# *N*-(3-Acyloxy-2-benzylpropyl)-*N*′-[4-(methylsulfonylamino)benzyl]thiourea Analogues: Novel Potent and High Affinity Antagonists and Partial Antagonists of the Vanilloid Receptor

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Isosteric replacement of the phenolic hydroxyl group in potent vanilloid receptor (VR1) agonists with the alkylsulfonamido group provides a series of compounds which are effective antagonists to the action of the capsaicin on rat VR1 heterologously expressed in Chinese hamster ovary (CHO) cells. In particular, compound **61**, *N*-[2-(3,4-dimethylbenzyl)-3-pivaloyloxypropyl]-*N*′-[3-fluoro-4-(methylsulfonylamino)benzyl]thiourea was a full antagonist against capsaicin, displayed a $K_i$ value of 7.8 nM (compared to 520 nM for capsazepine and 4 nM for 5-iodoRTX), and showed excellent analgesic activity in mice. Structure−activity analysis of the influence of modifications in the A- and C-regions of 4-methylsulfonamide ligands on VR1 agonism/antagonism indicated that 3-fluoro substitution in the A-region and a 4-*tert*-butylbenzyl moiety in the C-region favored antagonism, whereas a 3-methoxy group in the A-region and 3-acyloxy-2-benzylpropyl moieties in the C-region favored agonism.

## Introduction

The vanilloid receptor VR1[1] is a cation permeable ion channel present on polymodal nociceptors that is activated by protons, heat,[2] and ligands such as capsaicin (CAP),[3] the pungent ingredient in chilli peppers, and resiniferatoxin (RTX),[4] a natural diterpene isolated from the cactuslike succulent *Euphobia resinifera*. VR1 has been cloned from rat dorsal root ganglia (DRG)[5] and more recently from the human.[6] It is a member of the Trp family of channel proteins,[7] oligomerizing as a tetramer[8] and functioning as a relatively nonselective cation channel with some preference for calcium ions.[9] The vanilloid receptor is expressed predominantly on unmyelinated pain-sensing nerve fibers (C-fibers) and small A$\delta$ fibers in the dorsal root, trigeminal, and nodose ganglia. The activation of the vanilloid receptor by pungent agonists triggers cation influx resulting in excitation of primary sensory neurons, action potentials, and ultimately the central perception of pain. Following excitation, the C-fiber sensory neurons may become desensitized, and this desensitization forms a basis for the therapeutic use of vanilloid receptor agonists.[1,10] Potential therapeutic applications include chronic pain, such as that associated with diabetic neuropathy, postherpetic neuralgia, arthritis and cluster headache, urologic problems such as detrusor hyperreflexia and bladder hypersensitivity, and pruritus.[1]

A strategy complementary to desensitization of C-fiber sensory neurons by VR1 agonists is the blocking of pain-producing endogenous agonists by VR1 antagonists. Proof of principle for this approach has been provided with capsazepine and with some *N*-alkylglycine trimers.[11,12] Because of the long duration of desensitization following treatment with VR1 agonists, antagonists may be of particular utility where short-term blockade of VR1 is desired. Whereas several agonists are currently marketed (e.g., capsaicin) or undergoing clinical trial (e.g., resiniferatoxin,[13] DA-5018,[14] SDZ-249665[15]), only a few VR1 antagonists have been described so far. Capsazepine,[16] the most extensively characterized, has only modest potency and is somewhat nonspecific, also antagonizing voltage-sensitive calcium channels and the nicotinic cholinergic receptor.[17,18] The *N*-alkylglycine trimers appear to function as noncompetitive antagonists, presumably as channel blockers, with potencies around 1 $\mu$M.[12] Iodo-RTX, prepared semisynthetically from RTX by iodination, was also reported to display potent antagonism in rat and human VR1.[19]

The identification of RTX as a novel vanilloid with a binding potency approximately 4 orders of magnitude greater than that of capsaicin (RTX: $K_i$ = 0.13 nM, CAP: $K_i$ = 1,700 nM in CHO/VR1)[20] suggested a more complex pharmacophore for vanilloids than that appreciated previously. In the basis of part on previously published SAR studies on RTX,[21] we have proposed a hypothetical pharmacophore model for interaction with the capsaicin binding site of VR1 in which four func-

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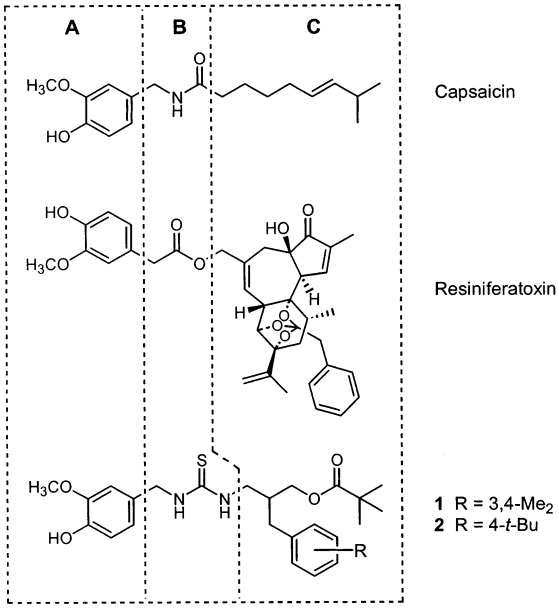



**Figure 1.**

tional groups, 4-hydroxy-3-methoxyphenyl, $C_{20}$-ester, $C_3$-keto, and orthophenyl, represent principal pharmacophores. On the basis of this model, we have synthesized and recently described the ultrapotent VR1 agonists **1** and **2**, with $K_i$ values of 19 nM and 11 nM, respectively, in a [$^3$H]RTX binding assay on DRG neurons; the compounds are thus approximately 280- and 480-fold more potent than capsaicin, respectively, for receptor binding.[22] The Novartis group[3,24] has designated the structural regions of vanilloids as regions A−C shown in Figure 1, and our current work involves replacement of the isodecenoyl moiety of the C-region with a 3-acyloxy-2-benzylpropyl moiety, which we designed based on our RTX-derived pharmacophore model and which represents a motif conferring significantly enhanced binding affinity in RTX competition for VR1 compared to capsaicin.

The series of compounds described here arise out of our ongoing program to find potent VR1 agonists and antagonists. This series is based on the structural modification on the A-region of the potent agonists, *N*-(3-acyloxy-2-benzylpropyl)-*N*′-(4-hydroxy-3-methoxybenzyl)-thioureas, which we had previously shown to have high affinities for rat VR1.[22] Extensive structure activity studies on the capsaicinoids have indicated that the 4-phenolic hydroxyl group represents a crucial pharmacophore although it also contributes to the metabolic lability in vivo.[23,24] We reasoned that isosteric replacement of the pungent phenolic OH group in our potent agonists might maintain their strong affinities, change their metabolic lability, and modify the biological characteristic of the ligands. The alkylsulfonylamido group has been utilized as a bioisostere of the phenolic OH because the acid dissociation constant of the NH group in alkylsulfonamides is close to that of the phenolic OH group (both are approximately p$K_a$ = 9). Its effectiveness as a bioisostere implies that the NH portion of the alkylsulfonamide hydrogen bonds with a receptor site by aligning itself in a manner closely approximating the phenolic OH group with respect to both bond distances and bond angles.[25] Consequently, the isosteric replacement of the phenolic OH group in our potent agonists with the alkylsulfonamido group led to a series of potent antagonists with high binding affinities. We have previously reported a different strategy for design of antagonists in which we incorporated our potent agonist templates with bicycles such as tetrahydrobenzazepine or tetrahydroisoquinoline as an A-region derived from capsazepine.[16] This approach afforded several novel antagonists with potencies similar to or modestly better than capsazepine; however, further modification of the structures was problematic because their agonistic/antagonistic character was very sensitive to small modifications.[26]

In the present study, we have synthesized and characterized in detail, using CHO cells heterologously expressing rat VR1, a series of the potent and selective VR1 antagonists having a *N*-4-alkylsulfonylaminobenzyl thiourea template. We have also analyzed SAR on the effect of A and C-regions in 4-alkylsulfonylamino ligands and describe significant substitution aspects on agonism/antagonism.

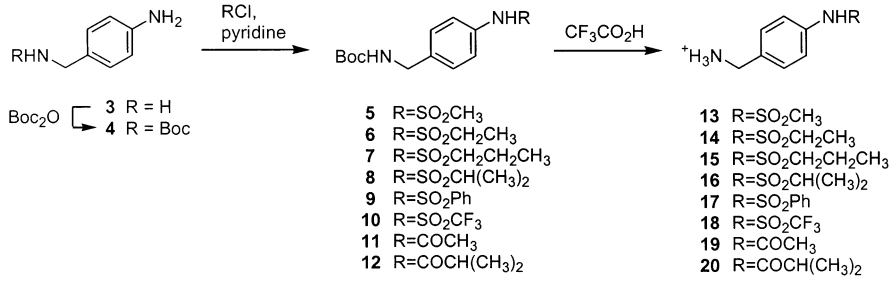
## Chemistry

The syntheses of target thioureas were achieved by the general coupling methods between the corresponding isothiocyanates and amines.[22] The syntheses of benzylamines with a different A-region were described in Schemes1−3. 4-Alkylsulfonylamino and 4-acylaminobenzylamines (**13**−**20**) were prepared from 4-aminobenzylamine (**3**) in three steps in good yields as shown in Scheme 1. 3-Methylsulfonylamino (**24**) and 4-(methylsulfonylamino)methylbenzylamines (**28**) were also prepared from the corresponding aminobenzyl alcohols (**21**, **25**) in a conventional manner as shown in Scheme 2. For the synthesis of 3-methoxy-4-(methylsulfonylamino)-benzylamine as outlined in Scheme 3, 3-methoxy-4-nitrobenzyl alcohol (**29**) was converted to the corresponding N−Boc amine (**31**) in three steps. The nitro group of **31** was reduced and then mesylated to furnish **33** whose protecting group was hydrolyzed to afford amine **34**. The synthesis of the 3-fluoro-4-(methylsulfonylamino)benzylamine (**35**) was described in a previous report.[27] For the synthesis of sulfonamide thiourea analogues, 2-(3,4-dimethylbenzyl)-3-pivaloyloxypropyl isothiocyanate (**39**) was coupled with the amines (**13**−**20**, **24**, **28**) to afford thioureas (**40**−**49**) as shown in Scheme 5. For the synthesis of different A/C region analogues as outlined in Scheme 6, 3-acyloxy-2-benzylpropyl azides (**50**−**53**)[26] were reduced under the Lindler's catalyzed hydrogenation and then coupled with appropriate isothiocyanates (**36**−**38**), which were prepared from the corresponding amines (**13**, **34**, **35**) by the reaction of 1,1′-thiocarbonyldiimidazole as shown in Scheme 4 to afford the desired thioureas (**54**−**61**).

