 3.01 (s, 3H, SO_2CH_3), 1.80 (m, 2H, $CHMe_2$ and OH), 0.95 (d, 3H, J = 6.8 Hz, CH_3), 0.83 (d, 3H, J = 6.8 Hz, CH_3).

5.1.19. *N*-[2-Fluoro-4-(1-hydroxy-2-phenylethyl)phenyl]-methanesulfonamide (25). 94% yield; yellow solid; mp = 123 °C; ^{1}H NMR ($CDCl_3$) δ 7.54 (t, 1H, J = 8.22 Hz, H-6), 7.1–7.35 (m, 7H, Ph and H-3,5), 6.44 (br s, 1H, $NHSO_2$), 4.89 (m, 1H, CHOH), 3.02 (s, 3H, SO_2CH_3), 2.98 (dd d of AB, 2H, CH_2Ph), 1.98 (d, 1H, J = 2.9 Hz, OH).

5.1.20. *N*-{2-Fluoro-4-[hydroxy(phenyl)methyl]phenyl}-methanesulfonamide (26). 99% yield; white solid; mp = 91 °C; ^{1}H NMR ($CDCl_3$) δ 7.52 (t, 1H, J = 8.25 Hz, H-6), 7.3–7.38 (m, 5H, Ph), 7.22 (dd, 1H, J = 1.6, 11.2 Hz, H-3), 7.17 (dd, 1H, J = 1.6 Hz, 8 Hz, H-5), 6.46 (br s, 1H, $NHSO_2$), 5.81 (s, 1H, CHOH), 3.00 (s, 3H, SO_2CH_3), 1.99 (br s, 1H, OH).

5.1.21. General procedure for coupling to thioureas (31–34). A cooled solution of the alcohol (1 mmol) in toluene (10 mL) at 0 °C was treated with diphenylphosphorylazide (0.26 mL, 1.2 mmol) followed by 1,8-diazabicyclo[5,4,0]undec-7-ene (0.18 mL, 1.2 mmol) and stirred for 2 h at 0 °C. After being further stirred for 20 h at room temperature, the reaction mixture was diluted with EtOAc. The organic layere was washed with 5% HCl (10 mL), water and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using EtOAc/hexanes (1:3) as eluant. A suspension of the azide (1 mmol) and 10% palladium on carbon (50 mg) in MeOH (10 mL) was hydrogenated under a balloon of hydrogen for 1 h. The reaction mixture was filtered and the filtrate was concentrated in vacuo. The residue was dissolved in DMF (3 mL) and then added 4-*tert*-butylbenzyl isothiocyanate (0.205 g, 1 mmol). After being stirred at room temperature for 3 h, the reaction mixture was diluted with water and extracted with EtOAc several times. The combined organic layers were washed with water and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:1) as eluant.

5.1.22. *N*-(4-*tert*-Butylbenzyl)-*N*′-{1-[4-(methylsulfonylamino)-3-fluorophenyl]propyl}thiourea (31). 82% yield; white solid; mp = 85 °C; ^{1}H NMR ($CDCl_3$) δ 7.45 (t, 1H, J = 8.04 Hz, H-5), 7.34 (d, 2H, J = 8.04 Hz), 7.12 (d, 2H, J = 8.04 Hz), 6.9–7.0 (m, 2H, H-2,6), 6.76 (br s, 1H, $NHSO_2$), 6.24 (br s, 2H, NH), 4.88 (br s, 1H, CHNH), 4.55 (br s, 2H, CH_2NH), 3.00 (s, 3H, SO_2CH_3), 1.7–1.8 (m, 2H, CH_2CH_3), 1.30 (s, 9H, $C(CH_3)_3$), 0.82 (t, 3H, J = 7.05 Hz, CH_2CH_3); MS (FAB) m/z 452 (MH^+); Anal. calcd for $C_{22}H_{30}FN_3O_2S_2$: C, 58.51; H, 6.70; N, 9.30; S, 14.20. Found: C, 58.72; H, 6.72; N, 9.27; S, 14.18.

