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Article

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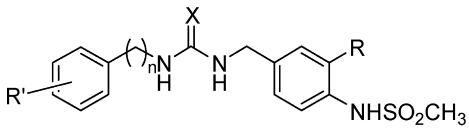

n = 0, 1, 2, 3

X = S, O, N

R = Cl, Br, I, NO_2, CO_2CH_3

R' = alkyl, aryl, halogen

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Novel Potent Antagonists of Transient Receptor Potential Channel, Vanilloid Subfamily Member 1: Structure−Activity Relationship of 1,3-Diarylalkyl Thioureas Possessing New Vanilloid Equivalents

Young-Ger Suh,*,† Yong-Sil Lee,† Kyung-Hoon Min,† Ok-Hui Park,† Jin-Kwan Kim,† Ho-Sun Seung,† Seung-Yong Seo,† Bo-Young Lee,† Yeon-Hee Nam,† Kwang-Ok Lee,† Hee-Doo Kim,‡ Hyeung-Geun Park,† Jeewoo Lee,† Uhtaek Oh,# Ju-Ok Lim,† Sang-Uk Kang,† Min-Jung Kil,† Jae-yeon Koo,# Song Seok Shin,§ Yung-Hyup Joo,§ Jin Kwan Kim,§ Yeon-Su Jeong,§ Sun-Young Kim,§ and Young-Ho Park§

College of Pharmacy, and Sensory Research Center, CRI, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, Korea, College of Pharmacy, Sookmyung Women's University, 53-12, Chungpa-Dong, Yongsan-Gu, Seoul 140-742, Korea, and R&D Center of AmorePacific Corp., 314-1, Bora-Ri, Kiheung-Eup, Yongin-Si, Kyounggi-Do 449-900, Korea

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Recently, 1,3-diarylalkyl thioureas have merged as one of the promising nonvanilloid TRPV1 antagonists possessing excellent therapeutic potential in pain regulation. In this paper, the full structure−activity relationship for TRPV1 antagonism of a novel series of 1,3-diarylalky thioureas is reported. Exploration of the structure−activity relationship, by systemically modulating three essential pharmacophoric regions, led to six examples of 1,3-dibenzyl thioureas, which exhibit Ca^{2+} uptake inhibition in rat DRG neuron with IC_{50} between 10 and 100 nM.

Introduction

Over the past few years, the amount of information from studies on pain transmission by transient receptor potential channel, vanilloid subfamily member 1 (TRPV1) binding ligands has dramatically increased, thus revealing novel targets for the advent of new pain therapies. Moreover, a gigantic step forward came with the identification of a protein called TRPV1, cloned in 1997, which is a ligand-gated nonselective cation channel vanilloid receptor 1 (VR1) with high Ca^{2+} permeability.[1] It is expressed on unmyelinated pain-sensing nerve fibers (C-fibers) and small Aδ fibers in the dorsal root, trigeminal, and nodose ganglia. TRPV1 is activated not only by vanilloid ligands such as capsaicin (**1**, Figure 1) and resiniferatoxin (**2**, Figure 1), noxious heat (>42 °C), and protons (extracelluar pH < 6) but also by endogenous mediators of inflammation such as cannabinoid anandamide and arachidonic metabolites.[2,3] Excitation of TRPV1 by agonists is followed by desensitization of a subset of primary neurons involved in nociception, neurogenic inflammation, and a variety of local regulatory functions. Thus, this desensitization has provided a basis for the therapeutic uses of TRPV1 agonists.[2a,4] However, the small therapeutic window between the analgesic effects and the excitatory side effects has limited the development of TRPV1 agonists as systemic agents.[5] Accordingly, the idea that TRPV1 functions as an integrator of multiple pain-producing stimuli implied that TRPV1 antagonists should have profound antinociceptive effects,[6a] especially in inflammatory pain models.[6b] Either the prevention of endovanilloid binding or the direct inhibitory action on TRPV1 is anticipated to provide therapeutically useful TRPV1 antagonists, which may function as potent analgesic agents by blocking the combined action of heat, protons, and endovanilloids on TRPV1. Taken together, these findings have led to considerable efforts in the development of novel TRPV1 antagonists.[7]

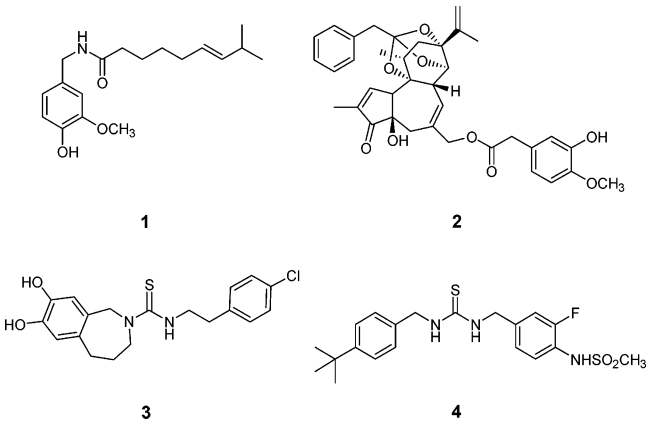

Figure 1.

Capsazepine (**3**, Figure 1), which has been extensively characterized, was the first reported competitive VR1 antagonist. However, capsazepine has only modest potency, with poor metabolic and pharmacokinetic properties, and is somewhat nonspecific.[8] Several other excellent TRPV1 antagonists have also appeared since capsazepine was reported.[7] In particular, new amide, urea, and thiourea series, which revealed the structural basis of capsaicin, resiniferatoxin, and capsazepine, have quite recently been described.[9] Some *N*-arylcinnamides were reported to exhibit potent TRPV1 antagonistic effects and good oral bioavailability in rats.[9a] The 2-pyridylpiperazine ureas[9b] were characterized in vitro and in vivo as potent, orally effective VR1 selective antagonists. A dibenzyl thiourea[9c] was also featured as a competitive antagonist of TRPV1.

* To whom correspondence should be addressed. Phone: 082-2-880-7875. Fax: 082-2-888-0649. E-mail: ygsuh@snu.ac.kr.
† College of Pharmacy, Seoul National University.
# Sensory Research Center, CRI, Seoul National University.
‡ College of Pharmacy, Sookmyung Women's University.
§ R&D Center of AmorePacific Corp.

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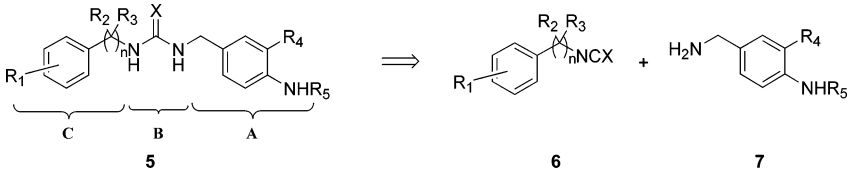
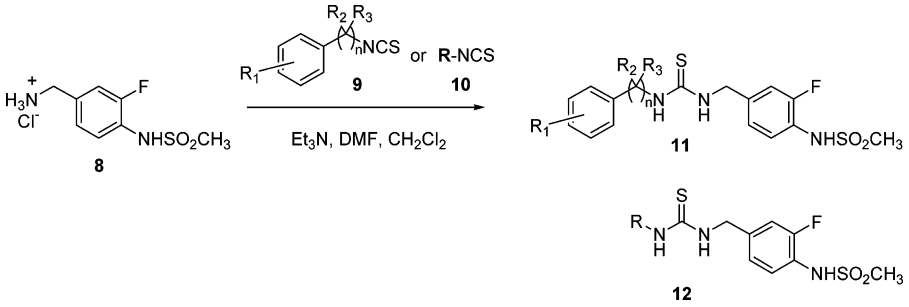
Scheme 1. Synthetic Strategy of 1,3-Diarylalkyl Thioureas and Ureas



Scheme 2



We also recently described some nonvanilloidal dibenzyl thioureas, including the thiourea **4** (Figure 1), which exhibited highly potent competitive antagonistic effects. Compound **4** inhibited [^{3}H]-RTX binding to TRPV1 expressed in Chinese hamster ovary cells, with an affinity of 54 nM, and antagonized capsaicin-induced calcium uptake with an EC_{50} of 8 nM, reflecting a 60-fold greater potency than capsazepine. Moreover, it blocked the response to heat and protons and exerted profound analgesic effect in a dose-dependent manner without pungency, which generally accompanies TRPV1 agonistic effects.

Since our development of compound **4**, a novel potent VR1 antagonist, based on the structures of endogenous, natural, and synthetic TRPV1 agonists and antagonists such as 12-HPETE, capsaicin and capsazepine,[10,11] respectively, we have continued to search for advanced TRPV1 antagonists on the basis of our previous elucidation of the TRPV1 ligand characteristics for agonistic or antagonistic effects.[11b] Thus, we explored the structure−activity relationship of the diarylalkyl thioureas by systematically modulating their three essential pharmacophoric regions. In this paper, we describe the synthesis and antagonistic activities of a series of potent TRPV1 antagonists, which were screened by a cell-based assay utilizing the Ca^{2+} permeability of TRPV1.[12] In addition, we report the structure−activity relationships of the diarylalkyl thioureas as well as the identification of six 1,3-dibenzyl thioureas, which are highly potent TRPV1 antagonists that exhibit Ca^{2+} uptake inhibition in rat DRG neurons with IC_{50}s of 10−100 nM.