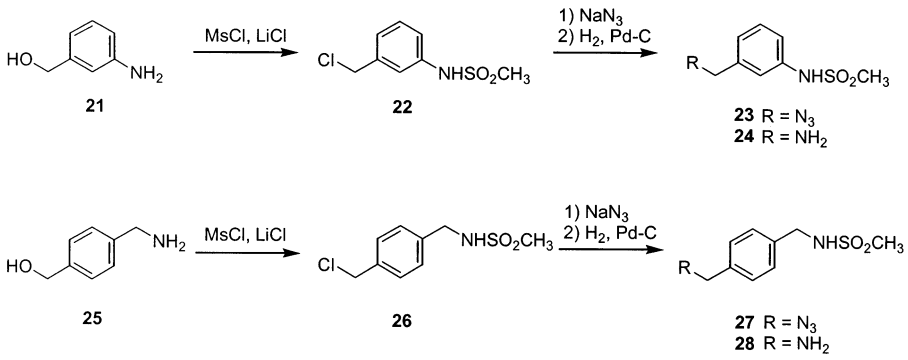
## Biological Results

The agonistic and antagonistic activities of the synthesized VR1 ligands were assessed in vitro by a $^{45}Ca^{2+}$ uptake assay, which was carried out using rat VR1 heterologously expressed in Chinese hamster ovary cells (CHO/VR1 cells) as previously described.[20,27,28] The in vitro antagonistic potencies of the compounds were evaluated by measuring antagonism of the $^{45}Ca^{2+}$ uptake induced by 50 nM capsaicin and expressed as the $K_i \pm$ SEM, respectively, correcting for competition

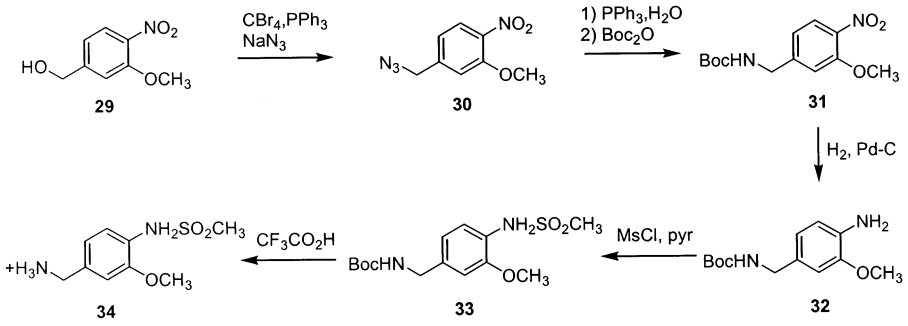
**Scheme 1**



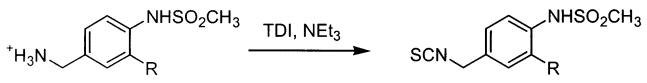

**Scheme 2**




**Scheme 3**




**Scheme 4**

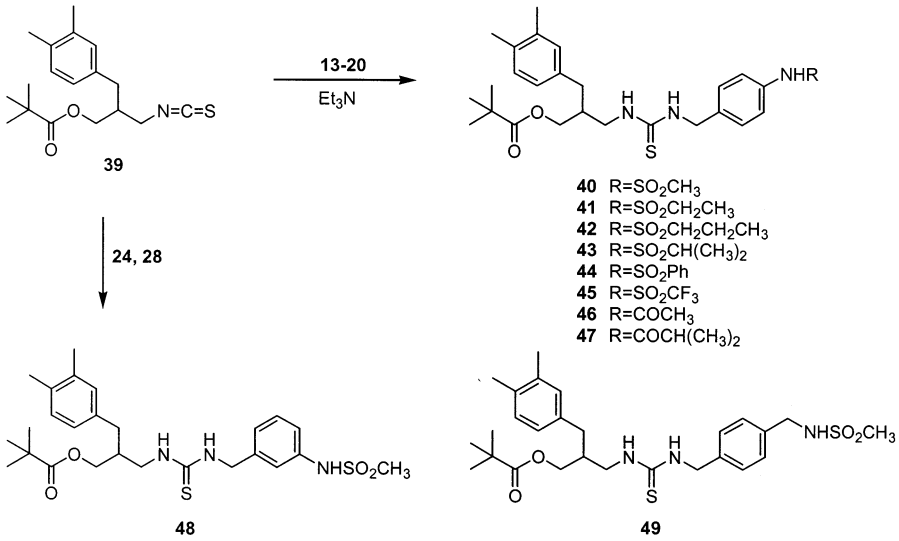



by capsaicin. All compounds were also evaluated as agonists. Potencies as agonists were expressed as $EC_{50}$ ± SEM, and absolute levels of $^{45}Ca^{2+}$ uptake were compared with that induced by a maximally effective concentration of capsaicin in this system, namely 300 nM. Receptor binding affinities were assessed in terms of the ability of the compounds to compete for specific binding of [$^3$H]RTX in the CHO/VR1 system and were expressed as the $K_i$ ± SEM. All values represent the mean of at least three experiments. The results are summarized in Table 1.

Replacement of the 4-phenolic OH group in our previously reported high affinity and potent agonist **1**[22] with a 4-methylsulfonamido group provided a potent antagonist **40** which inhibited the $^{45}Ca^{2+}$ uptake induced by capsaicin with a $K_i$ of 67 nM. The antagonistic potency of **40** was thus approximately 10-fold better than that of capsazepine ($K_i$ = 520 nM). Evaluated as an agonist, compound **40** proved not to be a true, full antagonist but rather retained weak efficacy as an agonist, yielding about 7% of the $^{45}Ca^{2+}$ uptake of a maximally effective dose of capsaicin (Table 1). The extent of the partial agonism depended on assay conditions. The detailed characterization of this weak partial agonism and its modulation by VR1 coregulators is presented elsewhere.[28] The receptor binding affinity of **40** yielded a $K_i$ value of 29.3 nM, which was significantly improved compared to capsazepine ($K_i$ = 1300 nM) and was almost unchanged compared to that of the corresponding agonist, **1**. Compound **40** established that replacement of the 4-hydroxyl with a 4-methylsulfonamido group in a vanilloid can change the biological behavior of the ligand from agonist to antagonist while preserving binding affinity.

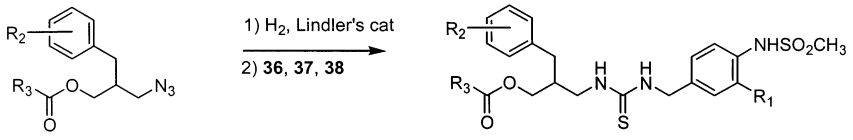
To determine the optimal size of the alkyl group of the 4-alkylsulfonamide, derivatives with alkyl groups of ethyl, propyl, isopropyl, and phenyl (**41**-**44**) were prepared. Their binding affinities and antagonistic potencies gradually decreased with increasing size although they showed partial agonism as did **40**. Indeed, for the 4-phenylsulfonylamino analogue (**44**), the antagonistic activity was completely abolished and the binding affinity was found to be weak. These results

**Scheme 5**




**40** R=$SO_2CH_3$
**41** R=$SO_2CH_2CH_3$
**42** R=$SO_2CH_2CH_2CH_3$
**43** R=$SO_2CH(CH_3)_2$
**44** R=$SO_2Ph$
**45** R=$SO_2CF_3$
**46** R=$COCH_3$
**47** R=$COCH(CH_3)_2$

**Scheme 6**




**50** $R_2$=4-t-Bu $R_3$=t-Bu
**51** $R_2$=4-t-Bu $R_3$=Ph
**52** $R_2$=3,4-$Me_2$ $R_3$=t-Bu
**53** $R_2$=3,4-$Me_2$ $R_3$=Ph

**54** $R_1$=H $R_2$=4-*t*-Bu $R_3$=*t*-Bu
**55** $R_1$=H $R_2$=3,4-$Me_2$ $R_3$=Ph
**56** $R_1$=H $R_2$=4-*t*-Bu $R_3$=Ph
**57** $R_1$=$OCH_3$ $R_2$=3,4-$Me_2$ $R_3$=*t*-Bu
**58** $R_1$=$OCH_3$ $R_2$=4-*t*-Bu $R_3$=*t*-Bu
**59** $R_1$=$OCH_3$ $R_2$=3,4-$Me_2$ $R_3$=Ph
**60** $R_1$=$OCH_3$ $R_2$=4-*t*-Bu $R_3$=Ph
**61** $R_1$=F $R_2$=3,4-$Me_2$ $R_3$=*t*-Bu

**Table 1.** Potencies of Vanilloid Ligands for Binding to Rat VR1 and for Inducing Calcium Influx in CHO/VR1 Cells

| | $K_i$ (nM) binding affinity | $EC_{50}$ (nM) agonism | $K_i$(nM) antagonism |
|---|---|---|---|
| capsaicin | 1800 (±270) | 44.8 (±3.8) | NE |
| capsazepine | 1300 (±150) | NE | 520 (±12) |
| **1** | 17.4 (±4.1) | 1.97 (±0.56) | NE[a] |
| **2** | 6.35 (±0.48) | 2.83 (±0.55) | NE |
| **40** | 29.3 (±7.6) | WE[b] | 67 (±25) |
| **41** | 108 (±11) | WE[b] | 309 (± 91) |
| **42** | 313 (±52) | WE[b] | 940 (±130) |
| **43** | 227 (±40) | 790 (±210)[c] | WE |
| **44** | 2500 (±660) | VWE | NE |
| **45** | 15.5% at 10 $\mu$M | VWE | NE |
| **46** | 1700 (±500) | 450 (±140) | NE |
| **47** | 2600 (±780) | 1140 (±260) | NE |
| **48** | 355 (±61) | 990 (±79) | NE |
| **49** | 3000 (±980) | 1700 (±150) | NE |
| **54** | 64 (±21) | WE[b] | 86 (±17) |
| **55** | 410 (±110) | 1730 (±450)[c] | NE |
| **56** | 239 (±69) | 2790 (±860)[b] | NE |
| **57** | 49 (±15) | 22 (±10)[c] | NE |
| **58** | 25.2 (±6.9) | 40 (±11) | NE |
| **59** | 148 (±19) | 166 (±15) | NE |
| **60** | 77.2 (±7.5) | 252(±13) | NE |
| **61** | 54 (±28) | NE | 7.8 (±3.0) |

[a] NE: not effective, VWE: very weakly effective, WE: weakly effective at 30 $\mu$M. [b] Only fractional calcium uptake compared to 300 nM capsaicin (**38**, 7%; **39**, 14%; **40**, 15%; **52**, 30%). [c] Only fractional calcium uptake compared to 300 nM capsaicin (23−59%).

suggest that the capsaicin binding site of the receptor probably possesses a small pocket for the phenolic OH group and is subject to steric interference by bulky groups. The 4-trifluoromethylsulfonylamino analogue (**45**) exhibited little binding affinity to the receptor. In this case, the size of the alkyl group was almost the same as that for the methylsulfonylamino group, and the dramatic loss in receptor potency may be attributed to the markedly reduced p$K_a$ value of the NH group in $NHSO_2CF_3$ (calculated p$K_a$ = 9.47 for $NHSO_2CH_3$; p$K_a$ = 4.83 for $NHSO_2CF_3$ in a substituted benzene) due to a strong withdrawing effect of $CF_3$. As another isostere of the phenolic OH, the amide analogues (**46**, **47**) of the potent agonist, **1**, were also characterized. However, they were devoid of any antagonism in blocking the entry of calcium induced by capsaicin and proved to be pure agonists with moderate binding affinities. To discern the positional importance of the 4-methylsulfonylamino in **40** for antagonism, the corresponding 3-methylsulfonylamino analogue (**48**) and one-carbon elongated analogue (**49**) were examined; they proved to be full agonists with binding affinities similar to capsaicin. These results indicate that the binding and antagonistic potency of **40** is attenuated by the above structural and positional modifications on the 4-methylsulfonylamino group.

Starting with the lead antagonist **40**, we explored structural modifications in the C region and elsewhere in the A region. Substitution of the 3,4-dimethylphenyl on **40** with the 4-*tert*-butylphenyl to provide **54** ($K_i$ = 86 nM) resulted in a little decrease in the antagonistic potencies. Likewise, the binding affinity of **54** with a $K_i$ value of 64 nM was reduced by 2-fold relative to **40**. This

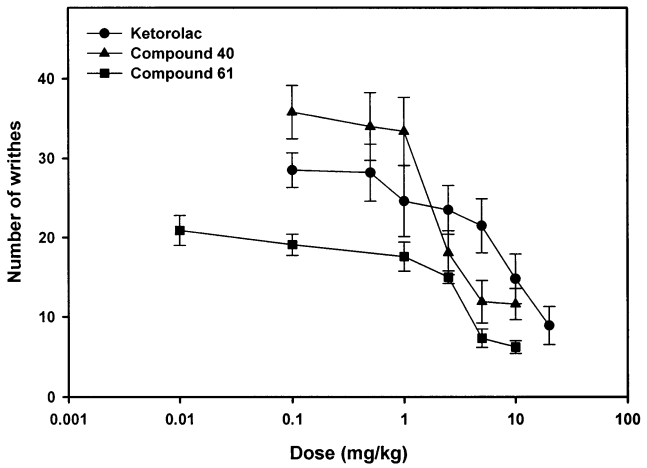



**Figure 2.** Comparison of the analgesic activities of compound **40** and **61** with ketorolac in the acetic acid-induced writhing model in mice.

finding is somewhat different from our previous observation[22] that a 4-*tert*-butylphenyl-substituted agonist, **2**, exhibited a higher affinity than the corresponding 3,4-dimethylphenyl-substituted derivative, **1,** as shown in Table 1. Like compound **40**, compound **54** showed partial agonism, 30% of the level of calcium uptake induced by 300 nM capsaicin. This difference of partial agonism is consistent with effects of C-region modifications on the pattern of antagonism for this template.