5.1.23. *N*′-(4-*tert*-Butylbenzyl)-*N*-{1-[4-(methylsulfonylamino)-3-fluorophenyl]-2-ethylpropyl}thiourea (32). 87% yield; white solid; mp = 84 °C; ^{1}H NMR ($CDCl_3$) δ 7.45 (t, 1H, J = 8.04 Hz, H-5), 7.36 (d, 2H, J = 8.04 Hz), 7.14 (d, 2H, J = 8.04 Hz), 6.85–6.95 (m, 2H, H-2,6), 6.78 (br s, 1H, $NHSO_2$), 6.25 (br s, 2H, NH), 4.81 (br s, 1H, CHNH), 4.53 (br s, 2H, CH_2NH), 3.01 (s, 3H, SO_2CH_3), 1.92 (m, 1H, $CHMe_2$), 1.30 (s, 9H, $C(CH_3)_3$), 0.77 (m, 6H, $2 \times CH_3$); MS (FAB) m/z 466 (MH^+); Anal. calcd for $C_{23}H_{32}FN_3O_2S_2$: C, 59.32; H, 6.93; N, 9.02; S, 13.77. Found: C, 59.54; H, 6.95; N, 9.00; S, 13.74.

5.1.24. *N*-(4-*tert*-Butylbenzyl)-*N*′-{1-[4-(methylsulfonylamino)-3-fluorophenyl]-2-phenylethyl}thiourea (33). 93% yield; white solid; mp = 116 °C; ^{1}H NMR ($CDCl_3$) δ 7.43 (t, 1H, J = 8.04 Hz, H-5), 7.33 (d, 2H, J = 8.04 Hz), 7.2–7.3 (m, 5H, Ph), 7.06 (d, 2H, J = 8.04 Hz), 6.9–7.0 (m, 2H, H-2,6), 6.63 (br s, 1H, $NHSO_2$), 6.11 (br s, 1H, NH), 5.45 (br s, 1H, CHNH), 4.43 (br s, 2H, CH_2NH), 3.06 (d, 2H, J = 5.6 Hz, CH_2Ph), 3.00 (s, 3H, SO_2CH_3), 1.31 (s, 9H, $C(CH_3)_3$); MS (FAB) m/z 514 (MH^+); Anal. calcd for $C_{27}H_{32}FN_3O_2S_2$: C, 63.13; H, 6.28; N, 8.18; S, 12.48. Found: C, 63.34; H, 6.30; N, 8.16; S, 12.45.

5.1.25. *N*-(4-*tert*-Butylbenzyl)-*N*′-{[4-(methylsulfonylamino)-3-fluorophenyl](phenyl)methyl}thiourea (34). 92% yield; white solid, mp = 191 °C; ^{1}H NMR ($CDCl_3$) δ 7.50 (t, 1H, J = 8.55 Hz, H-5), 7.25–7.4 (m, 7 H), 7.13 (d, 2H, J = 8.04 Hz), 6.9–7.0 (m, 2H, H-2,6), 6.51 (br s, 1H, $NHSO_2$), 6.30 (br s, 1H, NH), 6.23 (br s, 1H, CHNH), 4.58 (br s, 2H, CH_2NH), 3.02 (s, 3H, SO_2CH_3), 1.31 (s, 9H, $C(CH_3)_3$); MS (FAB) m/z 500 (MH^+); Anal. calcd for $C_{26}H_{30}FN_3O_2S_2$: C, 62.50; H, 6.05; N, 8.41; S, 12.83. Found: C, 62.71; H, 6.07; N, 8.38; S, 12.80.