Chemistry

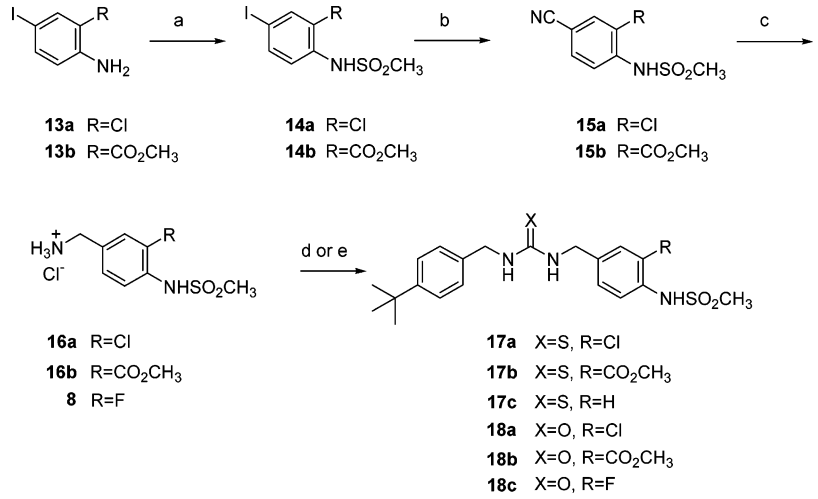
Our approach for the discovery of novel TRPV1 antagonists was based on the systematic investigation of three pharmacophoric regions of the 1,3-diarylalkyl thioureas, because our previous work revealed that even a slight modification of each pharmacophoric region sensitively changed the in vitro activity to TRPV1. Our previous work also implied that the combination of vanilloid equivalents possessing two H-bonding substituents at the 3- and 4-positions of the aromatic ring (A region), thiourea templates for the dipolar interacting region B, and appropriately substituted arylalkyl moieties for the hydrophobic C region is required for the high TRPV1 antagonistic activity. Thus, our study commenced with the progressive modification of those three pharmacophoric regions.

The general synthetic approach for the 1,3-diarylalkyl thioureas **5** features the coupling of the 3,4-disubstituted benzylamines **7** and the arylalkyl isothiocyanate **6**, as illustrated in Scheme 1. The amine **7** was prepared from the corresponding 3,4-disubstituted halobenzene by a sequence including cyanide substitution and reduction of the resulting nitrile.[10] The arylalkyl isothiocyanate **6** was conveniently prepared from the corresponding amines by a di-2-pyridiyl thiocarbonate (DPT) treatment.[10a]

The design and synthesis of the 1,3-diarylalkyl thioureas was initiated by optimizing the carbon chain length between the hydrophobic aromatic part and the thiourea, which had not been explored extensively because the previous studies focused mainly on variations for the TRPV1 agonistic effect.[13] The 1,3-diarylalkyl thioureas with a variety of linker lengths were synthesized, as shown in Scheme 2, by coupling 3-fluoro-4-methylsulfonamidobenzylamine[10] (**8**) with appropriate *tert*-butyl or arylalkyl isothiocyanates. The starting amines were either commercially available or readily prepared by the reduction of the appropriate cyanides, which were obtained by cyanide substitution of the corresponding halides.

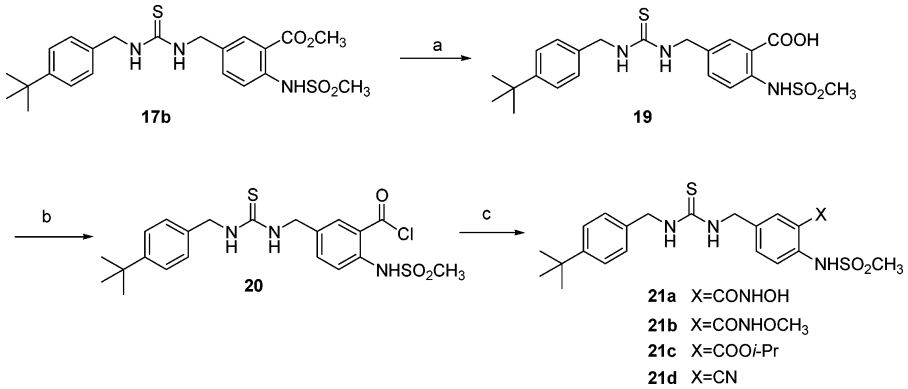
The design and synthesis of the multiple H-bonding region A was based on a variety of vanilloid equivalents possessing the H-bond donors at the 4-position and an additional H-bonding substituent at the 3-position. This rationale was implied from the antagonism, in response to capsaicin, of several diarylalkyl thioureas consisting of a 4-methylsulfonamido group and a 3-substituent, which turned out to be essential for the enhanced antagonistic activity of 1,3-diarylalkyl thioureas, as we previously reported.[10b,11b] Accordingly, our investigation of the A region focused on the incorporation of adequate H-bond donors or acceptors at the 3-position in the presence of a 4-methylsulfonamido group, which was identified as the best bioisostere to replace the phenolic group of the vanilloid moiety.[11b]

Scheme 3[a]



[a] Reagents and conditions: (a) CH_3SO_2Cl, pyridine, CH_2Cl_2; (b) CuCN, DMF, 130 °C; (c) H_2, Pd/C, *c*-HCl, MeOH; (d) 4-*tert*-butylbenzyl isothiocyanate for **17a** and **17b**, Et_3N, DMF, CH_2Cl_2; (e) 4-*tert*-butylbenzyl isocyanate for **18a**−**c**, Et_3N, DMF, CH_2Cl_2.

Scheme 4[a]



[a] Reagents and conditions: (a) LiOH, THF/H_2O (1:1); (b) $(COCl)_2$, C_6H_6, reflux; (c) $NH_2OH.HCl$, pyridinefor **21a**; NH_2OCH_3·HCl, pyridine for **21b**; *i*-PrOH for **21c**; $NH_2SO_2NH_2$, sulfolane,120 °C for **21d**.

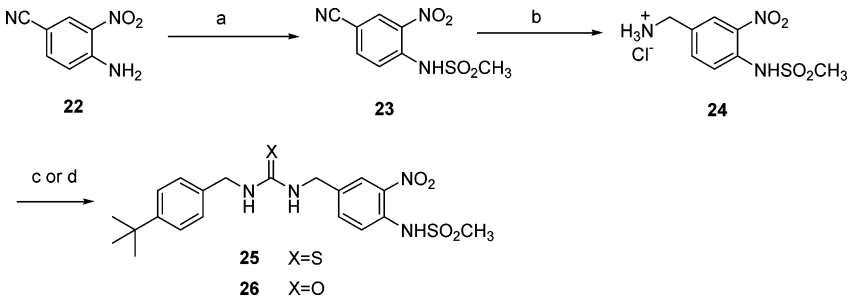
The 1,3-dibenzyl thioureas with a variety of 3,4-disubstituted benzyl moieties as a multiple H-bonding A region were synthesized by coupling *p-tert*-butylbenzyl isothiocyanate with the appropriate 3,4-disustituted benzylamines, as shown in Schemes 3−9. The starting 2-chloro-4-iodoaniline **13a** and 4-iodo-2-methoxycarbonylaniline **13b** were mesylated in the presence of pyridine and then substituted with CuCN, to give the 4-cyano substituted mesylates **15a** and **15b** (Scheme 3). Hydrogenation of the cyanides **15a** and **15b**, followed by coupling with *tert*-butylbenzyl isothiocyanate, afforded the thioureas **17a** and **17b**, the latter of which was hydrolyzed with LiOH to give the carboxylic acid **19**, as shown in Scheme 4. The carboxylic acid **19** was converted to the acid chloride **20** by oxalyl chloride treatment, and then the reaction of the acid chloride **20** with amines, alcohol or sulfonyl urea afforded the corresponding amides (**21a** and **21b**), the ester **21c** and the nitrile **21d**, as shown in Scheme 4.

The 3-nitro-4-methylsulfonamidobenzyl thiourea **25** was synthesized by the coupling of *tert*-butylbenzyl isothiocyanate with 3-nitro-4-sulfonamidobenzylamine **24**, which was prepared by mesylation of 2-nitro-4-cyanoaniline **22** followed by borane reduction of the resulting cyanide **23**, as shown in Scheme 5. By analogy with **25**, the 3-halo-4-methylsufonamidobenzyl thioureas **30a** and **30b** were synthesized from the 2-halo-4-cyanohaloanilines **28a** and **28b**, prepared by bromination or iodination of the 4-cyanoaniline **27**, as described in Scheme 6. In this particular series, the selective mesylation of aniline was carried out by Boc-protection of the benzylamine in advance of the aniline mesylation.

The 1,3-diarylalkyl thioureas possessing the bicyclic template for the A region were synthesized by the coupling of *tert*-butylbenzyl isothiocyanate and the corresponding bicyclo benzylamines, as shown in Scheme 7. The starting benzimidazole-5-carboxylic acid **31a** and benzotriazole-5-carboxylic acid **31b** were converted to the aldehydes **32a** and **32b** by LAH reduction, followed by TPAP oxidation of the resulting alcohols. Reaction of the aldehydes **32a** and **32b** with hydroxylamine and hydrogenation of the resulting oximes **33a** and **33b** afforded the bicyclic benzylamines **34a** and **34b**, which were coupled with *tert*-butylbenzyl isothiocyanate. Hydrogenation of the nitriles **37a** and **37b**, prepared by cyanide displacement of 5-bromobenzodioxole (**36**), afforded the benzylamines **38a** and **38b**, which were converted to the thioureas, **39a** and **39b**, respectively (Scheme 8).