Incorporation of a 3-methoxy group on compounds **40** and **54** provided **57** and **58**, which were found to be agonists, without antagonistic activity, and with good potency. The $EC_{50}$ values were 22 nM and 40 nM, respectively. The binding affinities of **57** and **58**, with $K_i$ values of 49 nM and 25.2 nM, were 2-fold weaker and 3-fold stronger than those of **40** and **54**, respectively. A structural comparison between **40** and **57** (or **54** and **58**) reveals that the 3-methoxy group on the A-region plays an important role in eliciting VR1 agonistic activity. It is noteworthy that in the structure−activity studies of a series of capsaicinoids, a 3-methoxy group on the A-region proved to be a crucial pharmacophore in terms of agonism in the calcium uptake assay. For example, the 3-demethoxy analogue of *N*-octylhomovanillic amide was ca. 20-fold less potent than that of the parent compound.[3] Replacement of 3-pivaloyl groups of **40**, **54**, **57**, and **58** with 3-benzoyl groups, compounds **55**, **56**, **59** and **60**, respectively, led to a decrease in their binding affinities and, for the first two, a shift toward greater agonism. It should be noted that several of the compounds in this series, namely **55**, **56**, and **57**, induced a lower level of calcium uptake than did 300 nM capsaicin. This did not represent partial agonism, in that no corresponding activity was observed in assays for antagonism. The mechanistic basis for this behavior remains under investigation.

When, as a replacement of the 3-methoxy group, a 3-fluoro group was introduced to produce compound **61**, there was little change in its affinity as measured in the binding assay ($K_i$ of 54 nM compared to 49 nM for **57**). On the other hand, consistent with the important role of the 3-methoxy group for agonism, its replacement with a 3-fluoro group generated a potent antagonist, with a $K_i$ of 7.8 nM. **61** thus represents a significant improvement in antagonist potency compared to compound **40**. Furthermore, unlike **40**, it did not show residual weak efficacy as an agonist. We conclude that the 3-fluoro-4-methylsulfonylaminophenyl group is a promising antagonistic pharmacophore in the A-region.

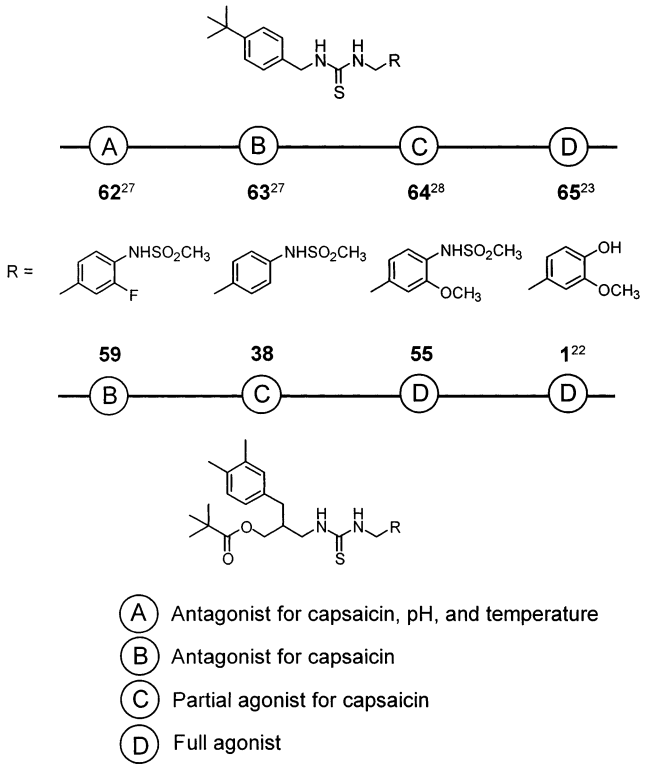



**Figure 3.** The SAR diagram of *N*-[4-(methylsulfonylamino)-benzyl]thiourea analogues.

The analgesic activities of potent antagonists (**40** and **61**) were evaluated in the acetic acid (AA)-induced writhing model in mice, following the previously reported procedure,[29] and were compared to that of the clinically used analgesic ketorolac (Figure 2). Whereas compound **40** showed a comparable potency ($ED_{50}$ = 2,620 ± 2380 $\mu$g/kg) relative to ketorolac ($ED_{50}$ = 2800 ± 1400 $\mu$g/kg), compound **61** exhibited dramatically enhanced analgesic potency, with an $ED_{50}$ of 7.43 ± 6.4 $\mu$g/kg that was approximately 350-fold more potent than that of compound **40**.

## Discussion

Recently, we have demonstrated detailed biochemical characterization of *N*-(4-*tert*-butylbenzyl)-*N′*-[4-(methylsulfonylamino)benzyl]thiourea, its 3-fluoro and 3-methoxy analogues as VR1 antagonists or partial agonists.[27,28] Structurally, they have the same A and B-regions relative to the VR1 ligands described above; however, their C-region contains a 4-*tert*-butylbenzyl group different from the 3-acyloxy-2-benzylpropyl moiety in above compounds. To investigate the effect on VR1 agonism/antagonism of 3-substituents in the A region and their modulation by the C-regions in 4-methylsulfonamide ligands, we compared the structure−activity relationships of two series of *N*-4-methylsulfonylamino thioureas. Their structures and biochemical properties as VR1 ligands are described in Figure 3. Their pattern of activity was consistent with our emerging understanding of vanilloid structure−activity relationships. Our findings can be summarized as follows:

(1) we have demonstrated that substitution of the 4-phenolic hydroxyl of the A-region in agonists with an 4-methylsulfonamido group shifted the ligands from agonism toward antagonism, and a 3-fluoro substitution further favored antagonism, whereas a 3-methoxy group favored agonism. (2) Although the A-region makes a major contribution to the extent of agonism/antagonism, it is clear that the C-region also contributes. Thus, the *N*-(4-*tert*-butylbenzyl) moiety (top series in Figure 3) tended to give more extensive antagonism than did the *N*-[2-(3,4-dimethylbenzyl)-3-(pivaloyloxy)propyl] moiety (bottom series in Figure 3).

In the 4-*tert*-butylbenzyl series (top series), the replacement of 4-hydroxy-3-methoxyphenyl in a potent and full agonist (**65**) previously reported by Wrigglesworth et al.[23] with 4-methylsulfonamide produced a full antagonist (**63**). Interestingly, compound **63** was a full antagonist of capsaicin stimulation but was only a partial antagonist for stimulation by temperature or pH.[27] The addition of a 3-fluoro substituent to the A-region in **63** further enhanced antagonism, as evidenced by compound **62** being a full antagonist for stimulation not only by capsaicin but also by temperature (44 °C) or pH 6.0.[27] It is noted that the 3-fluoro-4-hydroxyphenylacetamide analogue, generated by the replacement of 3-methoxy in **65** with 3-fluoro, was shown to have 10-fold less agonistic potency in calcium influx than the corresponding 3-methoxy analogue but was a weak partial agonist with ca. 40% efficacy.[3] Conversely, the incorporation of a 3-methoxy substituent to the A-region in **63** enhanced agonism, indicating that compound **64** proved to be a partial agonist like **40**.[28] Recently, we have reported in detail the biochemical characterization of **40** and compound **64** as partial agonists, including the demonstration that their fractional efficacy reflects their functioning as partial agonists with a corresponding degree of partial antagonism.[28]

In a similar manner, in the 2-(3,4-dimethylbenzyl)-3-(pivaloyloxy)propyl series (bottom series), in which **40** showed a low level of partial agonism, the corresponding derivative with a 4-hydroxy-3-methoxyphenyl group in the A-region was a full and potent agonist (compound **1**) as reported previously.[22] Conversely, the addition of a 3-fluoro group rendered it a full antagonist (**61**). The incorporation of a 3-methoxy substituent to **40** shifted its partial agonism to the full agonist **57**.

In conclusion, we investigated the isosteric replacement of the phenolic hydroxyl group in potent VR1 agonists with alkylsulfonamido groups and their structure−activity relationships as agonists and antagonists. We obtained compounds which are antagonists of capsaicin in CHO cells heterologously expressing VR1. Compound **61** with a 3-fluoro-4-methylsulfonylamino pattern of substitution in the A-region represents a potent and full VR1 antagonist. In vivo, it produced an excellent analgesic effect in the acetic-acid induced writhing test compared to ketorolac and **40** with partial agonism.

## Experimental Section

**General Method.** All chemical reagents were commercially available. Melting points were determined on a Melting Point Büchi 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, and were within 0.4% of the calculated values unless otherwise noted.

***tert*-Butyl *N*-(4-Aminobenzyl)carbamate (4).** A mixture of 4-aminobenzylamine (**3**) (10 g, 82 mmol) and di-*tert*-butyl dicarbonate (20 g, 90 mmol) in THF (100 mL) was stirred for 2 h at room temperature and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with hexane/ethyl acetate (3:2) as eluant to afford **4** as a yellow solid (17.5 g, 96%); mp = 74−75 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.06 (d, 2 H, $J$ = 8.3 Hz, H-2,6), 6.63 (dt, 2 H, $J$ = 2.7, 8.3 Hz, H-3,5), 4.73 (bs, 1 H, NHBoc), 4.18 (d, 2 H, $J$ = 5.6 Hz, $CH_2NH$), 3.60 (bs, 2 H, $NH_2$), 1.45 (s, 9 H, $C(CH_3)_3$).

**General Procedure for the Synthesis of 5−12.** A cooled solution of **4** (5 mmol) at 0 °C in pyridine (10 mL) was treated with the corresponding acyl chloride (6 mmol) and stirred for 16 h at room temperature. The reacton mixture was cooled in an ice-bath, carefully neutralized with 1 M hydrochloric acid solution, diluted with water, and extracted with dichloromethane several times. The combined organic layers were washed with water and brine, dried over magnesium sulfate, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:1) as eluant to afford **5**−**12**.

***tert*-Butyl *N*-[(4-methylsulfonylamino)benzyl]carbamate (5):** 92% yield, $^1$H NMR ($CDCl_3$) $\delta$ 7.28 (d, 2 H, $J$ = 8.3 Hz, H-2,6), 7.18 (dd, 2 H, $J$ = 2.2, 6.3 Hz, H-3,5), 6.76 (s, 1 H, $NHSO_2$), 4.88 (bs, 1 H, NHBoc), 4.28 (d, 2 H, $J$ = 5.6 Hz, $CH_2NH$), 2.99 (s, 3 H, $SO_2CH_3$), 1.46 (s, 9 H, $C(CH_3)_3$).

***tert*-Butyl *N*-[(4-ethylsulfonylamino)benzyl]carbamate (6):** 73% yield, $^1$H NMR ($CDCl_3$) $\delta$ 7.25 (d, 2 H, $J$ = 8.5 Hz, H-2,6), 7.17 (d, 2 H, $J$ = 8.5 Hz, H-3,5), 6.85 (s, 1 H, $NHSO_2$), 4.89 (bs, 1 H, NHBoc), 4.28 (d, 2 H, $J$ = 5.6 Hz, $CH_2NH$), 3.09 (q, 2 H, $J$ = 7.2 Hz, $SO_2CH_2CH_3$), 1.46 (s, 9 H, $C(CH_3)_3$), 1.34 (t, 3 H, $J$ = 7.2 Hz, $SO_2CH_2CH_3$).

***tert*-Butyl *N*-[(4-propylsulfonylamino)benzyl]carbamate (7):** 99% yield, $^1$H NMR ($CDCl_3$) $\delta$ 7.25 (d, 2 H, $J$ = 8.5 Hz, H-2,6), 7.17 (d, 2 H, $J$ = 8.5 Hz, H-3,5), 6.85 (s, 1 H, $NHSO_2$), 4.89 (bs, 1 H, NHBoc), 4.28 (d, 2 H, $J$ = 5.6 Hz, $CH_2NH$), 3.05 (t, 2 H, $J$ = 7.8 Hz, $SO_2CH_2CH_2$), 1.85 (m, 2 H, $SO_2CH_2CH_2$), 1.46 (s, 9 H, $C(CH_3)_3$), 1.01 (t, 3 H, $J$ = 7.6 Hz, $CH_3$).