5.1.26. General procedure for the synthesis of acids (44–49). A suspension of nitro compounds (5 mmol) and 10% palladium on carbon (150 mg) in MeOH (25 mL) was treated with concentrated hydrochloric acid (10 drops) and was hydrogenated under a balloon of hydrogen for 6 h. The reaction mixture was neutralized with solid $NaHCO_3$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography on silica gel using CH_2Cl_2/MeOH as eluant. A cooled solution of amine (10 mmol) in pyridine (10 mL) at 0 °C was treated with methanesulfonyl chloride (15 mmol) and stirred at room temperature for 2 h. The reaction mixture was diluted with H_2O and extracted with EtOAc several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash

column chromatography on silica gel using EtOAc/hexanes. A solution of ester (121 mg, 0.25 mmol) in THF (2 mL) was treated with LiOH (31 mg, 0.75 mmol) in H_2O (1 mL) and stirred for 48 h. The mixture was diluted and neutralized by adding 1 N HCl and concentrated. The residue was purified by column chromatography on silica with CH_2Cl_2/MeOH as eluant.

5.1.27. 2-[4-(methylsulfonylamino)phenyl]-2-methylpropionic acid (44). 92% yield; yellow solid; mp = 148–151 °C; ^{1}H NMR ($CDCl_3$) δ 7.39 (br d, 2H, Ar), 7.19 (br d, 2H, Ar), 6.44 (br s, 1H, $NHSO_2$), 3.02 (s, 3H, SO_2CH_3), 1.60 (s, 6H, $2 \times CH_3$).

5.1.28. 1-[4-(Methylsulfonylamino)phenyl]cyclopropanecarboxylic acid (45). 98% yield; yellow solid; mp = 220–224 °C; ^{1}H NMR (DMSO-d_6) δ 9.69 (br s, 1H, CO_2H), 7.26 (br d, 2H), 7.10 (br d, 2H), 2.96 (s, 3H, SO_2CH_3), 1.41 (dd, 2H, CH_2CCH_2), 1.08 (dd, 2H, CH_2CCH_2).

5.1.29. 2-[3-Fluoro-4-(methylsulfonylamino)phenyl]-2-methylpropionic acid (46). 88% yield; white solid; mp = 152–153 °C; ^{1}H NMR ($CDCl_3$) δ 7.53 (t, 1H, J = 8.3 Hz, H-5), 7.18–7.25 (m, 2H), 6.59 (br s, 1H, $NHSO_2$), 3.04 (s, 1H, SO_2CH_3), 1.59 (s, 6H, $2 \times CH_3$).

5.1.30. 2-(3-Methoxy-4-(methylsulfonylamino)phenyl)-propionic acid (47). 98% yield; white solid; mp = 98–99 °C; ^{1}H NMR ($CDCl_3$) δ 7.45 (d, 1H, J = 8.3 Hz, H-5), 6.90 (dd, 1H, J = 8.3 & 1.7 Hz, H-6), 6.85 (d, 1H, J = 1.7 Hz, H-2), 6.74 (br s, 1H, $NHSO_2$), 3.87 (s, 3H, OCH_3), 3.70 (q, 1H, J = 7.1 Hz, C*H*CH_3), 2.93 (s, 1H, SO_2CH_3), 1.49 (d, 1H, J = 7.1 Hz, CHC*H*$_3$).

5.1.31. 2-(3-Methoxy-4-(methylsulfonylamino)phenyl)-2-methylpropionic acid (48). 89% yield; white solid; mp = 122–124 °C; ^{1}H NMR ($CDCl_3$) δ 7.47 (d, 1H, J = 8.3 Hz, H-5), 7.00 (dd, 1H, J = 1.8, 8.3 Hz, H-6), 6.94 (d, 1H, J = 1.8 Hz, H-2), 6.78 (br s, 1H, $NHSO_2$), 3.88 (s, 3H, OCH_3), 2.96 (s, 3H, SO_2CH_3), 1.60 (s, 6H, $2 \times CH_3$).