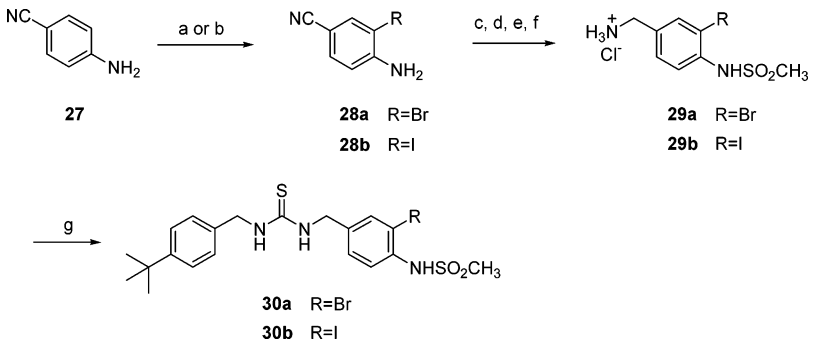
The thioureas **44a**−**h** were synthesized by the coupling of *tert*-butylbenzyl isothiocyanate and the benzylamines **43a**−**h,** as outlined in Scheme 9. The benzylamines **43a**−**h** were prepared, by analogy to **16a** and **16b** in Scheme 3, from the 2-fluoro-4-iodo-*N*-acyl(or

Scheme 5[a]



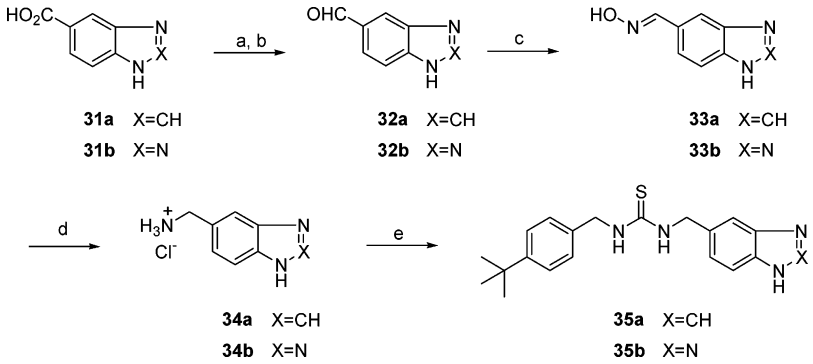
[a] Reagents and conditions: (a) CH_3SO_2Cl, KH, THF; (b) BH_3·THF, *c*-HCl; (c) 4-*tert*-butylbenzyl isothiocyanate for **25**, Et_3N, DMF, CH_2Cl_2; (d) 4-*tert*-butylbenzyl isocyanate for **26**, Et_3N, DMF, CH_2Cl_2.

Scheme 6[a]



[a] Reagents and conditions: (a) NBS, DMF; (b) I_2, H_2O_2, MeOH; (c) BH_3·THF, reflux, 2 *N*-HCl; (d) $(Boc)_2O$, DMAP, CH_2Cl_2, Et_3N; (e) CH_3SO_2Cl, pyridine, CH_2Cl_2; (f) *c*-HCl, MeOH; (g) 4-*t*-Bu-benyzl isothiocyanate, Et_3N, DMF, CH_2Cl_2.

Scheme 7[a]



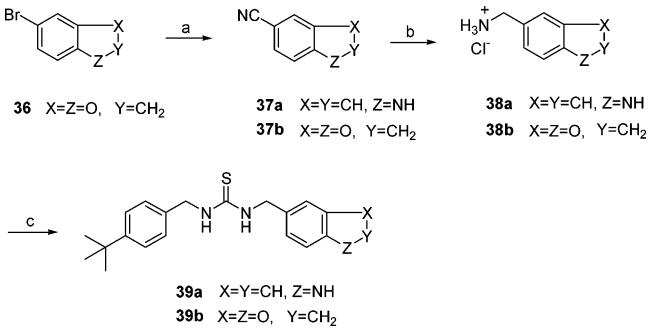
[a] Reagents and conditions: (a) LAH, THF; (b) TPAP, NMO, CH_2Cl_2, THF, DMF; (c) NH_2OH, NaOAc, MeOH; (d) H_2, *c*-HCl, Pd/C, MeOH; (e) 4-*tert*-butylbenzyl isohtiocyanate, Et_3N, CH_2Cl_2, DMF.

alkyl)aminobenzenes **41a**−**h**, obtained by *N*-acylation or *N*-alkylation of 2-fluoro-4-iodoaniline **40**. Hydrolysis of the ester **44g** with LiOH gave the carboxylic acid **44i**.

The B regions, such as thiourea, urea, or guanidine moieties, were investigated by a comparison of their bioisosteric effects on the antagonistic activity of the 1,3-diarylalkyl thioureas, although thiourea is known as one of the high binding templates in the TRPV1 active site.

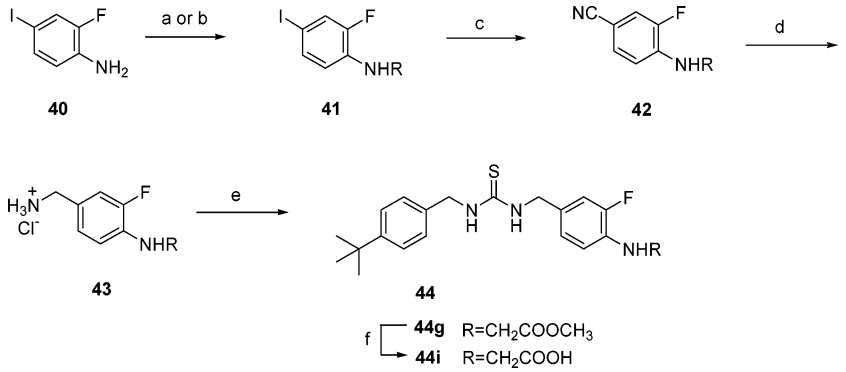
The 1,3-dibenzyl ureas **18a**−**c** and **26** in Schemes 3−5 were synthesized by analogy to the corresponding thioureas. Finally, the members of the guanidine series, **47a**−**c**, were synthesized as shown in Scheme 10. The guanidine **47a** was synthesized from *tert*-butylbenzylamine **45** by a reaction with ethyl isothiocyanate,[14] followed by a coupling of the resulting thiourea **46** with the benzylamine **8**. The cyanoguanidine **47b** was prepared by the reaction of *tert*-butylbenzyl isothiocyanate and sodium cyanamide,[15] followed by the addition of the benzylamine **8** in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI). The methoxy guanidine **47c** was readily prepared by the reaction of the thiourea **4** and methoxyamine.[16]

Scheme 8[a]



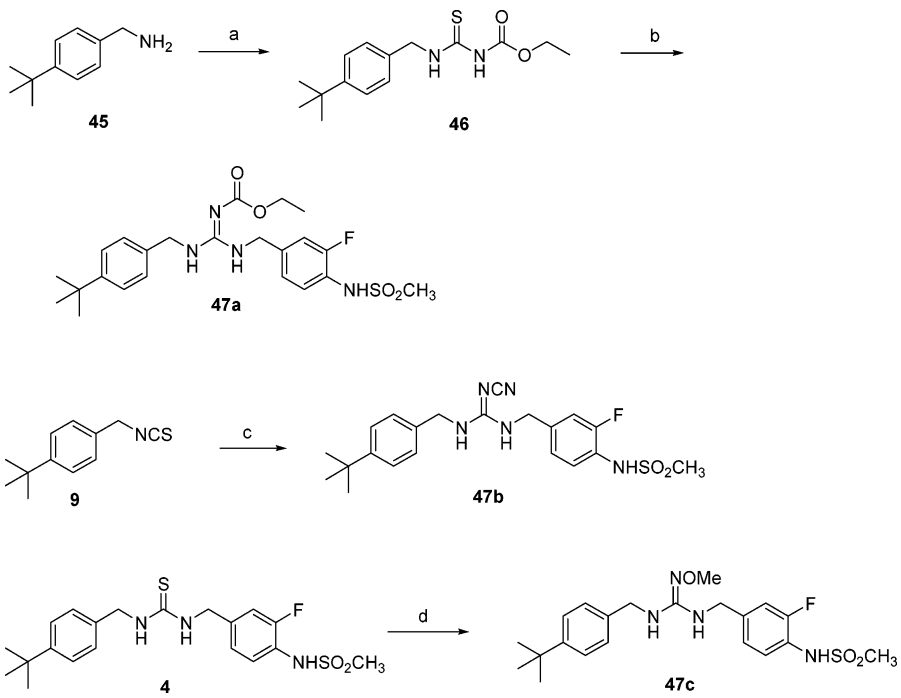
[a] Reagents and conditions: (a) CuCN, DMF, 130 °C; (a) H_2, Pd/C, *c*-HCl, MeOH or LAH, THF; (b) 4-*tert*-butylbenzyl isothiocyanate, Et_3N, DMF, CH_2Cl_2.

Scheme 9[a]



[a] Reagents and conditions: (a) RCl or RBr for **41b** − **41f**, pyridine, CH_2Cl_2; (b) $CHOCCO_2Et$, $NaBH(CN)_3$ for **41g** and **41h**, MeOH, HCl; (c) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 °C; (d) H_2, Pd/C, *c*-HCl, MeOH; (e) 4-*tert*-butyl isothiocyanate, Et_3N, DMF, CH_2Cl_2; (f) LiOH, THF/H_2O (1:1).