***tert*-Butyl *N*-[(4-isopropylsulfonylamino)benzyl]carbamate (8):** 78% yield, $^1$H NMR ($CDCl_3$) $\delta$ 7.25 (d, 2 H, $J$ = 8.3 Hz, H-2,6), 7.17 (d, 2 H, $J$ = 8.3 Hz, H-3,5), 6.85 (s, 1 H, $NHSO_2$), 4.87 (bs, 1 H, NHBoc), 4.27 (d, 2 H, $J$ = 5.6 Hz, $CH_2NH$), 3.28 (m, 1 H, $SO_2CH$), 1.46 (s, 9 H, $C(CH_3)_3$), 1.38 (d, 6 H, $J$ = 6.8 Hz, $CH(CH_3)_3$).

***tert*-Butyl *N*-[(4-phenylsulfonylamino)benzyl]carbamate (9):** 78% yield, $^1$H NMR ($CDCl_3$) $\delta$ 7.75 (d, 2 H, $J$ = 8.5 Hz, Ph), 7.54 (t, 1 H, $J$ = 7.3 Hz, Ph), 7.44 (t, 2 H, $J$ = 7.8 Hz, Ph), 7.15 (d, 2 H, $J$ = 8.5 Hz, H-2,6), 7.02 (d, 2 H, $J$ = 8.5 Hz, H-3,5), 6.68 (s, 1 H, $NHSO_2$), 4.80 (bt, 1 H, NHBoc), 4.23 (d, 2 H, $J$ = 5.8 Hz, $CH_2NH$), 1.44 (s, 9 H, $C(CH_3)_3$).

***tert*-Butyl*N*-[(4-trifluoromethylsulfonylamino)benzyl]-carbamate (10):** 15% yield, $^1$H NMR ($CDCl_3$) $\delta$7.46 (d, 2 H, $J$ = 8.3 Hz, H-2,6), 7.28 (d, 2 H, $J$ = 8.3 Hz, H-3,5), 4.98 (bs, 1 H, NHBoc), 4.30 (d, 2 H, $J$ = 5.8 Hz, $CH_2NH$), 1.47 (s, 9 H, $C(CH_3)_3$).

***tert*-Butyl *N*-[(4-acetylamino)benzyl]carbamate (11):** 96% yield, $^1$H NMR ($CDCl_3$) $\delta$ 7.45 (d, 2 H, $J$ = 8.3 Hz, H-2,6), 7.23 (d, 2 H, $J$ = 8.3 Hz, H-3,5), 4.81 (bs, 1 H, NHBoc), 4.27 (d, 2 H, $J$ = 5.1 Hz, $CH_2NH$), 2.18 (s, 3 H, $COCH_3$), 1.46 (s, 9 H, $C(CH_3)_3$).

***tert*-Butyl *N*-[(4-isobutylamino)benzyl]carbamate (12):** 99% yield, $^1$H NMR ($CDCl_3$) $\delta$ 7.48 (d, 2 H, $J$ = 8.3 Hz, H-2,6), 7.23 (d, 2 H, $J$ = 8.3 Hz, H-3,5), 7.11 (s, 1 H, $NHSO_2$), 4.79 (bs, 1 H, NHBoc), 4.26 (d, 2 H, $J$ = 5.1 Hz, $CH_2NH$), 2.50 (m, 1 H, COCH), 1.46 (s, 9 H, $C(CH_3)_3$), 1.25 (d, 6 H, $J$ = 6.8 Hz, $CH(CH_3)_2$).

**General procedure for the synthesis of 13−20.** A cooled solution of **5**−**12** (4 mmol) in dichloromethane (20 mL) in an ice-bath was slowly treated with trifluoroacetic acid (5 mL) and stirred for 1.5 h at 0 °C. The mixture was carefully concentrated in vacuo to give **13**−**20** in a quantitative yield. The amine salts were washed with ethyl ether and used for the next step without further purification.

***N*-[4-(Aminomethyl)phenyl]methanesulfonamide trifluoroacetate (13):** $^1$H NMR (DMSO-$d_6$) $\delta$ 9.87 (s, 1 H, $NHSO_2$), 8.14 (bs, 3 H, $NH_3$), 7.40 (d, 2 H, $J$ = 8.5 Hz, H-2,6), 7.22 (d, 2 H, $J$ = 8.5 Hz, H-3,5), 3.97 (s, 2 H, $CH_2$), 2.99 (s, 3 H, $SO_2CH_3$).

***N*-[4-(Aminomethyl)phenyl]ethanesulfonamide trifluoroacetate (14):** $^1$H NMR (DMSO-$d_6$) $\delta$ 9.92 (s, 1 H, $NHSO_2$), 8.21 (bs, 3 H, $NH_3$), 7.39 (d, 2 H, $J$ = 8.5 Hz, H-2,6), 7.22 (d, 2 H, $J$ = 8.5 Hz, H-3,5), 3.96 (s, 2 H, $CH_2$), 3.08 (q, 2 H, $J$ = 7.3 Hz, $SO_2CH_2CH_3$), 1.17 (t, 3 H, $J$ = 7.3 Hz, $SO_2CH_2CH_3$).

***N*-[4-(Aminomethyl)phenyl]-1-propanesulfonamide trifluoroacetate (15):** $^1$H NMR (DMSO-$d_6$) $\delta$ 9.92 (s, 1 H, $NHSO_2$), 8.21 (bs, 3 H, $NH_3$), 7.39 (d, 2 H, $J$ = 8.5 Hz, H-2,6), 7.21 (d, 2 H, $J$ = 8.5 Hz, H-3,5), 3.96 (s, 2 H, $CH_2$), 3.06 (t, 2 H, $J$ = 7.5 Hz, $SO_2CH_2CH_2$), 1.66 (m, 2 H, $SO_2CH_2CH_2$), 0.91 (t, 3 H, $J$ = 7.3 Hz, $SO_2CH_2CH_3$).

***N*-[4-(Aminomethyl)phenyl]-2-propanesulfonamide trifluoroacetate (16):** $^1$H NMR (DMSO-$d_6$) $\delta$ 9.88 (s, 1 H, $NHSO_2$), 8.17 (bs, 3 H, $NH_3$), 7.38 (d, 2 H, $J$ = 8.4 Hz, H-2,6), 7.24 (d, 2 H, $J$ = 8.4 Hz, H-3,5), 3.95 (s, 2 H, $CH_2$), 3.20 (m, 1 H, $SO_2CH$), 1.22 (d, 6 H, $J$ = 6.6 Hz, $CH(CH_3)_2$).

***N*-[4-(Aminomethyl)phenyl]benzenesulfonamide trifluoroacetate (17):** $^1$H NMR (DMSO-$d_6$) $\delta$ 8.11 (bs, 3 H, $NH_3$), 7.78 (dd, 2 H, $J$ = 1.5, 6.8 Hz, Ph), 7.5−7.65 (m, 3 H, Ph), 7.28 (d, 2 H, $J$ = 8.4 Hz, H-2,6), 7.11 (d, 2 H, $J$ = 8.4 Hz, H-3,5), 3.89 (s, 2 H, $CH_2$).

***N*-[4-(Aminomethyl)phenyl]trifluoromethanesulfonamide trifluoroacetate (18):** $^1$H NMR (DMSO-$d_6$) $\delta$ 8.12 (bs, 3 H, $NH_3$), 7.28 (d, 2 H, $J$ = 8.4 Hz, H-2,6), 7.11 (d, 2 H, $J$ = 8.4 Hz, H-3,5), 3.98 (s, 2 H, $CH_2$).

***N*-[4-(Aminomethyl)phenyl]acetamide trifluoroacetate (19):** $^1$H NMR ($D_2O$) $\delta$ 7.50 (d, 2 H, $J$ = 8.5 Hz, H-2,6), 7.46 (d, 2 H, $J$ = 8.5 Hz, H-3,5), 4.18 (s, 2 H, $CH_2$), 2.70 (m, 1 H, $SO_2CH$), 1.21 (d, 6 H, $J$ = 7.1 Hz, $CH(CH_3)_2$).

***N*-[4-(Aminomethyl)phenyl]-2-methylpropanamide trifluoroacetate (20):** $^1$H NMR ($D_2O$) $\delta$ 7.52 (d, 2 H, $J$ = 8.5 Hz, H-2,6), 7.46 (d, 2 H, $J$ = 8.5 Hz, H-3,5), 4.18 (s, 2 H, $CH_2$), 2.19 (s, 3 H, $COCH_3$).

***N*-[3-(Chloromethyl)phenyl]methanesulfonamide (22).** A cooled suspension of 3-aminobenzyl alcohol (**21**) (0.2 g, 1.6 mmol), lithium chloride (0.136 g, 3.2 mmol), and 2,6-lutidine (0.410 g, 3.52 mmol) in DMF (5 mL) at 0 °C was treated dropwise with methanesulfonyl chloride (0.272 g, 3.52 mmol) and stirred for 10 min. After being stirred for 5 h at room temperature, the reaction mixture was diluted with $H_2O$ and extracted with ether several times. The combined organic layer was washed successively with $H_2O$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (3:1) as eluent to afford **22** (0.27 g, 76%) as a white solid: mp = 99.5 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.15−7.4 (m, 4 H, Ar), 6.38 (bs, 1 H, NH), 4.57 (s, 2 H, $CH_2Cl$), 3.04 (s, 3 H, $SO_2CH_3$); IR (KBr) 3258 (NH), 1326, 1149 ($SO_2N$) cm$^{-1}$

***N*-[3-(Azidomethyl)phenyl]methanesulfonamide (23).** A mixture of **22** (0.279 g, 1.27 mmol) and sodium azide (0.247 g, 3.81 mmol) in DMF (5 mL) was refluxed for 7 h. The reaction mixture was cooled to room temperature, diluted with $H_2O$ and extracted with ethyl acetate several times. The combined organic layer was washed successively with $H_2O$ and brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (3:1) as eluent to afford **23** (0.273 g, 95%) as an oil.;$^1$H NMR ($CDCl_3$) $\delta$ 7.15−7.4 (m, 4 H, Ar), 6.46 (bs, 1 H, NH), 4.36 (s, 2 H, $CH_2N_3$), 3.04 (s, 3 H, $SO_2CH_3$); IR (neat) 3265 (NH), 2101 ($N_3$), 1326, 1150 ($SO_2N$) cm$^{-1}$

***N*-[3-(Aminomethyl)phenyl]methanesulfonamide (24).** A suspension of **23** (100 mg, 0.442 mmol) and palladium on carbon (20 mg) in MeOH (5 mL) was hydrogenated under a balloon of hydrogen for 2 h. The reaction mixture was filtered, and the filtrate was concentrated in vacuo to give the corresponding amine (88 mg, 99%) as a white solid which was pure enough to be used for the next step.

**[4-(Aminomethyl)phenyl]methanol (25).** To a suspension of lithium aluminum hydride (0.58 g, 15.25 mmol) in THF (40 mL) was added dropwise a solution of 4-cyanobenzaldehyde (0.5 g, 3.8 mmol) in THF (10 mL), and the mixture was refluxed for 5 h. The reaction mixture was cooled in an ice bath, quenched by successive addition of $H_2O$ (15 mL), 15% aqueous NaOH (3 mL), and $H_2O$ (20 mL), and stirred for 30 min. The resulting suspension was filtered, and the residue was washed with $CH_2Cl_2$. The combined filtrate and washings were washed successively with $H_2O$ and brine, dried over $MgSO_4$, and concentrated in vacuo to give **25** as a solid (0.5 g, 96%), which was used for the next step without further purification: $^1$H NMR ($CDCl_3$) $\delta$ 7.33 (dd, 4 H, Ar), 4.69 (s, 2 H, $CH_2OH$), 3.88 (s, 2 H, $CH_2NH_2$).