5.1.32. 1-[3-Methoxy-4-(methylsulfonylamino)phenyl]-cyclopropanecarboxylic acid (49). 96% yield; white solid; mp = 192–193 °C; ^{1}H NMR ($CDCl_3$) δ 7.44 (d, 1H, J = 8.1 Hz H-5), 7.6.94 (dd, 1H, J = 1.8, 8.1 Hz, H-6), 6.91 (d, 1H, J = 1.8 Hz, H-2), 6.77 (br s, 1H, $NHSO_2$), 3.88 (s, 3H, OCH_3), 2.95 (s, 3H, SO_2CH_3), 1.68 (dd, 2H, CH_2CCH_2), 1.26 (dd, 2H, CH_2CCH_2).

5.1.33. General procedure for coupling reaction to thiourea (56–61). A solution of acid (1 mmol) in toluene (6 mL) was treated with 4 A molecular sieve (200 mg), triethylamine (1.3 mmol), and diphenylphosphoryl azide (1.3 mmol), and heated at 110 °C for 1 h. The mixture was cooled to room temperature and BnOH (20 mmol) was added. After the mixture was heated to 110 °C for 12 h and concentrated in vacuo, the residue was purified by column chromatography on silica gel with EtOAc/hexanes as eluant. A suspension of carbamate (0.5 mmol) and 5% palladium on carbon (100 mg) in MeOH (10 mL) was hydrogenated under a rubber balloon of hydrogen for 1 h. After the solvent was evaporated by rotary evaporator, the residue was dissolved in DMF (5 mL) and treated with 4-*tert*-butylbenzyl isothiocyanate (0.5 mmol). After being stirred overnight, the mixture went to aqueous work-up and the residue was purified by column chromatography on silica gel with EtOAc/hexanes as eluant.

5.1.34. *N*-(4-*tert*-Butylbenzyl)-*N*′-{1-methyl-1-[4-(methylsulfonylamino)phenyl]ethyl}thiourea (56). 94% yield; white solid; mp = 161–164 °C; ^{1}H NMR ($CDCl_3$) δ 7.42 (d, 2H, Ar), 7.22 (dd, 4H, Ar), 6.83 (br s, 1H, $NHSO_2$), 6.80 (d, 2H, Ar), 6.63 (br s, 1H, NH), 5.23 (bt, 1H, NH), 4.58 (d, 2H, J = 4.9 Hz, $ArCH_2NH$), 2.97 (s, 3H, SO_2CH_3), 1.65 (s, 6H, $2 \times CH_3$), 1.28 (s, 9H, $C(CH_3)_3$); MS (FAB) *m*/*z* 434 (MH^+); Anal. calcd for $C_{22}H_{31}N_3O_2S_2$: C, 60.94; H, 7.21; N, 9.69; S, 14.79. Found: C, 61.10; H, 7.23; N, 9.66; S, 14.76.

5.1.35. *N*-(4-*tert*-Butylbenzyl)-*N*′-{1-[4-(methylsulfonylamino)phenyl]cyclopropyl}thiourea (57). 78% yield; white solid; mp = 110–113 °C; ^{1}H NMR ($CDCl_3$) δ 7.33 (d, 2H, Ar), 7.17 (m, 4H, Ar), 7.05 (d, 2H, Ar), 4.58 (m, 2H, $ArCH_2NH$), 3.01 (s, 3H, SO_2CH_3), 1.7–1.9 (m, 2H, CCH_2CH_2C), 0.85 (t, 2H, J = 7.5 Hz, CCH_2CH_2C); MS (EI) *m*/*z* 433 (M^++2); Anal. calcd for $C_{22}H_{29}N_3O_2S_2$: C, 61.22; H, 6.77; N, 9.74; S, 14.86. Found: C, 61.49; H, 6.79; N, 9.72; S, 14.82.