Scheme 10[a]



[a] Reagents and conditions: (a) ethyl isothiocyanate, CH_2Cl_2; (b) **8**, EDCI, Et_3N, DMF; (c) NaNHCN, EtOH, then **8**, EDCI, Et_3N, DMF; (d) HgO, $MeONH_2$, Et_3N, DMF.

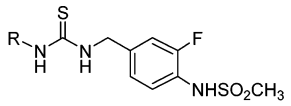
The design and synthesis of the aromatic hydrophobic region C focused on the substituted benzyl moiety, because the benzylic template was superior to the resiniferatoxin-based 3-acyloxy-2-benzylpropyl template in terms of both the in vitro and in vivo activities.[10,11b] Thus, our studies on this series included the effects of the substitution positions and the substituents' sizes on the antagonistic activity. The 1,3-dibenzyl thioureas consisting of the 3-fluoro-4-methylsulfonamidobenzyl moiety as the A region and a variety of substituted benzyl moieties as the C region were synthesized by coupling the benzylamine **8** and the corresponding benzyl isothiocyanate, as described in Scheme 2.

Results and Discussion

We measured the inhibition of Ca^{2+} uptake by the synthesized compounds in rat DRG neurons.[12] As shown in Table 1, methylene was found to be the length-optimized linker between the hydrophobic aromatic part and the thiourea moiety. The antagonistic activity of the 1,3-diarylalkyl thioureas decreased as the length of the linker increased or decreased from that of methylene. In addition, the removal of the aromatic ring or the incorporation of methyl substituents at the methylene linker eliminated the antagonistic effect. These results implied the necessity of having a benzene ring for the hydrophobic region C of the 1,3-diarylalkyl thiourea series. Subsequently, we fully explored the structure−activity relationships of the 1,3-diarylalkyl thioureas, with the methylene chain as the length-optimized linker.

The antagonistic effects of the 1,3-dibenzyl thioureas with the 4-methanesulfonamido group in A-region were highly dependent on the nature of the 3-position substituent of the aromatic template, as summarized in Table 2. The 3-methoxycarbonyl analogue **17b** exhibited an antagonistic activity with an IC_{50} of 720 nM, while the analogue **21c**, with the bulkier isopropyloxycarbonyl group, showed a significantly reduced antagonistic activity, although both substituents are not beneficial

Table 1. Ca^{2+} Uptake Inhibition by the Linker-Diversified Diarylalkyl Thioureas



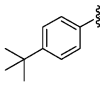
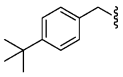
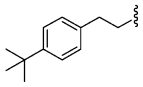
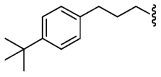
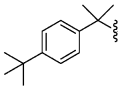
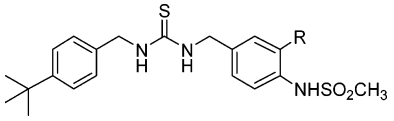
compound	R	antagonist activity IC_{50} (nM)
11a		1,600 ± 40
4		37 ± 6
11b		370 ± 30
11c		2,000 ± 30
11d		>30,000
12a		>30,000

Table 2. Ca^{2+} Uptake Inhibition by the 3-Substitutent-Diversified Dibenzyl Thioureas

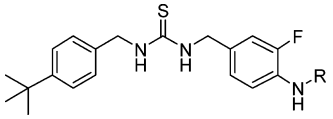


compd	R	antagonist activity, IC_{50} (nM)
4	F	37 ± 6
17a	Cl	8.4 ± 2.4
17b	$COOCH_3$	720 ± 460
17c	H	110 ± 10
21a	CONHOH	>30000
21b	$CONHOCH_3$	>30000
21c	COO*i*-Pr	>30000
21d	CN	5900 ± 240
25	NO_2	71 ± 6
30a	Br	80 ± 20
30b	I	41 ± 5

as compared to hydrogen (**17c**).[10b] The introduction of the alkoxyamido groups (**21a** and **21b**) as the ester isosteres was detrimental to the antagonistic activity. Interestingly, the analogues with H-bond acceptors, such as cyano (**21d**), nitro (**25**) and halogen groups (**4**, **17a**, **30a**, and **30b**), at the 3-position were preferred and exhibited highly potent antagonistic effects. In particular, the 3-chloro analogue **17a** showed an excellent antagonistic effect, with an IC_{50} of 8.4 nM. However, the antagonistic activities of the 3-halogen substituted analogues did not quantitatively correlate to the electronegativities of the halides.

After we identified the ideal H-bonding groups at the 3-position of the aromatic template, we turned our attention to the 4-alkylamino or amido substituents, which are capable of replacing the methansulfonamido group. Thus, we introduced a variety of alkylamino or amido groups at the 4-position of the aromatic system

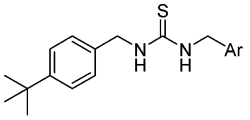
Table 3. Ca^{2+} Uptake Inhibition by the *N*-Acyl- and Alkyl-Substituted Dibenzyl Thioureas

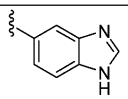
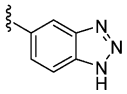
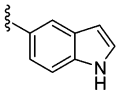
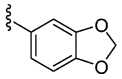


compd	R	antagonist activity, IC_{50} (nM)	agonist activity
44a	H	NE[a]	36% at 10 μM
44b	$COCH_3$	1500 ± 240	
44c	$COCF_3$	>30000	
44d	$COOCH_3$	NE	72% at 10 μM
44e	$COOCH_2CH_3$	>30000	
44f	$COCH_2OCH_3$	10900 ± 1200	
44g	CH_2COOCH_3	3600 ± 20	
44h	$CH_2COOCH_2CH_3$	>30000	
44i	CH_2COOH	NE	19% at 30 μM

[a] NE: not effective.

Table 4. Ca^{2+} Uptake Inhibition by the Thioureas Possessing Bicyclic Vanilloid Equivalents



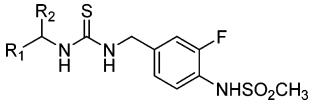
compound	Ar	antagonist activity IC_{50} (nM)
35a		790 ± 120
35b		50 ± 7
39a		1,800 ± 1100
39b		NE[a]

[a] NE: not effective.

of the A-region, with the fluoro substituent fixed at the 3-position, as shown in Table 3. None of them exhibited better antagonistic activity than the 4-methansulfonamido analogue **4**. Instead, the amine **44a** and the amides **44d** and **44i** revealed agonistic effects. The acidity increase of NH by incorporating a stronger electron withdrawing group such as $COCF_3$ (**44c**) or by appending additional H-bond donors or acceptors (**44f**−**i**) significantly reduced the antagonistic activity. In conclusion, changing the size of the alkyl group or introducing modifications, which are likely to change the NH acidity, sensitively reduce or eliminate the antagonistic activity of the 1,3-dibenzyl thioureas.

In consideration of the optimized substitution pattern of the vanilloid equivalent (A region), we further examined the replacement of the benzene ring with bicyclic templates, which mimic the 3,4-disubstituted benzene moieties. Thus, five-membered heterocyclic systems possessing H-bonding groups at the corresponding 3- and 4-positions of the benzene ring were fused to the existing benzene moiety, as shown in Table 4. As we anticipated, the bicyclic analogues (**35a**, **35b**, and **39a**)

Table 5. Ca^{2+} Uptake Inhibition by the Hydrophobic Aromatic Part-Diversified Thioureas



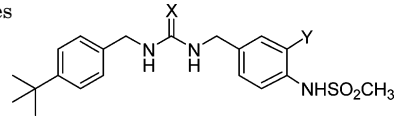
	R_1	R_2	antagonist activity, IC_{50} (nM)	agonist activity
11e	phenyl	H	9700 ± 20	
11f	4-CH_3-phenyl	H	1500 ± 160	
11g	2-CH_3-phenyl	H	670 ± 50	
11h	3-CH_3-phenyl	H	1300 ± 240	
11i	4-Et-phenyl	H	1100 ± 1000	
11j	4-*n*-Pr-phenyl	H	460 ± 30	
11k	4-*n*-Bu-phenyl	H	470 ± 20	
11l	4-*i*-Pr-phenyl	H	520 ± 230	
11m	2-*t*-Bu-phenyl	H	NE[a]	28% at 10 μM
11n	3,5-di-*t*-Bu-phenyl	H	200 ± 60	
11o	4-Cl-phenyl	H	340 ± 10	
11p	4-Br-phenyl	H	570 ± 250	
11q	4-I-phenyl	H	630 ± 390	
11r	phenyl	Ph	2500 ± 730	
11s	4-Ph-phenyl	H	>30000	
12b	1-naphthyl	H	2600 ± 730	
12c	9-anthracenyl	H	1100 ± 800	

[a] NE: not effective.

with the H-bond donors at the 4-position exhibited highly potent or moderate antagonistic activities, while the analogue **39b**, with only a H-bond acceptor exhibited no antagonism. In particular, the bicyclic analogue **35b,** with an additional H-bond acceptor at the 3-position, were 36-fold more potent than the analogue **39a** without an H-bond acceptor at the 3-position. The higher potency of the benzotriazole analogue (**35b**), as compared to that of the benzimidazole analogue (**35a**), is likely to be due to the appropriate NH acidity of the benzotriazole template (**35b**).[17]