***N*-[4-(Chloromethyl)benzyl]methanesulfonamide (26).** The compound was prepared from **25** by following the procedure described for the synthesis of **22** as a white solid in 52% yield: mp = 75 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.35 (dd, 4 H, Ar), 4.58 (s, 2 H, $CH_2Cl$), 4.34 (s, 2 H, $CH_2NH_2SO_2$), 2.91 (s, 3 H, $NHSO_2CH_3$); IR (KBr) 3235 (NH), 1302, 1134 ($NHSO_2$)

***N*-[4-(Azidomethyl)benzyl]methanesulfonamide (27).** The compound was prepared from **26** by following the procedure described for the synthesis of **23** as an oil in 78% yield: $^1$H NMR ($CDCl_3$) $\delta$ 7.35 (dd, 4 H, Ar), 4.35 (m, 4 H, $CH_2N_3$ and $CH_2NH_2SO_2$), 2.90 (s, 3 H, $NHSO_2CH_3$); IR (KBr) 3266 (NH), 2101 ($N_3$), 1305, 1424 ($NHSO_2$)

***N*-[4-(Aminomethyl)benzyl]methanesulfonamide (28).** The compound was prepared from **27** by following the procedure described for the synthesis of **24** as a solid in a quantitative yield and was directly used for the next step.

**3-Methoxy-4-nitrobenzyl Azide (30).** A cooled solution of 3-methoxy-4-nitrobenzyl alcohol (**29**, 1 g, 5.46 mmol) and triphenylphosphine (2.146 g, 8.19 mmol) in DMF (30 mL) at 0 °C was treated with carbon tetrabromide (2.716 g, 8.19 mmol) and stirred for 10 min at 0 °C. The reaction mixture was treated with sodium azide (1.065 g, 16.38 mmol) and stirred for 24 h at room temperature. The mixture was diluted with water and extracted with ethyl acetate several times. The combined organic layers were washed with water and brine, dried over magnesium sulfate, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:2) as eluant to afford **30** as a yellow solid (1.055 g, 93%): mp = 35−37 °C, $^1$H NMR ($CDCl_3$) $\delta$ 7.87 (d, 1 H, $J$ = 8.3 Hz, H-5), 7.04 (s, 1 H, H-2), 6.97 (dd, 1 H, $J$ = 1.7, 8.3 Hz, H-6), 4.45 (s, 2 H, $CH_2N_3$), 3.99 (s, 3 H, $OCH_3$).

***tert*-Butyl *N*-(3-Methoxy-4-nitrobenzyl)carbamate (31).** A mixture of **30** (0.833 g, 4 mmol), triphenylphosphine (1.573 g, 6 mmol), and water (0.144 g, 8 mmol) in tetrahydrofuran (30 mL) was stirred for 24 h at room temperature and concentrated in vacuo. The residue was dissolved in ethanol (20 mL) and treated with di-*tert*-butyl dicarbonate (1.746 g, 8 mmol). After being stirred for 2 h at room temperature, the mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:2) as eluant to afford **31** as a yellow oil (0.971 g, 86%): $^1$H NMR ($CDCl_3$) $\delta$ 7.84 (d, 1 H, $J$ = 8.3 Hz, H-5), 7.02 (s, 1 H, H-2), 6.93 (dd, 1 H, $J$ = 8.3 Hz, H-6), 4.99 (bs, 1 H, NHBoc), 4.35 (d, 2 H, $J$ = 6.1 Hz, $CH_2NH$), 3.96 (s, 3 H, $OCH_3$), 1.47 (s, 9 H, $C(CH_3)_3$).

***tert*-Butyl *N*-(4-Amino-3-methoxybenzyl)carbamate (32).** A suspension of **31** (0.847 g, 3 mmol) and 10% palladium on carbon (0.085 g) in methanol (20 mL) was hydrogenated under a balloon of hydrogen for 1 h at room temperature. The mixture was filtered and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:1) as eluant to afford **32** as a white solid

(0.684 g, 90%): mp = 76−77.5 °C; $^1$H NMR ($CDCl_3$) $\delta$ 6.6−6.75 (m, 3 H, Ar), 4.20 (d, 2 H, $J$ = 5.6 Hz, $CH_2NH$), 3.84 (s, 3 H, $OCH_3$), 1.46 (s, 9 H, $C(CH_3)_3$).

***tert*-Butyl *N*-[3-Methoxy-4-(methylsulfonylamino)benzyl]carbamate (33).** A cooled solution of **32** (0.634 g, 2.5 mmol) in pyridine (5 mL) at 0 °C was treated with methanesulfonyl chloride (0.23 mL, 3 mmol) and stirred for 16 h at room temperature. The reaction mixture was cooled in an ice-bath, carefully neutralized with 1 M hydrochloric acid solution, diluted with water, and extracted with dichloromethane several times. The combined organic layers were washed with water and brine, dried over magnesium sulfate, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:1) as eluant to afford **33** as a white solid (0.595 g, 72%): mp = 125.5−127.5 °C; $^1$H NMR ($CDCl_3$)) $\delta$ 7.46 (d, 1 H, $J$ = 8.5 Hz, H-5), 6.86 (m, 2 H, H-2,6), 6.74 (s, 1 H, $NHSO_2$), 4.88 (bs, 1 H, NHBoc), 4.28 (d, 2 H, $J$ = 5.8 Hz, $CH_2NH$), 3.88 (s, 3 H, $OCH_3$), 2.94 (s, 3 H, $SO_2CH_3$), 1.47 (s, 9 H, $C(CH_3)_3$).

**3-Methoxy-4-(methylsulfonylamino)benzylamine Trifluoroacetate (34).** A cooled solution of **33** (1 mmol) in dichloromethane (4 mL) in an ice-bath was slowly treated with trifluoroacetic acid (1 mL) and stirred for 1.5 h at 0 °C. The mixture was carefully concentrated in vacuo to give **34** as a white solid in a quantitative yield. The amine salts were washed with ethyl ether and used for the next step without further purification, respectively: mp = 161−163.5 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 8.16 (bs, 3 H, $NH_3$), 7.28 (d, 1 H, $J$ = 8.0 Hz, H-5), 7.20 (s, 1 H, H-2), 6.99 (d, 1 H, $J$ = 8.0 Hz, H-6), 4.00 (s, 2 H, $CH_2NH_3$), 3.82 (s, 3 H, $OCH_3$), 2.95 (s, 3 H, $SO_2CH_3$).

**3-Fluoro-4-(methylsulfonylamino)benzylamine (35).** This compound was prepared from 2-fluoro-4-iodoaniline in three steps by our previously reported procedure.[27]

**General Procedure for the Synthesis of Isothiocyanate. Method A**. A solution of amine salt (10 mmol) in DMF (5 mL) was treated with triethylamine (1.4 mL, 10 mmol), stirred for 1 h at room temperature, and added with 1,1′-thiocarbonyldi-2(1*H*)-pyridone (2.326 g, 10 mmol). After being stirred for 24 h at room temperature, the mixture was diluted with water and extracted with ethyl acetate. The combined organic layers were washed with water and brine, dried over magnesium sulfate, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography on silica gel with EtOAc/hexanes (1:1) as eluant to afford isothiocyanate.

**Method B**. To a solution of 1,1′-thiocarbonyl diimidazole (12 mmol) dissolved completely in DMF (20 mL) at 50 °C was added dropwise a preprepared solution of amine salt (10 mmol) and triethylamine (10 mmol) in DMF (3 mL) for 5 min. After the mixture was stirred for 20 min at room temperature, the usual workup and purification described above afforded isothiocyanate.

**4-(Methylsulfonylamino)benzyl isothiocyanate (36):** 63% yield, white solid, mp =122−124 °C; $^1$H NMR($CDCl_3$) $\delta$ 7.32 (d, 2 H, $J$ = 8.5 Hz) 7.24 (d, 2 H, $J$ = 8.5 Hz), 6.62 (s, 1 H, $NHSO_2$), 4.70 (s, 2 H, $CH_2$) 3.04 (s, 3 H, $SO_2CH_3$).

**3-Methoxy-4-(methylsulfonylamino)benzyl isothiocyanate (37):** 59% yield, white solid, mp = 100−103 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.46 (d, 1 H, $J$ = 8.5 Hz, H-5), 6.86 (m, 2 H, H-2,6), 6.74 (s, 1 H, $NHSO_2$), 4.68 (s, 2 H, $CH_2$), 3.88 (s, 3 H, $OCH_3$), 2.98 (s, 3 H, $SO_2CH_3$).

**3-Fluoro-4-(methylsulfonylamino)benzyl isothiocyanate (38):** 62% yield, white solid, mp = 93−96 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.61 (t, 1 H, $J$ = 8.3 Hz, H-5), 7.1−7.2 (m, 2 H, H-2,6), 6.58 (bs, 1 H, $NHSO_2$), 4.71 (s, 2 H, $CH_2$), 3.05 (s, 3 H, $SO_2CH_3$).