5.1.36. *N*-(4-*tert*-Butylbenzyl)-*N*′-{1-methyl-1-[3-fluoro-4-(methylsulfonylamino)phenyl]ethyl}thiourea (58). 80% yield; white solid; mp = 83–85 °C; ^{1}H NMR ($CDCl_3$) δ 7.52 (t, 1H, J = 8.2 Hz, H-5), 7.18–7.3 (m, 4H, Ar), 6.86 (d, 2H, J = 7.9 Hz, Ar), 6.50 (br s, 1H, $NHSO_2$), 5.20 (br s, 1H, NH), 4.59 (d, 2H, J = 4.8 Hz, $ArCH_2NH$), 2.98 (s, 3H, SO_2CH_3), 1.65 (s, 6H, CH_3CCH_3), 1.29 (s, 9H, $C(CH_3)_3$); MS *m*/*z* 486 (MNa^+); Anal. calcd for $C_{22}H_{30}FN_3O_2S_2$: C, 58.51; H, 6.70; N, 9.30; S, 14.20. Found: C, 58.78; H, 6.72; N, 9.27; S, 14.16.

5.1.37. *N*-(4-*tert*-Butylbenzyl)-*N*′-{1-[3-methoxy-4-(methylsulfonylamino)phenyl]ethyl}thiourea (59). 91% yield; white solid; mp = 80–82 °C; ^{1}H NMR ($CDCl_3$) δ 7.46 (d, 1H, J = 8.04 Hz, H-5), 7.31 (d, 2H, Ar), 7.03 (d, 2H, Ar), 6.75–6.85 (m, 3H, Ar and $NHSO_2$), 6.14 (br s, 2H, NH), 5.80 (br s, 2H, NH), 4.93 (br s, 1H, NHC*H*Me), 4.58 (dd d of AB, 2H, CH_2NH), 3.83 (s, 3H, OCH_3), 2.94 (s, 3H, SO_2CH_3), 1.49 (d, 3H, J = 6.6 Hz, $CHCH_3$), 1.30 (s, 9H, $C(CH_3)_3$); MS (FAB) *m*/*z* 450 (MH^+); Anal. calcd for $C_{22}H_{31}N_3O_3S_2$: C, 58.77; H, 6.95; N, 9.35; S, 14.26. Found: C, 58.99; H, 6.97; N, 9.32; S, 14.22.

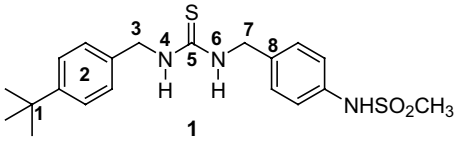
5.1.38. *N*-(4-*tert*-Butylbenzyl)-*N*′-{1-methyl-1-[3-methoxy-4-(methylsulfonylamino)phenyl]ethyl}thiourea (60). 69% yield; white solid; mp = 148–150 °C; ^{1}H NMR ($CDCl_3$) δ 7.47 (d, 1H, J = 8.2 Hz, H-5), 7.23 (d, 1H, Ar), 6.94–7.0 (m, 2H), 6.80 (d, 3H, Ar and $NHSO_2$), 6.50 (br s, 1H, NH), 5.31 (t, 1H, NH), 4.57 (d, 2H, J = 5.1 Hz, $ArCH_2NH$), 3.77 (s, 3H, OCH_3), 2.89 (s, 3H, SO_2CH_3), 1.65 (s, 6H, CH_3CCH_3), 1.29 (s, 9H, $C(CH_3)_3$); MS (FAB) *m*/*z* 464 (MH^+); Anal. calcd for

$C_{23}H_{33}N_3O_3S_2$: C, 59.58; H, 7.17; N, 9.06; S, 13.83. Found: C, 59.88; H, 7.15; N, 9.03 S, 13.80.