The alkyl substituted aromatic templates of the lipophilic part C were evaluated by a comparison of the antagonistic activities of the 1,3-dibenzyl thioureas with a variety of substituents at the aromatic ring, which are characterized by their steric or electronic effects (Table 5). The 3-fluoro-4-methansulfonamidobenzyl thiourea system for the A and B regions was employed as the standard template. The incorporation of aliphatic substituents in the lipophilic aromatic templates was beneficial for the antagonistic activity, regardless of the substitution position. The antagonistic activity increased as the size of the aliphatic substituent at the para position increased up to that of a *tert*-butyl group (**11f, 11i**−***l***, and **4**), while it dropped as the substituent size exceeded that of a *tert*-butyl group (**11r**, **11s**, **12b**, and **12c**). The *tert*-butyl group at the para position was superior to any of the other substituents, in terms of antagonistic activity. In contrast, the analogue **11m** with the *tert*-butyl substituent at the ortho position exhibited an agonistic effect, while the 3,5-disubstituted analogue **11n** showed 5-fold less potent antagonistic activity as compared to the *p-tert*-butyl substituted analogue **4**. The replacement of the benzene ring (**11e**) with naphthalene (**12b**) or anthracene (**12c**) increased the antagonistic activity. However, the biphenyl (**11s**) substitution of the benzene ring significantly dropped the antagonistic activity, which implies the intolerance for a highly bulky aromatic template. The analogues (**11o**−**q**) with a halogen at the para position exhibited equipotent antagonistic activities to those of the *p*-

Table 6. Ca^{2+} Uptake Inhibition by the 1,3-Dibenzyl Ureas and Guanidines



	X	Y	antagonist activity, IC_{50} (nM)
18a	O	Cl	240 ± 20
18b	O	$COOCH_3$	3700 ± 30
18c	O	F	300 ± 30
26	O	NO_2	240 ± 20
47a	$NCO_2CH_2CH_3$	F	6200 ± 10
47b	NCN	F	730 ± 30
47c	$NOCH_3$	F	3100 ± 300

propyl (**11j**), butyl (**11k**), and isopropyl (**11*l***) analogues. Thus, the electronegativity of the para substituent does not affect the antagonistic potency. Some unusual similarity of the structure−activity relationship, for the lipophilic part C, between that for agonist[13b] and antagonist is noteworthy.

Finally, the thiourea template of the B region was evaluated by replacing it with a urea or guanidine template, as shown in Table 6. The analogues (**18a**−**c**, and **26**) with the urea template, which replaced the thiourea template of the representative thiourea antagonists, displayed about 5 to 30-fold lower potencies as compared to the corresponding thioureas (**17a**, **17b**, **4**, and **25**). In addition, the guanidine analogues (**47a**−**c**) exhibited lower antagonistic activities than the corresponding urea analogue **18c**. Taken together, thiourea seems to serve as the best template for the B region, although the urea and guanidine templates might still be utilized as its bioisosteres.

In conclusion, we have discovered novel TRPV1 antagonists, of which six (**17a**, **17c**, **25**, **30a**, **30b**, and **35b**) exhibited Ca^{2+} uptake inhibition in rat DRG neuron with IC_{50} between 10 and 100 nM. Moreover, the structure−activity relationship of the diarylalkyl thiourea series for the TRPV1 antagonistic effect was fully established.

In the 1,3-diarylalkyl thiourea series, methylene turned out to be the optimal linker between thiourea and the aromatic part of the hydrophobic region C, which is essential for the TRPV1 antagonistic effect. The methansulfonamido group was validated as the best substituent at the 4-position of the H-bonding aromatic part, while changing the methylsulfonyl group to other electron-withdrawing groups reduced the antagonistic activity, regardless of the size or electronic character. For the 3-substituent of the H-bonding aromatic region A, the H-bond acceptors were highly preferred. The introduction of alkyl or halogen substituents to the aromatic ring of the C-region is beneficial for the higher antagonistic activity. The antagonistic activity generally increased as the size of the substituent at the para position increased up to that of a *tert*-butyl group, while bulkier substituents were detrimental or intolerable. The thiourea template for the dipolar interacting B-region was superior to the urea and guanidine templates (Figure 2).

These SAR study results provide an important basis for the further identification of advanced TPRV1 antagonists, which would be useful for clinical applications. The highly potent antagonists with novel vanilloid

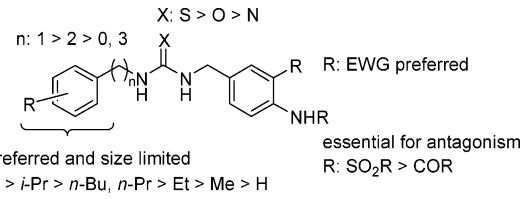


Figure 2. Summary of SAR.

equivalents are currently undergoing in vivo evaluation, and the results will be reported in due course.

Experimental Section

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. ^{1}H NMR spectra were recorded on a JEOL JNM-LA 300 at 300 MHz. Chemical shifts are reported in ppm units from an internal Me_4Si 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.

***N*-4-[([4-(*tert*-Butyl)anilino]carbothioylamino)methyl]-2-fluorophenylmethanesulfonamide (11a).** To a solution of 1,1′-thiocarbonyl diimidazole (200 mg, 1.1 mmol) in CH_2Cl_2 (7 mL) was added 4-*tert*-butylaniline (0.2 mL, 1.3 mmol) dropwise. After stirring for 8 h at ambient temperature, a solution of amine **8** (290 mg, 1.1 mmol) and Et_3N (0.23 mL, 1.7 mmol) in a mixture of DMF (0.5 mL) and CH_2Cl_2 (2 mL) was added dropwise using a cannula to the reaction mixture, which was stirred for 24 h. The reaction mixture was acidified with 2 N aqueous HCl and extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:2) afforded 200 mg (45%) of the thiourea **11a** as a white solid: mp = 65 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.00 (bs, 1H), 7.50 (t, 1H, J = 8.3 Hz), 7.43 (d, 2H, J = 9.2 Hz) 7.15−7.11 (m, 3H), 7.06 (d, 1H, J = 8.4 Hz), 6.78 (bs, 1H), 6.33 (t, 1H, J = 5.8 Hz), 4.86 (d, 2H, J = 5.9 Hz), 3.00 (s, 3H), 1.29 (s, 9H); LRMS (FAB) *m/z* 410 (M + H$^+$). Anal. ($C_{19}H_{24}FN_3O_2S_2$) C, H, N, S.

Representative Procedure for the Synthesis of 11b−12d. *N*-(4-[([4-(*tert*-Butyl)phenethyl]aminocarbothioyl)amino]methyl-2-fluorophenyl)methanesulfonamide (11b). To a solution of **8**[10] (150 mg, 0.7 mmol) in DMF (0.5 mL) and CH_2Cl_2 (3 mL) were added 4-*tert*-butylphenethyl isothiocyanate (180 mg, 0.8 mmol) and Et_3N (0.19 mL, 1.4 mmol). The reaction mixture was stirred for 2 h at ambient temperature and was diluted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:1) afforded 297 mg (100%) of **11b** as a white solid: mp = 128 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46 (t, 1H, J = 8.3 Hz), 7.01−7.31 (m, 6H), 6.68 (bs, 1H), 4.60 (bs, 2H), 3.67 (bs, 2H), 2.99 (s, 3H), 2.85 (bs, 2H), 1.27 (s, 9H); LRMS (EI) *m/z* 437 (M$^+$). Anal. ($C_{21}H_{28}FN_3O_2S_2$) C, H, N, S.

***N*-[4-([(3-[4-(*tert*-Butyl)phenyl]propylamino)carbothioyl]aminomethyl)-2-fluorophenyl]methanesulfonamide (11c).** Yield: 55%. White solid: mp = 113−115 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.41−7.46 (t, 1H, J = 8.0 Hz), 7.26−7.28 (d, 2H, J = 8.0 Hz), 7.04−7.14 (m, 4H), 6.81 (bs, 1H), 4.62 (bs, 2H), 3.38 (bs, 2H), 2.97 (s, 3H), 2.58−2.62 (t, 2H, J = 7.3 Hz), 1.90 (s, 2H), 1.26 (s, 9H); LRMS (EI) *m/z* 451 (M$^+$). Anal. ($C_{22}H_{30}FN_3O_2S_2$) C, H, N, S

***N*-[4-([(1-[4-(*tert*-Butyl)phenyl]-1-methylethylamino)carbothioyl]aminomethyl)-2-fluorophenyl]methanesulfonamide (11d).** Yield: 34%. White solid: mp = 154−155 °C. ^{1}H NMR (acetone-d_6, 300 MHz) δ 7.32−7.39 (m, 5H), 7.22 (bs, 1H), 6.77−6.88 (m, 2H), 4.68 (d, 2H, J = 5.9 Hz), 3.00 (s, 3H), 1.71 (s, 6H), 1.27 (s, 9H); LRMS (FAB) *m/z* 452 (M + H$^+$). Anal. ($C_{22}H_{30}FN_3O_2S_2$) C, H, N, S

***N*-[4-({[(Benzylamino)carbothioyl]amino}methyl)-2-fluorophenyl]methanesulfonamide (11e).** Yield: 90%. Pale yellow solid: mp = 134−135 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.14−7.48 (m, 8H), 4.83 (d, 2H, J = 7.1 Hz), 4.80 (d, 2H, J = 4.1 Hz), 3.02 (s, 3H); LRMS (EI) *m/z* 367 (M$^+$). Anal. ($C_{16}H_{18}FN_3O_2S_2$) C, H, N, S.