**General Procedure for the Synthesis of Thiourea (40−49).** A solution of amine salt (**13**−**20**, 0.5 mmol) in dimethylformamide (1 mL) was treated with triethylamine (0.5 mmol) (not needed for free amine **24** and **28**) and stirred for 30 min at room temperature. To the mixture was added {[2-(3,4-dimethylbenzyl)-3-pivaloyloxy]propyl} isothiocyanate (**39**) (0.5 mmol). After being stirred for 24 h at room temperature, the mixture was diluted with water and extracted with ethyl acetate several times. The combined organic layers were washed with water and brine, dried over magnesium sulfate, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:1) as eluant to afford the thiourea in 85−95% yields.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[4-(methylsulfonylamino)benzyl]thiourea (40):** white solid, mp = 47 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.29 (d, 2 H, $J$ = 8.3 Hz), 7.18 (d, 2 H, $J$ = 8.3 Hz), 6.85−7.05 (m, 3 H), 6.68 (s, 1 H, $NHSO_2$), 6.34 (m, 1 H, NHCS), 6.09 (bs, 1 H, NHCS), 4.51 (bs, 2 H, $CSNHCH_2Ar$), 4.17 (m, 1 H, $CH_2OCO$), 3.7−3.85 (m, 2 H, $CH_2OCO$ and $CHCH_2NHCS$), 3.22 (m, 1 H, $CHCH_2NHCS$), 2.99 (s, 3 H, $SO_2CH_3$), 2.5−2.7 (m, 2 H, $CH_2Ar$), 2.2−2.4 (m, 7 H, 2 × $CH_3$ and CH), 1.23 (d, 9 H, $J$ = 3.9 Hz, $C(CH_3)_3$); MS (FAB) $m/z$ 520 ($MH^+$); Anal. ($C_{26}H_{37}N_3O_4S_2$) C, H, N, S.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[4-(ethylsulfonylamino)benzyl]thiourea (41):** white solid, mp = 48 °C; $^1$H NMR($CDCl_3$) $\delta$ 7.25 (d, 2 H, $J$ = 8.6 Hz), 7.16 (d, 2 H, $J$ = 8.6 Hz), 6.85−7.1 (m, 3 H), 6.40 (m, 1 H, NH), 6.21 (bs, 1 H, NH), 4.50 (bs, 2 H, $CSNHCH_2Ar$), 4.15 (m, 1 H, $CH_2OCO$), 3.65−3.85 (m, 2 H, $CH_2OCO$ and $CHCH_2NHCS$), 3.24 (m, 1 H, $CHCH_2NHCS$), 3.09 (q, 3 H, $J$ = 7.2 Hz, $SO_2CH_2CH_3$), 2.5−2.7 (m, 2 H, $CH_2Ar$), 2.15−2.4 (m, 7 H, 2 × $CH_3$ and CH), 1.34 (t, 3 H, $J$ = 7.2 Hz, $SO_2CH_2CH_3$), 1.24 (d, 9 H, $J$ = 3.9 Hz, $C(CH_3)_3$); MS (FAB) $m/z$ 534 ($MH^+$); Anal. ($C_{27}H_{39}N_3O_4S_2$) C, H, N, S.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[4-(propylsulfonylamino)benzyl]thiourea (42):** white solid, mp = 54 °C; $^1$H NMR($CDCl_3$) $\delta$ 7.25 (d, 2 H, $J$ = 8.6 Hz), 7.15 (d, 2 H, $J$ = 8.6 Hz), 6.85−7.1 (m, 3 H), 6.45 (bs, 1 H, NH), 6.30 (bs, 1 H, NH), 4.51 (bs, 2 H, $CSNHCH_2Ar$), 4.15 (m, 1 H, $CH_2OCO$), 3.7−3.85 (m, 2 H, $CH_2OCO$ and $CHCH_2NHCS$), 3.24 (m, 1 H, $CHCH_2NHCS$), 3.04 (t, 3 H, $SO_2CH_2CH_2CH_3$), 2.5−2.7 (m, 2 H, $CH_2Ar$), 2.15−2.4 (m, 7 H, 2 × $CH_3$ and CH), 1.82 (m, 2 H, $SO_2CH_2CH_2CH_3$), 1.24 (d, 9 H, $J$ = 3.7 Hz, $C(CH_3)_3$), 1.00 (t, 3 H, $SO_2CH_2CH_2CH_3$); MS (FAB) $m/z$ 548 ($MH^+$); Anal. ($C_{28}H_{41}N_3O_4S_2$) C, H, N, S.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[4-(isopropylsulfonylamino)benzyl]thiourea (43):** white solid, mp = 48 °C; $^1$H NMR($CDCl_3$) $\delta$ 7.34 (s, 1 H, $NHSO_2$), 7.22 (d, 2 H, $J$ = 8.5 Hz), 7.16 (d, 2 H, $J$ = 8.5 Hz), 6.8−7.05 (m, 3 H), 6.48 (bs, 1 H, NH), 6.40 (bs, 1 H, NH), 4.50 (bs, 2 H, $CSNHCH_2Ar$), 4.13 (m, 1 H, $CH_2OCO$), 3.65−3.85 (m, 2 H, $CH_2OCO$ and $CHCH_2NHCS$), 3.2−3.35 (m, 2 H, $CHCH_2NHCS$ and $SO_2CH(CH_3)_2$), 2.5−2.7 (m, 2 H, $CH_2Ar$), 2.15−2.4 (m, 7 H, 2 × $CH_3$ and CH), 1.36 (s, 3 H, $SO_2CH(CH_3)_2$), 1.34 (s, 3 H, $SO_2CH(CH_3)_2$), 1.23 (d, 9 H, $J$ = 3.6 Hz, $C(CH_3)_3$); MS (FAB) $m/z$ 548 ($MH^+$); Anal. ($C_{28}H_{41}N_3O_4S_2$) C, H, N, S.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[4-(phenylsulfonylamino)benzyl]thiourea (44):** white solid, mp = 66 °C; $^1$H NMR($CDCl_3$) $\delta$ 7.76 (d, 2 H, $J$ = 7.3 Hz), 7.51 (t, 1 H), 7.41 (t, 2 H), 7.14 (d, 2 H, $J$ = 8.3 Hz), 6.8−7.1 (m, 5 H), 6.40 (bs, 1 H, NH), 6.19 (bs, 1 H, NH), 4.43 (bs, 2 H, $CSNHCH_2Ar$), 4.13 (m, 1 H, $CH_2OCO$), 3.65−3.85 (m, 2 H, $CH_2OCO$ and $CHCH_2NHCS$), 3.20 (m, 1 H, $CHCH_2NHCS$), 2.5−2.7 (m, 2 H, $CH_2Ar$), 2.1−2.4 (m, 7 H, 2 × $CH_3$ and CH), 1.23 (d, 9 H, $J$ = 3.8 Hz, $C(CH_3)_3$); MS (FAB) $m/z$ 582 ($MH^+$); Anal. ($C_{31}H_{39}N_3O_4S_2$) C, H, N, S.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[4-(trifluoromethylsulfonylamino)benzyl]thiourea (45):** white solid, mp = 51−52 °C; $^1$H NMR($CDCl_3$) $\delta$ 7.28 (d, 2 H, $J$ = 8.7 Hz), 7.22 (d, 2 H, $J$ = 8.7 Hz), 6.85−7.05 (m, 3 H), 6.36 (bs, 1 H, NH), 6.16 (bs, 1 H, NH), 4.51 (bs, 2 H, $CSNHCH_2Ar$), 4.08 (dd, 1 H, $CH_2OCO$), 3.65−3.85 (m, 2 H, $CH_2OCO$ and $CHCH_2NHCS$), 3.20 (m, 1 H, $CHCH_2NHCS$), 2.5−2.7 (m, 2 H, $CH_2Ar$), 2.15−2.3 (m, 7 H, 2 × $CH_3$ and CH), 1.22 (d, 9 H, $J$ = 3.8 Hz, $C(CH_3)_3$); MS (FAB) $m/z$ 574 ($MH^+$); Anal. ($C_{26}H_{34}F_3N_3O_4S_2$) C, H, N, S.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[4-(acetylamino)benzyl]thiourea (46):** white solid, mp = 74 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.40 (d, 2 H, $J$ = 8.3 Hz), 7.23 (d, 2

H, $J$ = 8.3 Hz), 6.8−7.05 (m, 3 H), 6.28 (m, 1 H, NH), 6.08 (bs, 1 H, NH), 4.44 (bs, 2 H, CSNHC$H_2$Ar), 4.17 (m, 1 H, C$H_2$OCO), 3.7−3.85 (m, 2 H, C$H_2$OCO and CHC$H_2$NHCS), 3.27 (m, 1 H, CHC$H_2$NHCS), 2.5−2.7 (m, 2 H, C$H_2$Ar), 2.1−2.4 (m, 10 H, 2 × C$H_3$, COC$H_3$ and CH), 1.23 (d, 9 H, $J$ = 3.9 Hz, C(C$H_3$)$_3$) MS (FAB) $m/z$ 484 ($MH^+$); Anal. ($C_{27}H_{37}N_3O_3S$) C, H, N, S.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[4-(isobutylcarbonylamino)benzyl]thiourea (47):** white solid, mp = 65 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.50 (d, 2 H, $J$ = 8.3 Hz), 7.24 (d, 2 H, $J$ = 8.3 Hz), 6.8−7.05 (m, 3 H), 6.25 (m, 1 H, NH), 6.00 (bs, 1 H, NH), 4.42 (bs, 2 H, CSNHC$H_2$Ar), 4.12 (m, 1 H, C$H_2$OCO), 3.7−3.85 (m, 2 H, C$H_2$OCO and CHC$H_2$-NHCS), 3.25 (m, 1 H, CHC$H_2$NHCS), 2.5−2.7 (m, 3 H, C$H_2$Ar and CHMe$_2$), 2.15−2.3 (m, 7 H, 2 × C$H_3$ and CH), 1.25 (m, 15 H, C(C$H_3$)$_3$ and CH(C$H_3$)$_2$); MS (FAB) $m/z$ 512 ($MH^+$); Anal. ($C_{29}H_{41}N_3O_3S$) C, H, N, S.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[3-(methylsulfonylamino)benzyl]thiourea (48):** white solid, mp = 51−54 °C; $^1$H NMR ($CDCl_3$) $\delta$ 6.85−7.35 (m, 7 H, Ar), 6.3−6.4 (bs, 3 H, NH), 4.57 (bs, 2 H, NHC$H_2$Ar), 4.12 (m, 1 H, O=COC$H_2$), 3.85 (m, 1 H, O=COC$H_2$), 3.71 (m, 1 H, CHC$H_2$-NHC=S), 3.29 (m, 1 H, CHC$H_2$NHC=S), 2.99 (s, 3 H, $SO_2CH_3$), 2.55−2.64 (m, 2 H, C$H_2$Ph), 2.2−2.3 (m, 7 H, 2 × C$H_3$ and C$H$CH$_2$NHC=S), 1.24 (s, 9 H, (C$H_3$)$_3$CO$_2$); IR (KBr) 3364 (NH), 3250 (NH), 1715 (C=O), 1326 ($SO_2$N), 1286 (N−CS−N), 1150 ($SO_2$N) cm$^{-1}$; MS (FAB) $m/z$ 520 ($MH^+$); Anal. ($C_{26}H_{37}N_3O_4S_2$) C, H, N, S.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-{[4-(methylsulfonylamino)methyl]benzyl}thiourea (49):** white solid, mp = 39 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.25−7.35 (m, 4 H), 6.85−7.05 (m, 3 H, aromatic), 6.31 (bs, 1 H, NH), 6.22 (bs, 1 H, NH), 5.04 (bs, 1 H, NH), 4.49 (bs, 2 H, NHC$H_2$Ar), 4.35 (d, 2 H, $J$ = 5.7 Hz, C$H_2$NHSO$_2$CH$_3$), 3.92 (m, 1 H, O=COC$H_2$), 3.72 (m, 1 H, O=COC$H_2$), 3.48 (m, 1 H, CHC$H_2$NHC=S), 3.22 (m, 1 H, CHC$H_2$NHC=S), 2.89 (s, 3 H, $SO_2CH_3$), 2.5−2.64 (m, 2 H, C$H_2$Ph), 2.2−2.3 (m, 7 H, 2 × C$H_3$ and C$H$CH$_2$NHC=S), 1.22 (s, 9 H, (C$H_3$)$_3$CO$_2$); IR (KBr) 3355 (NH), 1716 (C=O), 1318 ($SO_2$N), 1286 (N−CS−N), 1150 ($SO_2$N) cm$^{-1}$; MS (EI) $m/z$ 532 ($M^+ - 1$); Anal. ($C_{27}H_{39}N_3O_4S_2$) C, H, N, S.

**General Procedure for the Synthesis of Thioureas (54−61).** A suspension of azide (**50**−**53**) (0.5 mmol) and Lindlar catalyst (50 mg) in ethanol (5 mL) was hydrogenated under a balloon of hydrogen for 2 h. The mixture was filtered, and the filtrate was concentrated in vacuo. The residue was dissolved in dichloromethane (5 mL) and added with isocyanate (**36**−**38**) (0.5 mmol). After being stirred for 24 h at room temperature, the mixture was concentrated in vacuo, and the residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:1) as eluant to afford thiourea.

***N*-[2-(4-*tert*-Butylbenzyl)-3-pivaloyloxypropyl]-*N*′-[4-(methylsulfonylamino)benzyl]thiourea (54):** white solid, mp = 61 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.30 (d, 4 H, $J$ = 8.5 Hz), 7.18 (d, 2 H, $J$ = 8.6 Hz), 7.11 (d, 2 H, $J$ = 8.6 Hz), 6.51 (s, 1 H, NHSO$_2$), 6.33 (m, 1 H, NH), 6.02 (bs, 1 H, NH), 4.53 (bs, 2 H, CSNHC$H_2$Ar), 4.16 (dd, 1 H, C$H_2$OCO), 3.7−3.85 (m, 2 H, C$H_2$OCO and CHC$H_2$NHCS), 3.22 (m, 1 H, CHC$H_2$NHCS), 2.99 (s, 3 H, $SO_2CH_3$), 2.6−2.7 (m, 2 H, C$H_2$Ar), 2.32 (m, 1 H, CH), 1.29 (s, 9 H, C(C$H_3$)$_3$), 1.23 (s, 9 H, C(C$H_3$)$_3$); MS (FAB) $m/z$ 548 ($MH^+$); Anal. ($C_{28}H_{41}N_3O_4S_2$) C, H, N, S.

***N*-[3-Benzoyloxy-2-(3,4-dimethylbenzyl)propyl]-*N*′-[4-(methylsulfonylamino)benzyl]thiourea (55):** white solid, mp = 46 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.0 (m, 2 H), 7.59 (m, 1 H), 7.45 (m, 2 H), 7.29 (bs, 1 H, NHSO$_2$), 7.24 (d, 2 H, $J$ = 8.3 Hz), 7.15 (d, 2 H, $J$ = 8.3 Hz), 6.85−7.05 (m, 3 H), 6.40 (m, 1 H, NH), 6.09 (bs, 1 H, NH), 4.52 (bs, 2 H, CSNHC$H_2$Ar), 4.37 (m, 1 H, C$H_2$OCO), 4.10 (m, 1 H, C$H_2$OCO), 3.80 (m, 1 H, CHC$H_2$-NHCS), 3.41 (m, 1 H, CHC$H_2$NHCS), 2.94 (s, 3 H, $SO_2CH_3$), 2.6−2.8 (m, 2 H, C$H_2$Ar), 2.44 (m, 1 H, CH), 2.2−2.4 (m, 6 H, 2 × C$H_3$); MS (FAB) $m/z$ 540 ($MH^+$); Anal. ($C_{28}H_{33}N_3O_4S_2$) C, H, N, S.