5.1.39. *N*-(4-*tert*-Butylbenzyl)-*N*′-{1-[3-methoxy-4-(methylsulfonylamino)phenyl]cyclopropyl}thiourea (61). 86% yield; white solid; mp = 100–103 °C; ^{1}H NMR ($CDCl_3$) δ 7.46 (d, 1H, Ar), 7.31 (d, 1H, Ar), 7.02 (d, 2H, Ar), 6.7–6.85 (m, 3H, Ar),6.20 (br s, 1H, NH), 5.78 (br s, 1H, NH), 4.58 (dd d, 2H, $ArCH_2NH$), 3.83 (s, 3H, OCH_3), 2.94 (s, 3H, SO_2CH_3), 1.7–1.9 (m, 2H, CCH_2CH_2C), 1.30 (s, 9H, $C(CH_3)_3$), 0.88 (t, 2H, J = 7.5 Hz, CCH_2CH_2C); MS (EI) *m/z* 463 (M^++2); Anal. calcd for $C_{23}H_{31}N_3O_3S_2$: C, 59.84; H, 6.77; N, 9.10; S, 13.89. Found: C, 60.05; H, 6.79; N, 9.07; S, 13.85.

5.2. Molecular modeling

The structures of tested compounds were built with Concord and energy minimized using the MMFF94s force field (method: powell, termination: gradient 0.05 kcal/mol Å, and max iterations: 1,000,000) implemented in the SYBYL molecular modeling program (Tripos, Inc.). The conformational analyses of thioureas (for example, S=C_5–N_4–H, S=C_5–N_6–H, and C_5–N_6–C_7–C_8 in compound **1** below) were performed using the SYBYL Grid Search method (force field: MMFF94s, charges: MMFF94). In the analyses, 48 unique conformations of each molecule were found, and the lowest energy conformation was chosen and solvated with water (shape of the cluster: box; radii type: Tripos; and number of solvent layers: 20). The solvated conformers were energy minimized using the MMFF94s force field as described above. Then, the resulting lowest energy conformers were overlaid using the fit atoms function in SYBYL. All computational studies were performed with Tripos SYBYL molecular modeling program package, version 7.3, on a Linux (RHEL 4.0 Intel Xeon processor 5050) workstation.



1

Acknowledgments

This work was supported by Grant R01-2004-000-10132-0 from the Basic Research Program of the Korea Science & Engineering Foundation and by Grant No. R15-2006-020 from the National Core Research Center (NCRC) program of the Ministry of Science & Technology (MOST) through the Center for Cell Signaling & Drug Discovery Research at Ewha Womans University. This work was also supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. We thank Richard Tran and Matthew A. Morgan for technical assistance.