***N*-2-Fluoro-4-[([(4-methylbenzyl)amino]carbothioylamino)methyl]phenylmethanesulfonamide (11f).** Yield: 58%. White solid: mp = 164−166 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.41 (t, 1H, J = 8.0 Hz), 7.07−7.18 (m, 6H), 4.72 (s, 2H), 4.64 (s, 2H), 2.95 (s, 3H), 2.30 (s, 3H); LRMS (FAB) *m/z* 382 (M + H$^+$). Anal. ($C_{17}H_{20}FN_3O_2S_2$) C, H, N, S.

***N*-2-Fluoro-4-[([(2-methylbenzyl)amino]carbothioylamino)methyl]phenylmethanesulfonamide (11g).** Yield: 81%. White solid: mp = 157−158 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45 (t, 1H, J = 8.3 Hz), 7.02−7.20 (m, 6H), 6.58 (s, 1H), 4.68 (s, 2H), 4.47 (s, 2H), 2.97 (s, 3H), 2.28 (s, 3H); LRMS (FAB) *m/z* 382 (M + H$^+$). Anal. ($C_{17}H_{20}FN_3O_2S_2$) C, H, N, S.

***N*-2-Fluoro-4-[([(3-methylbenzyl)amino]carbothioylamino)methyl]phenylmethanesulfonamide (11h).** Yield: 93%. White solid: mp = 107−110 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.48 (t, 1H, J = 8.1 Hz), 6.96−7.24 (m, 6H), 6.48 (s, 1H), 4.70 (s, 2H), 4.44 (s, 2H), 2.99 (s, 3H), 2.31 (s, 3H); LRMS (FAB) *m/z* 382 (M + H$^+$). Anal. ($C_{17}H_{20}FN_3O_2S_2$) C, H, N, S.

***N*-{4-[({[(4-Ethylbenzyl)amino]carbothionyl}methyl)-2-luorophenyl]methanesulfonamide (11i).** Yield: 62%. White solid: mp = 148−149 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.08−7.42 (m, 7H), 2.94 (s, 4H), 2.61 (q, 2H, J = 7.5 Hz), 1.20 (t, 3H, J = 7.5 Hz); LRMS (ESI) *m/z* 396.2 (M + H$^+$). Anal. ($C_{18}H_{22}FN_3O_2S_2$) C, H, N, S.

***N*-{2-Fluoro-4-[({[(4-propylbenzyl)amino]carbothioyl}amino)methyl]phenyl}methanesulfonamide (11j).** Yield: 40%. White solid: mp = 135 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.43 (t, 1H, J = 8.1 Hz), 7.18 (d, 2H, J = 7.6 Hz), 7.13 (d, 2H, J = 7.9 Hz), 7.03−6.96 (m, 2H), 6.61 (s, 1H), 4.69 (s, 2H), 4.54 (s, 2H), 2.98 (s, 3H), 2.54 (t, 2H, J = 7.6 Hz), 1.60 (qd, 2H, J = 7.4, 7.6 Hz), 0.90 (t, 3H, J = 7.3 Hz); LRMS (ESI) *m/z* 410.2 (M + H$^+$). Anal. ($C_{19}H_{24}FN_3O_2S_2$) C, H, N, S.

***N*-{4-[({[(4-Butylbenzyl)amino]carbothioyl}amino)methyl]-2-fluorophenyl}methanesulfonamide (11k).** Yield: 84%. White solid: mp = 133 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.94−7.37 (m, 7H), 4.63 (s, 2H), 4.49 (s, 2H), 2.93 (s, 3H), 2.52 (t, 2H, J = 7.7 Hz), 1.45−1.55 (m, 2H), 1.16−1.33 (m, 2H), 0.85 (t, 3H, J = 7.2 Hz); LRMS (ESI) *m/z* 424.3 (M + H$^+$). Anal. ($C_{20}H_{26}FN_3O_2S_2$) C, H, N, S.

***N*-2-Fluoro-4-[([(4-isopropylbenzyl)amino]carbothioylamino)methyl]phenylmethanesulfonamide (11*l*).** Yield: 91%. White solid: mp = 113−115 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.46 (t, 1H, J = 8.0 Hz), 7.24−7.30 (m, 3H), 7.08 (t, 2H, J = 9.0 Hz), 6.85 (s, 1H), 4.72 (s, 2H), 4.59 (s, 2H), 3.02 (s, 3H), 2.85−2.97 (m, 1H), 1.25−1.27 (d, 6H, J = 6.8 Hz); LRMS (FAB) *m/z* 410 (M + H$^+$). Anal. ($C_{19}H_{24}FN_3O_2S_2$) C, H, N, S.

***N*-(4-{[({[(2-*tert*-Butyl)benzyl]amino}carbothioyl)amino]methyl}-2-fluorophenyl)methanesulfonamide (11m).** Yield: 57%. Pale yellow solid: mp = 102−104 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.00−7.50 (m, 7H), 4.81 (s, 2H), 4.65 (s, 2H), 3.00 (s, 3H), 1.35 (s, 9H); LRMS (EI) *m/z* 423 (M$^+$). Anal. ($C_{20}H_{26}FN_3O_2S_2$) C, H, N, S.

***N*-(4-[([3,5-di(*tert*-Butyl)benzyl]aminocarbothioyl)amino]methyl-2-fluorophenyl)methanesulfonamide (11n).** Yield: 72%. White solid: mp = 80 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44 (t, 1H, J = 7.8 Hz), 7.36 (s, 1H), 7.11 (s, 2H), 7.04−7.00 (m, 2H), 6.61 (bs, 1H), 4.73 (s, 2H), 4.54 (s, 2H), 2.98 (s, 3H), 1.27 (s, 18H); LRMS (FAB) *m/z* 480 (M + H$^+$). Anal. ($C_{24}H_{34}FN_3O_2S_2$) C, H, N, S.

***N*-(4-Chlorobenzyl)-*N*′-(3-fluoro-4-[methyl(dimethylene)-6-sulfanyl]aminobenzyl)thiourea (11o).** Yield: 52%. White solid: mp = 134−135 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.44 (t, 1H, J = 8.0 Hz), 7.18−7.29 (m, 4H), 7.05 (t, 2H, J = 9.2 Hz), 6.60 (s, 1H), 4.67 (s, 2H), 4.60 (s, 2H), 2.98 (s, 3H); LRMS (EI) *m/z* 401 (M$^+$). Anal. ($C_{16}H_{17}ClFN_3O_2S_2$) C, H, N, S.

***N*-(4-Bromobenzyl)-*N'*-(3-fluoro-4-[methyl(dimethylene)-6-sulfanyl]aminobenzyl)thiourea (11p).** Yield: 73%. White solid: mp = 153 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42−7.51 (m, 4H), 7.30 (d, 2H, J = 8.3 Hz), 7.14−7.22 (m, 2H), 4.83 (t, 4H, J = 6.3 Hz), 3.02 (s, 3H); LRMS (EI) m/z 444 (M^+). Anal. ($C_{16}H_{17}BrFN_3O_2S_2$) C, H, N, S.

***N*-2-Fluoro-4-[([(4-iodobenzyl)amino]carbothioylamino)methyl]phenylmethanesulfonamide (11q).** Yield: 57%. White solid: mp = 171 °C. ^{1}H NMR (acetone-d_6, 300 MHz) δ 8.39 (s, 1H), 7.69 (d, 2H, J = 8.3 Hz), 7.42−7.51 (m, 2H), 7.14−7.22 (m, 4H), 4.83 (dd, 4H, J = 5.6, 11.2 Hz), 3.02 (s, 3H); LRMS (EI) m/z 492 (M^+). Anal. ($C_{16}H_{17}FIN_3O_2S_2$) C, H, N, S.

***N*-4-[([(1,1′-Biphenyl]-4-ylmethyl)amino]carbothioylamino)methyl]-2-fluorophenylmethanesulfonamide (11r).** Yield: 57%. White solid: mp = 171−172 °C. ^{1}H NMR (acetone-d_6, 300 MHz) δ 8.38 (s, 1H), 7.31−7.65 (m, 9H), 7.24 (d, 1H, J = 11.5 Hz), 7.18 (d, 1H, J = 8.3 Hz), 4.83 (d, 2H, J = 7.1 Hz), 4.80 (s, 2H), 3.00 (s, 3H); LRMS (EI) m/z 443 (M^+). Anal. ($C_{22}H_{22}FN_3O_2S_2$) C, H, N; S.

***N*-[4-([(Benzhydrylamino)carbothioyl]aminomethyl)-2-fluorophenyl]methanesulfonamide (11s).** Yield: 85%. White solid: mp = 175 °C. ^{1}H NMR (acetone-d_6, 300 MHz) δ 8.38 (s, 1H), 7.85 (d, 1H, J = 7.6 Hz), 7.55 (s, 1H), 7.46 (t, 1H, J = 8.3 Hz), 7.13−7.35 (m, 8H), 6.89 (d, 1H, J = 8.0 Hz), 4.86 (s, 2H) 4.85 (s, 2H), 3.01 (s, 3H); LRMS (EI) m/z 443 (M^+). Anal. ($C_{22}H_{22}FN_3O_2S_2$) C, H, N, S.

***N*-[4-([(*tert*-Butylamino)carbothioyl]aminomethyl)-2-fluorophenyl]methanesulfonamide (12a).** Yield: 49%. White solid: mp = 158−159 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.45 (t, 1H, J = 8.2 Hz), 7.11−7.03 (m, 2H), 6.68 (bs, 1H), 4.77 (s, 2H), 2.73 (s, 3H), 1.36 (s, 9H); LRMS (FAB) m/z 334 (M + H^+). Anal. ($C_{13}H_{20}FN_3O_2S_2$) C, H, N, S.