***N*-[3-Benzoyloxy-2-(4-*tert*-butylbenzyl)propyl]-*N*′-[4-(methylsulfonylamino)benzyl]thiourea (56):** white solid, mp = 68 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.02 (d, 2 H), 7.60 (t, 1 H) 7.47 (t, 2 H), 7.25−7.35 (m, 4 H), 7.1−7.2 (m, 4 H), 6.54 (s, 1 H, NHSO$_2$), 6.39 (m, 1 H, NH), 6.12 (bs, 1 H, NH), 4.53 (bs, 2 H, CSNHC$H_2$Ar), 4.40 (m, 1 H, C$H_2$OCO), 4.08 (m, 1 H, C$H_2$OCO), 3.80 (m, 1 H, CHC$H_2$NHCS), 3.38 (m, 1 H, CHC$H_2$-NHCS), 2.97 (s, 3 H, $SO_2CH_3$), 2.6−2.75 (m, 2 H, C$H_2$Ar), 2.48 (m, 1 H, CH), 1.29 (s, 9 H, C(C$H_3$)$_3$); MS (FAB) $m/z$ 568 ($MH^+$); Anal. ($C_{30}H_{37}N_3O_4S_2$) C, H, N, S.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[3-methoxy-4-(methylsulfonylamino)benzyl]thiourea (57):** white solid, mp = 49 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.48 (d, 1 H, $J$ = 8.0 Hz), 6.85−7.1 (m, 5 H), 6.77 (s, 1 H, NHSO$_2$), 6.31 (m, 1 H, NH), 5.98 (bs, 1 H, NH), 4.49 (bs, 2 H, CSNHC$H_2$Ar), 4.18 (m, 1 H, C$H_2$OCO), 3.7−3.85 (m, 2 H, C$H_2$OCO and CHC$H_2$-NHCS), 3.88 (s, 3 H, OC$H_3$), 3.22 (m, 1 H, CHC$H_2$NHCS), 2.93 (s, 3 H, $SO_2CH_3$), 2.5−2.7 (m, 2 H, C$H_2$Ar), 2.2−2.4 (m, 7 H, 2 × C$H_3$ and CH), 1.23 (d, 9 H, $J$ = 4.1 Hz, C(C$H_3$)$_3$); MS (FAB) $m/z$ 550 ($MH^+$); Anal. ($C_{27}H_{39}N_3O_5S_2$) C, H, N, S.

***N*-[2-(4-*tert*-Butylbenzyl)-3-pivaloyloxypropyl]-*N*′-[3-methoxy-4-(methylsulfonylamino)benzyl]thiourea (58):** white solid, mp = 62 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.43 (d, 1 H, $J$ = 8.1 Hz), 7.30 (d, 2 H, $J$ = 8.3 Hz), 7.10 (d, 2 H, $J$ = 8.3 Hz), 6.93 (s, 1 H), 6.88 (d, 1 H, $J$ = 8.1 Hz), 6.81 (s, 1 H, NHSO$_2$), 6.39 (t, 1 H, NH), 6.24 (bs, 1 H, NH), 4.52 (bs, 2 H, CSNHC$H_2$Ar), 4.15 (dd, 1 H, $J$ = 3.6, 11.2 Hz, C$H_2$OCO), 3.7−3.9 (m, 2 H, C$H_2$OCO and CHC$H_2$NHCS), 3.87 (s, 3 H, OC$H_3$), 3.25 (m, 1 H, CHC$H_2$NHCS), 2.92 (s, 3 H, $SO_2CH_3$), 2.5−2.7 (m, 2 H, C$H_2$Ar), 2.30 (m, 1 H, CH), 1.29 (s, 9 H, C(C$H_3$)$_3$), 1.23 (s, 9 H, C(C$H_3$)$_3$); MS (FAB) $m/z$ 578 ($MH^+$); Anal. ($C_{29}H_{43}N_3O_5S_2$) C, H, N, S.

***N*-[3-Benzoyloxy-2-(3,4-dimethylbenzyl)propyl]-*N*′-[3-methoxy-4-(methylsulfonylamino)benzyl]thiourea (59):** white solid, mp = 62 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.02 (m, 2 H), 7.60 (m, 1 H), 7.4−7.5 (m, 3 H), 6.8−7.1 (m, 5 H), 6.77 (s, 1 H, NHSO$_2$), 6.38 (m, 1 H, NH), 6.10 (bs, 1 H, NH), 4.50 (bs, 2 H, CSNHC$H_2$Ar), 4.42 (m, 1 H, C$H_2$OCO), 4.05 (m, 1 H, C$H_2$OCO), 3.82 (m, 1 H, CHC$H_2$NHCS), 3.85 (s, 3 H, OC$H_3$), 3.37 (m, 1 H, CHC$H_2$NHCS), 2.92 (s, 3 H, $SO_2CH_3$), 2.6−2.8 (m, 2 H, C$H_2$Ar), 2.45 (m, 1 H, CH), 2.2−2.3 (m, 6 H, 2 × C$H_3$); MS (FAB) $m/z$ 570 ($MH^+$); Anal. ($C_{29}H_{35}N_3O_5S_2$) C, H, N, S.

***N*-[3-Benzoyloxy-2-(4-*tert*-butylbenzyl)propyl]-*N*′-[3-methoxy-4-(methylsulfonylamino)benzyl]thiourea (60):** white solid, mp = 57 °C; $^1$H NMR ($CDCl_3$) $\delta$ 8.02 (dd, 2 H, $J$ = 7.1, 1.5 Hz), 7.60 (m, 1 H), 7.47 (m, 3 H), 7.32 (d, 2 H, $J$ = 8.3 Hz), 7.15 (d, 2 H, $J$ = 8.3 Hz), 6.85−6.95 (m, 2 H), 6.77 (s, 1 H, NHSO$_2$), 6.38 (t, 1 H, NH), 6.08 (bs, 1 H, NH), 4.51 (bs, 2 H, CSNHC$H_2$Ar), 4.42 (dd, 1 H, $J$ = 3.9, 11.7 Hz, C$H_2$OCO), 4.05 (m, 1 H, C$H_2$OCO), 3.82 (m, 1 H, CHC$H_2$NHCS), 3.86 (s, 3 H, OC$H_3$), 3.37 (m, 1 H, CHC$H_2$NHCS), 2.92 (s, 3 H, $SO_2CH_3$), 2.5−2.7 (m, 2 H, C$H_2$Ar), 2.45 (m, 1 H, CH), 1.29 (s, 9 H, C(C$H_3$)$_3$); MS (FAB) $m/z$ 598 ($MH^+$); Anal. ($C_{31}H_{39}N_3O_5S_2$) C, H, N, S.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N*′-[3-fluoro-4-(methylsulfonylamino)benzyl]thiourea (61):** white solid, mp = 56 °C; $^1$H NMR ($CDCl_3$) $\delta$ 7.45 (d, 2 H, $J$ = 8.3 Hz), 6.85−7.15 (m, 5 H), 6.53 (bs, 2 H, NHCS), 4.58 (bs, 2 H, CSNHC$H_2$Ar), 4.14 (dd, 1 H, $J$ = 3.9, 11.7 Hz, C$H_2$OCO), 3.6−3.9 (m, 2 H, C$H_2$OCO and CHC$H_2$NHCS), 3.25 (m, 1 H, CHC$H_2$NHCS), 2.99 (s, 3 H, $SO_2CH_3$), 2.5−2.7 (m, 2 H, C$H_2$Ar), 2.2−2.4 (m, 7 H, 2 × C$H_3$ and CH), 1.23 (d, 9 H, $J$ = 3.9 Hz, C(C$H_3$)$_3$); MS (FAB) $m/z$ 538 ($MH^+$); Anal. ($C_{26}H_{36}FN_3O_4S_2$) C, H, N, S.

**VR1 Binding Assays. Cell Culture**. The pUHG102 VR1 plasmid was transfected into CHO cells containing the pTet Off regulatory plasmid (Clontech). In these cells, expression of the VR1 is repressed in the presence of tetracycline but is induced upon removal of the antibiotic. Stable clones were isolated in culture medium containing puromycin (10 $\mu$g/mL) and maintained in HAM F12 medium supplemented with tetracycline (1 $\mu$g/mL), 5 $\mu$g/mL geniticin, 25 mM HEPES, 10% FBS. Cells utilized for assays were grown in culture medium without antibiotic for 48 h before use. Cells were seeded in T75 cell culture flasks in media without antibiotics and grown to approximately 90% confluence. The flasks were then washed with PBS and harvested in 0.25% trypsin, 1 mM EDTA. The

cells were pelleted by gentle centrifugation and stored at −20 °C until assay.

**Competition Binding Assay**. Binding studies with [$^3$H]resiniferatoxin (RTX) were carried out as described previously with minor modifications (Szallasi et al., 1992). Binding assay mixtures were set up on ice and contained 50−100 pM [$^3$H]RTX, various concentrations of competing ligands, 0.25 mg/mL BSA (Cohn fraction V), and about $5 \times 10^5$ VR1-transfected cells. The final volume was adjusted to 350 $\mu$L with DPBS with $Ca^{2+}$ and $Mg^{2+}$ and 0.25 mg/mL bovine serum albumin. Nonspecific binding was determined in the presence of 100 nM nonradioactive RTX. The binding reaction was initiated by transferring the assay mixtures to a 37 °C water bath and was terminated after a 60 min incubation period by cooling the tubes on ice. To reduce nonspecific binding, 200 $\mu$g/mL α-glycoprotein was added. Membrane-bound RTX was then separated from the free by pelleting the membranes in a Beckman 12 benchtop centrifuge (15 min, maximal velocity), the tips of the tubes containing the pellets were cut off, and the radioactivity was determined by scintillation counting. Equilibrium binding parameters ($K_i$ and cooperativity) were determined by fitting the Hill equation to the measured values with the aid of the program MicroCal Origin 6.0.

**Compound Preparation.** Initial stocks were dissolved in DMSO. For the binding assays, compounds were diluted in with DPBS with $Ca^{2+}$ and $Mg^{2+}$ and 0.25 mg/mL bovine serum albumin. For the calcium uptake assays, compounds were diluted in DMEM with 0.25 mg/mL bovine serum albumin.

**Functional Characterization for Agonist/Antagonist Activity. $^{45}Ca^{2+}$ Uptake.** Molecules were characterized to determine whether they were full agonists, partial agonists, or antagonists. For studies of $^{45}Ca^{2+}$ uptake by CHO/VR1 cells (Tet-off cells), the cells were plated in 24-well plates to yield a cell density 20−40% of that required to produce confluence. The next day the medium was changed to remove the tetracycline and induce VR1 expression. Experiments were performed approximately 36−40 h after induction. For $^{45}Ca^{2+}$ uptake assay, cells were incubated for 5 min at 37 °C in a total volume of 400 $\mu$L of serum free DMEM (containing 1.8 mM $CaCl_2$) in the presence of 0.25 mg/mL BSA (Sigma), 1 $\mu$Ci/mL $^{45}Ca^{2+}$ (5−30 Ci/g from ICN, CA), and increasing concentrations of the compound to be tested. Immediately after the incubation, extracellular $^{45}Ca^{2+}$ was removed by washing the cells three times with cold DPBS (containing 1.8 mM $CaCl_2$). Then 400 $\mu$L of RIPA buffer (50 mM Tris pH 7.4; 150 mM NaCl; 1% Triton X-100; 0.1% SDS; 1% sodium deoxycholate) was added to each well in order to lyse the cells. Plates were shaken slowly for 20 min; then 300 $\mu$L of cell lysate was transferred from each well into a scintillation vial and radioactivity was determined by scintillation counting. For each data point in each experiment, four wells were assayed. Data from these experiments were analyzed by computer fit to the Hill equation. At least three separate experiments were carried out for each compound. To determine antagonist activity, studies were performed in exactly the same fashion with the exception that 50 nM capsaicin was added to the assay mixture to stimulate $^{45}Ca^{2+}$ uptake.