References and notes

1. Szallasi, A.; Blumberg, P. M. *Pharmacol. Rev.* **1999**, *51*, 159.
2. Montell, C.; Birnbaumer, L.; Flockerzi, V.; Bindels, R. J.; Bruford, E. A.; Caterina, M. J.; Clapham, D. E.; Harteneck, C.; Heller, S.; Julius, D.; Kojima, I.; Mori, Y.; Penner, R.; Prawitt, D.; Scharenberg, A. M.; Schultz, G.; Shimizu, N.; Zhu, M. X. *Mol. Cell* **2002**, *9*, 229.
3. Tominaga, M.; Caterina, M. J.; Malmberg, A. B.; Rosen, T. A.; Gilbert, H.; Skinner, K.; Raumann, B. E.; Basbaum, A. I.; Julius, D. *Neuron* **1998**, *21*, 531.
4. Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. *Nature* **1997**, *389*, 816.
5. Zygmunt, P. M.; Petersson, J.; Andersson, D. A.; Chuang, H.-H.; Sorgard, M.; Di Marzo, V.; Julius, D.; Hogestatt, E. D. *Nature* **1999**, *400*, 452.
6. Hwang, S. W.; Cho, H.; Kwak, J.; Lee, S. Y.; Kang, C. J.; Jung, J.; Cho, S.; Min, K. H.; Suh, Y. G.; Kim, D.; Oh, U. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6155.
7. Walpole, C. S. J.; Wrigglesworth, R. *Capsaicin in the Study of Pain*; Academic Press: San Diego, CA, 1993, pp. 63.
8. Appendino, G.; Szallasi, A. *Life Sci.* **1997**, *60*, 681.
9. Breitenbucher, J. G.; Chaplan, S. R.; Carruthers, N. I. *Ann. Rep. Med. Chem.* **2005**, *40*, 185.
10. Wang, Y.; Szabo, T.; Welter, J. D.; Toth, A.; Tran, R.; Lee, J.; Kang, S. U.; Lee, Y.-S.; Min, K. H.; Suh, Y.-G.; Park, M.-K.; Park, H.-G.; Park, Y.-H.; Kim, H.-D.; Oh, U.; Blumberg, P. M.; Lee, J. *Mol. Pharm.* **2002**, *62*, 947, published erratum appears in *Mol. Pharmacol*. **2003**, 63, 958.
11. Lee, J.; Lee, J.; Kang, M.; Shin, M.-Y.; Kim, J.-M.; Kang, S.-U.; Lim, J.-O.; Choi, H.-K.; Suh, Y.-G.; Park, H.-G.; Oh, U.; Kim, H.-D.; Park, Y.-H.; Ha, H.-J.; Kim, Y.-H.; Toth, A.; Wang, Y.; Tran, R.; Pearce, L. V.; Lundberg, D. J.; Blumberg, P. M. *J. Med. Chem.* **2003**, *46*, 3116.
12. Suh, Y.-G.; Lee, Y.-S.; Min, K.-H.; Park, O.-H.; Kim, J.-K.; Seung, H.-S.; Seo, S.-Y.; Lee, B.-Y.; Nam, Y.-H.; Lee, K.-O.; Kim, H.-D.; Park, H.-G.; Lee, J.; Oh, U.; Ju-Ok, Lim; Kang, S.-U.; Kil, M.-J.; Koo, J.-Y.; Shin, S. S.; Joo, Y.-H.; Kim, J. K.; Jeong, Y.-S.; Kim, S.-Y.; Park, Y.-H. *J. Med. Chem.* **2005**, *48*, 5823.
13. Wang, Y.; Toth, A.; Tran, R.; Szabo, T.; Welter, J. D.; Blumberg, P. M.; Lee, J.; Kang, S.-U.; Lim, J.-O.; Lee, J. *Mol. Pharm.* **2003**, *64*, 325.
14. Borg, G. B.; Cogan, D. A.; Ellman, J. A. *Tetrahedron Lett.* **1999**, *40*, 6709.
15. Lee, J.; Kang, S.-U.; Choi, H.-K.; Lee, J.; Lim, J.-O.; Kil, M.-J.; Jin, M.-K.; Kim, K.-P.; Sung, J.-H.; Chung, S.-J.; Ha, H-J.; Kim, Y.-H.; Pearce, L. V.; Tran, R.; Lundberg, D. J.; Wang, Y.; Toth, A.; Blumberg, P. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2291.
16. Lee, J.; Kang, S.-U.; Kil, M.-J.; Shin, M.; Lim, J.-O.; Choi, H.-K.; Jin, M.-K.; Kim, S. Y.; Kim, S.-E.; Lee, Y.-S.; Min, K.-H.; Kim, Y.-H.; Ha, H.-J.; Tran, R.; Welter, J. D.; Wang, Y.; Szabo, T.; Pearce, L. V.; Lundberg, D. J.; Toth, A.; Pavlyukovets, V. A.; Morgan, M. A.; Blumberg, P. M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4136.
17. Ryu, C. H.; Jang, M. J.; Jung, J. W.; Park, J-H.; Choi, H. Y.; Suh, Y.-G.; Oh, U.; Park, H.-G.; Lee, J.; Koh, H.-J.; Mo, J.-H.; Joo, Y. H.; Park, Y-H.; Kim, H.-D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1751.