***N*-2-Fluoro-4-[([(1-naphthylmethyl)amino]carbothioylamino)methyl]phenylmethanesulfonamide (12b).** Yield: 95%. White solid: mp = 65−67 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.74−7.92 (m, 3H), 7.43−7.52 (m, 2H), 7.34−7.39 (m, 2H), 7.21−7.27 (m, 1H), 6.99 (dd, 1H, J = 1.3, 11.0 Hz), 6.90 (d, 1H, J = 8.3 Hz), 6.80 (s, 1H), 4.98 (s, 2H), 4.55 (s, 2H), 2.84 (s, 3H); LRMS (EI) m/z 417 (M^+). Anal. ($C_{20}H_{20}FN_3O_2S_2$) C, H, N, S.

***N*-4-[([(1,4-Dihydro-9-anthracenylmethyl)amino]carbothioylamino)methyl-2-fluorophenylmethanesulfonamide (12c).** Yield: 47%. Yellow solid: mp = 188−189 °C. ^{1}H NMR (THF-d_8, 400 MHz) δ 10.79 (s, 1H), 8.68 (s, 1H), 8.47 (s, 1H), 8.40 (d, 2H, J = 8.8 Hz), 8.02 (d, 2H, J = 8.4 Hz), 7.53 (td, 2H, J = 1.3, 6.5 Hz), 7.40−7.46 (m, 3H), 7.18 (dd, 1H, J = 1.4, 11.5 Hz), 7.07 (d, 2H, J = 9.2 Hz), 6.84 (bs, 1H), 5.70 (d, 2H, J = 4.2 Hz), 4.76 (d, 2H, J = 5.6 Hz), 4.68 (s, 1H), 2,85 (s, 3H); LRMS (EI) m/z 467 (M^+). Anal. ($C_{24}H_{22}FN_3O_2S_2$) C, H, N, S.

***N*-(2-Chloro-4-iodophenyl)methanesulfonamide (14a).** To a solution of 2-chloro-4-iodoaniline (1.2 g, 4.7 mmol) in CH_2Cl_2 (12 mL) at 0 °C were added pyridine (1.9 mL, 23.7 mmol) and methanesulfonyl chloride (1.8 mL, 23.7 mmol). The reaction mixture was stirred for 1 h at ambient temperature, and the reaction was quenched by an addition of 1.5 N aqueous HCl. The resulting mixture was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:2) afforded 1.46 g (93%) of **14a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.74 (d, 1H, J = 2.0 Hz), 7.60 (dd, 1H, J = 2.0, 8.5 Hz), 7.38 (d, 1H, J = 8.6 Hz), 6.73 (bs, 1H), 3.00 (s, 3H); LRMS (FAB) m/z 331 (M + H^+). Anal. ($C_7H_7ClINO_2S$) C, H, N, S

***N*-(4-Cyano-2-chlorophenyl)methanesulfonamide (15a).** To a solution of **14a** (1.46 g, 4.4 mmol) in DMF (10 mL) were added $Zn(CN)_2$ (620 mg, 5.3 mmol) and $Pd(PPh_3)_4$ (138 mg, 0.1 mmol). The reaction mixture was stirred for 1.5 h at 80 °C, cooled to ambient temperature, and then diluted with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 2:1) afforded 1.02 g (100%) of **15a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.75 (d, 1H, J = 8.5 Hz), 7.71 (d, 1H, J = 1.7 Hz), 7.59 (dd, 1H, J = 1.7, 8.6 Hz), 7.14 (bs, 1H), 3.11 (s, 3H); LRMS (FAB) m/z 230 (M + H^+). Anal. ($C_8H_7ClN_2O_2S$) C, H, N, S

***N*-(4-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)amino]-methyl-2-chlorophenyl)methanesulfonamide (17a).** To a solution of **15a** (1.0 g, 4.3 mmol) in MeOH (40 mL) were added 10% Pd on carbon (300 mg) and *c*-HCl (3 mL). The reaction mixture was stirred under an H_2 atmosphere using a balloon for 1 h (TLC check) and filtered through a pad of Celite, which was then rinsed with Et_2O. The filtrate was concentrated and dried on a vacuum pump to give 1.16 g (99%) of the crude **16a**, which was directly used for the next step.

To a solution of **16a** (1.2 g, 4.3 mmol) in DMF (3 mL) and CH_2Cl_2 (14 mL) were added 4-*tert*-butylbenzyl isothiocyanate (1.1 g, 5.2 mmol) and Et_3N (1.2 mL, 8.6 mmol). The reaction mixture was stirred for 2 h at ambient temperature and diluted with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:1) afforded 643 mg (34%) of **17a** as a white solid: mp = 79−82 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.50 (d, 1H, J = 8.3 Hz), 7.37 (d, 2H, J = 8.3 Hz), 7.35 (d, 1H, J = 2.0 Hz), 7.23 (d, 2H, J = 8.3 Hz), 7.13 (d, 1H, J = 7.1 Hz), 6.92 (bs, 1H), 4.69 (s, 2H), 4.58 (s, 2H), 2.98 (s, 3H), 1.30 (s, 9H); LRMS (FAB) m/z 440 (M + H^+). Anal. ($C_{20}H_{26}ClN_3O_2S_2$) C, H, N, S.

Methyl 5-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)-amino]methyl-2-[(methylsulfonyl)amino]benzenecarboxylate (17b). The methyl ester **17b** was prepared from **13b** by the procedure described for **17a** (78% yield). White solid: mp = 165−166 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.4 (bs, 1H), 7.99 (s, 1H), 7.57 (d, 1H, J = 8.5 Hz), 7.41 (d, 1H, J = 8.4 Hz), 7.36 (d, 2H, J = 8.0 Hz), 7.23 (d, 2H, J = 8.0 Hz), 4.71(s, 2H), 4.62 (s, 2H), 3.93 (s, 3H), 2.84 (s, 3H), 1.31 (s, 9H); LRMS (FAB) m/z 464 (M + H^+). Anal. ($C_{22}H_{29}N_3O_4S_2$) C, H, N, S.

***N*-(4-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)amino]-methylphenyl)methanesulfonamide (17c).** The synthetic procedure for the methyl ester **17c** is described in the refs 10.

***N*-(4-[([4-(*tert*-Butyl)benzyl]aminocarbonyl)amino]-methyl-2-chlorophenyl)methanesulfonamide (18a).** To a solution of amine **16a** (325 mg, 1.2 mmol) in CH_2Cl_2 (3 mL) were added Et_3N (0.25 mL, 1.8 mmol), DMF (1 mL), and 4-*tert*-butylbenzyl isocyanate (340 mg, 1.8 mmol) dropwise. The reaction mixture was stirred for 24 h at ambient temperature, acidified with 2 N aqueous HCl, and then extracted with CH_2Cl_2. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:1) afforded 50 mg (10%) of the urea **18a** as a white solid: mp = 60−61 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.57 (d, 1H, J = 8.3 Hz), 7.36−7.31 (m, 3H), 7.23−7.15 (m, 3H), 6.70 (bs, 1H), 4.64 (bs, 2H), 4.35(d, 4H, J = 5.9 Hz), 2.97 (s, 3H), 1.29 (s, 9H); LRMS (ESI) m/z 424.0 (M + H^+). Anal. ($C_{20}H_{26}ClN_3O_3S$) C, H, N, S.

Methyl 5-[([4-(*tert*-Butyl)benzyl]aminocarbonyl)amino]methyl-2-[(methylsulfonyl)amino]benzenecarboxylate (18b). The urea **18b** was prepared (54% yield) from **13b** by the procedure described for **18a**: White solid: mp = 140 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.3 (bs, 1H), 7.92 (d, 1H, J = 2.0 Hz), 7.61 (d, 1H, J = 8.4 Hz), 7.41 (dd, 1H, J = 2.4, 8.6 Hz), 7.32 (d, 2H, J = 8.4 Hz), 7.19 (d, 2H, J = 8.6 Hz), 4.98 (dt, 2H, J = 5.7, 20 Hz), 4.00 (t, 2H, J = 5.9 Hz), 3.89 (s, 3H), 2.99 (s, 3H), 1.27 (s, 9H); LRMS (ESI) m/z 448.1 (M + H^+). Anal. ($C_{22}H_{29}N_3O_5S$) C, H, N, S.

***N*-(4-[([4-(*tert*-Butyl)benzyl]aminocarboyl)amino]-methyl-2-fluorophenyl)methanesulfonamide (18c).** The urea **18c** was prepared (53% yield) from **8** by the procedure described for **18a**. White solid: mp = 95 °C. ^{1}H NMR ($CDCl_3$ + CD_3OD, 300 MHz) δ 7.34 (t, 1H, J = 8.3 Hz), 7.26 (d, 2H, J = 8.4 Hz), 7.13 (d, 2H, J = 8.2 Hz), 6.99−6.93 (m, 2H), 4.24 (s, 2H), 4.22 (s, 2H), 2.91 (s, 3H), 1.23 (s, 9H); LRMS (FAB) m/z 408 (M + H^+). Anal. ($C_{20}H_{26}FN_3O_3S$) C, H, N, S.