**Writhing Test.** Experimental protocols involving animals in this study were reviewed by the Animal Care and Use Committee of the College of Pharmacy, Seoul National University, according to the NIH guidelines (NIH publication number 85-23, revised 1985) of "Principles of Laboratory Animal Care". Male ICR mice (Bio Genomics, Korea), weighing ∼25 g, were maintained on a 12 h light-dark cycle (light on between 6:00 p.m. and 6:00 a.m.) and allowed free access to food and water. The temperature and humidity of the animal room were maintained at 22 ± 2 °C and 50 ± 5%, respectively. Mice were allowed to habituate for ∼30 min in the testing room on the day of experimentation. Animals then received an intraperitoneal injection of 0.3 mL of an acetic acid solution (1.2%, diluted in 0.9% saline) and were placed in a transparent acrylic cage. Five minutes later the number of writhing movements (abnormal stretching) was counted for a 20 min period. Animals (10 animals/dose) were pretreated with test compounds or vehicle (0.2 mL, i.p.) 30 min before the injection of acetic acid. Test compounds were dissolved in either ethanol/Tween-80/saline (10/10/80) mixture or Cremophor EL/DMSO/distilled water (10/10/80) mixture. The effect of each compound was tested at 4−7 different doses. A reduction in the number of writhing movements compared to the vehicle-treatment group (the mean number of writhing movements in this group was 35) was considered to be indicative of an antinociceptive effect of a compound. The percentage antinociceptive efficiency (eff) was calculated as follows: % eff = 100-[(# of writhing movements/# of writhing movement control) × 100].

Data are expressed as $ED_{50}$ values that indicate the concentration at which a given compound reduces the number of writhing by 50% compared to that of a vehicle-treatment group. $ED_{50}$ values were obtained based on dose−response curves using mean data and fitted to by nonlinear regression analysis (Winnonlin version 3.1, Pharsight Corp., Mountainview, CA) on a PC.

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## References

(1) Szallasi, A.; Blumberg, P. M. Vanilloid (Capsaicin) Receptors and Mechanisms. *Pharmacol. Rev.* **1999**, *51*, 159−211.
(2) Kress, M.; Zeihofer, H. U. Capsaicin, Protons and Heat: New Excitement about Nociceptors. *TIPS* **1999**, *20*, 112−118.
(3) Walpole C. S. J.; Wrigglesworth, R. *Capsaicin in the Study of Pain*; Academic Press: San Diego, CA, 1993; pp 63−82.
(4) Appendino, G.; Szallasi, A. Euphorbium: Modern Research on Its Active Principle, Resiniferatoxin, Revives an Ancient Medicine. *Life Sci.* **1997**, *60*, 681−696.
(5) Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. The Capsaicin Receptor: a Heat-activated Ion Channel in the Pain Pathway. *Nature* **1997**, *389*, 816−824.
(6) Hayes, P.; Meadows, H. J.; Gunthorpe, M. J.; Harries, M. H.; Duckworth, D. M.; Cairns, W.; Harrision, D. C.; Clarke, C. E.; Ellington, K.; Prinjha, R. K.; Barton, A. J. L.; Medhurst, A. D.; Smith, G. D.; Topp, S.; Murdock, P.; Sanger, G. J.; Terrett, J.; Jenkins, O.; Benham, C. D.; Randall, A. D.; Gloger, I. S.; Davis, J. B. Cloning and Functional Expression of a Human Orthologue of Rat Vanilloid Receptor-1. *Pain* **2000**, *88*, 205−215.
(7) Gunthorpe, M. J.; Benham, C. D.; Randall, A.; Davis, J. B. The Diversity of the Vanilloid Receptor Family of Ion Channels. *Trends in Pharmacol. Sci.* **2002**, *23*, 183−191.
(8) Kedei, N.; Szabo, T.; Lile, J. D.; Treanor, J. J.; Olah, Z.; Iadarola, M. J.; and Blumberg, P. M. Analysis of the Native Quaternary Structure of the Vanilloid Receptor 1. *J. Biol. Chem.* **2001**, *276*, 28613−28619.
(9) Oh, U.; Hwang, S. W.; Kim, D. Capsaicin Activates a Nonselective Cation Channel in Cultured Neonatal Rat Dorsal Root Ganglion Neurons. *J. Neurosci.* **1996**, *16*, 1659−1667.
(10) Szallasi, A.; Blumberg, P. M.; Mechanisms and Therapeutic Potential of Vanilloids (Capsaicin-like Molecules). *Adv. Pharmacol.* **1993**, *24*, 123−155.
(11) Kwak, J.; Jung, J. Y.; Hwang, S. W.; Lee, W. T.; and Oh, U. A Capsaicin-Receptor Antagonist, Capsazepine, Reduces Inflammation-induced Hyperalgesic Responses in the Rat: Evidence for an Endogenous Capsaicin-like Substance. *Neuroscience* **1998**, *86*, 619−626.
(12) Garcia-Martinez, C.; Humet, M.; Planells-Cases, R.; Gomis, A.; Caprini, M.; Viana, F.; De le Pena, E.; Sanchez-Baeza, F.; Carbonell, T.; De Felipe, C.; Perez-Paya, E.; Belmonte, C.; Messeguer, A.; and Ferrer-Montiel, A. Attenuation of Thermal Nociception and Hyperalgesia by VR1 Blockers. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 2374−2379.
(13) Lazzeri, M.; Beneforti, P.; Spinelli, M.; Zanollo, A.; Barbagli, G.; Turini, D. Intravesical Resiniferatoxin for the Treatment of Hypersensitive Disorder: a Randomized Placebo Controlled Study. *J. Urol.* **2000**, *164*, 676−679.
(14) Park, N. S.; Seong, C. M.; Jung, Y. S.; Kim, W. B.; Kim, S. H. DA-5018 (Capsavanil, KR-25018). *Drugs Future* **2000**, *25*, 1131−1137.
(15) Urban, L.; Campbell, E. A.; Panesar, M.; Patel, S.; Chaudhry, N.; Kane, S.; Buchheit, K.-H.; Sandells, B.; James, I. F. In Vivo Pharmacology of SDZ 249−665, a Novel, Nonpungent Capsaicin Analogue. *Pain* **2000**, *89*, 65−74.

(16) Walpole, C. S. J.; Bevan, S.; Bovermann, G.; Boelsterli, J. J.; Breckenridge, R.; Davies, J. W.; Hughes, G. A.; James, I.; Oberer, L.; Winter, J.; Wrigglesworth, R. The Discovery of Capsazepine, the First Competitive Antagonist of the Sensory Neuron Excitants Capsaicin and Resiniferatoxin. *J. Med. Chem*. **1994**, *37*, 1942−1954.

(17) Docherty, R. J.; Yeats, J. C.; Piper A. S. Capsazepine Block of Voltage-gated Calcium Channels in Adult Rat Dorsal Root Ganglion Neurons in Culture. *Br. J. Pharmacol*. **1997**, *121*, 1461−1467.

(18) Liu, L.; Simon, S. A. Capsazepine, a Vanilloid Receptor Antagonist, Inhibits Nicotinic Acetylcholine-Receptors in Rat Trigeminal Ganglia. *Neurosci. Lett*. **1997**, *228*, 29−32.

(19) (a) Wahl, P.; Foged, C.; Tullin, S.; Thomsen, C. Iodo-Resiniferatoxin, a New Potent Vanilloid Receptor Antagonist. *Mol. Pharmacol*. **2001**, *59*, 9−15. (b) Seabrook, G. R.; Sutton, K. G.; Jarolimek, W.; Hollingworth, G. J.; Teague, S.; Webb, J.; Clark, N.; Boyce, S.; Kerby, J.; Ali, Z.; Chou, M.; Middleton, R.; Kaczorowski, G.; Jones, A. B. Functional Properties of the High-Affinity TRPV1 (VR1) Vanilloid Receptor Antagonist (4-Hydroxy-5-iodo-3-methoxyphenylacetate ester) Iodo-Resiniferatoxin. *J. Pharmacol. Exp. Ther*. **2002**, *303*, 1052−1060. *Other Iodo-RTX*: (c) McDonnell, M. E.; Zhang, S.-P.; Dubin, A. E.; Dax, S. L. Synthesis and In Vitro Evaluation of a Novel Iodinated Resiniferatoxin Derivatives that is an Agonist at the Human Vanilloid VR1 Receptor. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 1189−1192.

(20) Szallasi, A.; Blumberg, P. M.; Annicelli, L. L.; Krause, J. E.; Cortright, D. N. The Cloned Rat Vanilloid Receptor VR1 Mediates Both R-type Binding and C-type Calcium Response in Dorsal Root Ganglion Neurons. *Mol. Pharmacol*. **1999**, *56*, 581−587.

(21) Walpole, C. S. J.; Bevan, S.; Bloomfield, G.; Breckenridge, R.; James, I. F.; Ritchie, T.; Szallasi, A.; Winter, J.; Wrigglesworth, R. Similarities and Differences in the Structure−Activity Relationships of Capsaicin and Resiniferatoxin Analogues. *J. Med. Chem*. **1996**, *39*, 2939−2952.

(22) Lee, J.; Lee, J.; Kim, J.; Kim, S. Y.; Chun, M. W.; Cho, H.; Hwang, S. W.; Oh, U.; Park, Y. H.; Marquez, V. E.; Beheshti, M.; Szabo, T.; Blumberg, P. M. N−(3-Acyloxy-2-benzylpropyl)-N′-(4-hydroxy-3-methoxybenzyl)thiourea Derivatives as Potent Vanilloid Receptor Agonists and Analgesics. *Bioorg. Med. Chem*. **2001**, *9*, 19−32.

(23) Wriggleworth, R.; Walpole, C. S. J.; Bevan, S.; Campell, E. A.; Dray, A.; Hughes, G. A.; James, I.; Masdin, K. J.; Winter, J. Analogues of Capsaicin with Agonist Activity as Novel Analgesic Agents: Structure−Activity Studies. 4. Potent, Orally Active Analgesics. *J. Med. Chem*. **1996**, *39*, 4942−4951.

(24) Walpole, C. S. J.; Wrigglesworth, R.; Bevan, S.; Campbell, E. A.; Dray, A.; James, I. F.; Perkins, M. N.; Reid, D. J.; Winter, J. Analogues of Capsaicin with Agonist Activity as Novel Analgesic Agents; Structure−Activity Studies. 1. The Aromatic "A-Region". *J. Med. Chem*. **1993**, *36*, 2362−2372.

(25) Larsen, A. A.; Lish, P. M. A New Bio-isostere: Alkylsulphonamidophenethanolamines. *Nature* **1964**, *203*, 1283−1284.

(26) Lee, J.; Lee, J.; Szabo, T.; Gonzalez, A. F.; Welter, J. D.; Blumberg, P. M. N-(3-Acyloxy-2-benzylpropyl)-N-Dihydroxy Tetrahydrobenzazepine and Tetrahydroisoquinoline Thiourea Analogues as Vanilloid Receptor Ligands. *Bioorg. Med. Chem*. **2001**, *9*, 1713−1720.

(27) 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. High Affinity Antagonists of the Vanilloid Receptor. *Mol. Pharmacol*. **2002**, *62*, 947−956. (Published erratum appears in *Mol. Pharmacol*. **2003**, *63*, 958.)

(28) The biochemical behavior of **38** and compound **III** will be described in Wang, Y, Toth, A, Tran, R.; Szabo, T.; Welter, J. D.; Blumberg, P. M.; Lee, J.; Kang, S.-U.; Lim, J.-O.; Lee, J. High Affinity Partial Agonists of Vanilloid Receptor, **2003**, *Mol. Pharm*. In press.

(29) Lee, J.; Lee, J.; Kang, M.-S.; Kim, K.-P.; Chung, S.-J.; Blumberg, P. M.; Yi, J.-B.; Park, Y. H. Phenolic Modification as an Approach to Improve the Pharmacology of the 3-Acyloxy-2-benzylpropyl Homovanillic Amides and Thioureas, a Promising Class of Vanilloid Receptor Agonist and Analgesics. *Bioorg. Med. Chem*. **2002**, *10*, 1171−1179.

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