5-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)amino]-methyl-2-[(methylsulfonyl)amino]benzenecarboxylic Acid (19). To a solution of **17b** (1.08 g, 2.3 mmol) in acetone (20

mL) was added 2.5 M aqueous LiOH solution (15 mL). The reaction mixture was stirred for 5 h at ambient temperature, acidified to pH 4 with 5% HCl, and then extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:1) afforded 980 mg (94%) of **19** as a white solid: mp = 180 °C. ^{1}H NMR (CD_3CD, 300 MHz) δ 8.07 (d, 1H, J = 2.2 Hz) 7.63 (d, 1H, J = 8.5 Hz), 7.51 (d, 1H), 7.34 (d, 2H, J = 8.5 Hz), 7.20 (d, 2H, J = 8.0 Hz), 4.73 (s, 2H), 4.66 (s, 2H), 3.03 (s, 3H), 1.29 (s, 9H); LRMS (FAB) *m/z* 450 (M + H$^+$). Anal. ($C_{21}H_{27}N_3O_4S_2$) C, H, N, S.

5-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)amino]-methyl-*N*-hydroxy-2-[(methylsulfonyl)amino]benzenecarboxamide (21a). To a solution of **19** (70 mg, 0.2 mmol) in benzene (2 mL) was added oxalyl chloride (0.13 mL, 1.6 mmol) dropwise. The reaction mixture was refluxed for 2 h and concentrated in vacuo and then NH_2OH·HCl (111 mg, 1.6 mmol) and pyridine (2 mL) were added to the crude **20**. The reaction mixture was concentrated *in* vacuo and the residue was purified by flash column chromatography (EtOAc only) to afford 52 mg (73%) of **21a** as a pale yellow solid: mp = 199−200 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ 8.09 (d, 1H, J = 2.0 Hz), 7.51 (d, 1H, J = 8.3 Hz), 7.44 (dd, 1H, J = 2.2, 8.6 Hz), 7.31 (m, 4H), 5.05 (s, 4H), 2.92 (s, 3H), 1.27 (s, 9H). LRMS (ESI) *m/z* 465.2 (M + H$^+$). Anal. ($C_{21}H_{28}N_4O_4S_2$) C, H, N, S.

5-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)amino]-methyl-*N*-methoxy-2-[(methylsulfonyl)amino]benzenecarboxamide (21b). The thiourea **21b** was prepared (30% yield) from **20** by the procedure described for **21a**. Yellow solid: mp = 136−137 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.14 (s, 1H), 9.38 (s, 1H), 7.55 (m, 3H), 7.32 (m, 4H), 5.04 (s, 2H), 5.01 (s, 2H), 3.82 (s, 3H), 3.00 (s, 3H), 1.25 (s, 9H). LRMS (ESI) *m/z* 478.2 (M + H$^+$).Anal. ($C_{22}H_{30}N_4O_4S_2$) C, H, N, S.

Isopropyl 5-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)-amino]methyl-2-[(methylsulfonyl)amino]benzenecarboxylate (21c). The thiopurea **21c** was prepared (50% yield) from **20** by the procedure described for **21a**. Pale yellow solid: mp = 159−160 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.5 (s, 1H), 8.06 (d, 1H, J = 2.2 Hz), 7.60 (d, 1H, J = 8.5 Hz), 7.53 (dd, 1H, J = 2.2, 8.5 Hz) 7.32−7.25 (m, 4H), 5.18 (m, 1H), 5.01 (s, 4H), 2.97 (s, 3H), 1.31 (d, 6H, J = 6.1 Hz), 1.21 (s, 9H). LRMS (EI) *m/z* 491 (M$^+$). Anal. ($C_{24}H_{33}N_3O_4S_2$) C, H, N, S.

***N*-(4-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)amino]-methyl-2-cyanophenyl)methanesulfonamide (21d).** To a solution of **19** (50 mg, 1.2 mmol) in benzene (3 mL) was added oxalyl chloride (0.1 mL, 1.2 mmol) dropwise, and the reaction mixture was refluxed for 3 h. The reaction mixture was concentrated in vacuo, and a mixture of sulfide (106 mg) and sulfolane (2 mL) was added. The resulting mixture was refluxed for 3 h at 120 °C, cooled to room temperature, and quenched by 1 N aqueous NaOH. The mixture was extracted with ether, and the combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:1) afforded 8 mg (16%) of **21d** as a pale yellow solid: mp = 142 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.8 (s, 1H), 7.65 (m, 2H), 7.58 (m, 1H), 7.33 (d, 4H), 5.05(s, 4H), 3.01 (s, 3H), 1.24 (s, 9H). LRMS (ESI) *m/z* 431.1 (M + H$^+$). Anal. ($C_{21}H_{26}N_4O_2S_2$) C, H, N, S.

***N*-(4-Cyano-2-nitrophenyl)methanesulfonamide (23).** To a suspension of KH (600 mg, 6.1 mmol) in THF (15 mL) at −78 °C was added dropwise a solution of 4-amino-3-nitrobenzonitrile **22** (500 mg, 3.1 mmol) in THF (10 mL). The reaction mixture was stirred for 30 min, and methanesulfonyl chloride (0.35 mL, 4.6 mmol) was added. After the resulting solution was stirred for 15 h, the reaction mixture was quenched with water and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated *in* vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:5) afforded 120 mg (16%) of **23**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.59 (d, 1H, J = 2.0 Hz), 8.03 (d, 1H, J = 8.8 Hz), 7.90 (dd, 1H, J = 2.0, 8.8 Hz), 3.24 (s, 3H); LRMS (FAB) *m/z* 242 (M + H$^+$). Anal. ($C_8H_7N_3O_4S$) C, H, N, S.

***N*-(4-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)amino]-methyl-2-nitrophenyl)methanesulfonamide (25).** To a solution of **23** (120 mg, 0.5 mmol) in THF (5 mL) was added BH_3−THF (1 M solution in THF, 1.5 mL, 1.5 mmol) dropwise. The reaction mixture was refluxed for 2 h and 2 N HCl (1.0 mL) was added. The resulting mixture was refluxed for an additional 1 h and then concentrated and dried on a vacuum pump to give 48 mg (39%) of **24**, which was directly used for the next step.

The sulfonamide **25** was prepared (80% yield) from **24** by the procedure described for **17a**. Yellow solid: mp = 74−75 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.09 (s, 1H), 7.76 (d, 1H, J = 8.7 Hz), 7.75 (d, 1H, J = 8.5 Hz), 7.11−7.40 (m, 4H), 4.79 (d, 2H, J = 8.3 Hz), 4.55 (s, 2H), 3.11 (s, 3H), 1.26 (s, 9H); LRMS (EI) *m/z* 450 (M$^+$). Anal. ($C_{20}H_{26}N_4O_4S_2$) C, H, N, S.

***N*-(4-[([4-(*tert*-Butyl)benzyl]aminocarbonyl)amino]-methyl-2-nitrophenyl)methanesulfonamide (26).** The sulfonamide **26** was prepared (10% yield) from **24** by the procedure described for **18a**. White solid: mp = 177−178 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.64 (bs, 1H), 8.12 (s, 1H), 7.81 (d, 1H, J = 8.8 Hz), 7.58 (d, 1H, J = 6.6 Hz), 7.36 (d, 2H, J = 8.4 Hz), 7.23 (d, 2H, J = 8.3 Hz), 4.81 (bs, 1H), 4.73 (bs, 1H), 4.40 (d, 2H, J = 5.9 Hz), 4.35 (d, 2H, J = 5.7 Hz), 3.10 (s, 3H), 1.29 (s, 9H); LRMS (EI) *m/z* 435 (M + H$^+$). Anal. ($C_{20}H_{26}N_4O_5S$) C, H, N, S.

4-Amino-3-bromobenzenecarbonitrile (28a). To a solution of 4-aminobenzonitrile (216 mg, 1.8 mmol) in DMF (4 mL) was added NBS (391 mg, 2.2 mmol). The reaction mixture was stirred for 30 min and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated i*n* vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:3) afforded 248 mg (69%) of **28a**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.66 (d, 1H, J = 2.0 Hz), 7.34 (dd, 1H, J = 2.0, 8.3 Hz), 6.72 (d, 1H, J = 8.3 Hz), 4.59 (bs, 2H); LRMS (FAB) *m/z* 196 (M + H$^+$). Anal. ($C_7H_5BrN_2$) C, H, N.

4-Amino-3-iodobenzenecarbonitrile (28b). To a solution of 4-aminobenzonitrile (300 mg, 2.5 mmol) in MeOH (2 mL) were added I_2 (387 mg, 1.5 mmol) and H_2O_2 (30% solution, 0.25 mL). The reaction mixture was stirred for 12 h and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:3) afforded 412 mg (67%) of the aniline **28b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.87 (d, 1H, J = 2.0 Hz), 7.36 (dd, 1H, J = 2.0, 8.6 Hz), 6.67 (d, 1H, J = 8.3 Hz), 4.59 (bs, 1H); LRMS (FAB) *m/z* 244 (M + H$^+$).Anal. ($C_7H_5IN_2$) C, H, N.

***tert*-Butyl *N*-(4-Amino-3-bromobenzyl)carbamate.** To a solution of **28a** (100 mg, 0.4 mmol) in THF (2 mL) was added BH_3−THF (1 M solution in THF, 0.5 mL, 0.5 mmol) dropwise. The reaction mixture was refluxed for 2 h, and then 2 N HCl (0.6 mL) was added. The resulting mixture was refluxed for an additional 1 h, concentrated in vacuo, and dried on a vacuum pump to give 412 mg (67%) of the benzylamine, which was directly used for the next step.

To a solution of the above benzylamine (113 mg, 0.6 mmol) in CH_2Cl_2 (5 mL) were added DMAP (14 mg, 0.1 mmol), Et_3N (0.2 mL, 1.4 mmol), and $(Boc)_2O$ (382 mg, 1.8 mmol). The reaction mixture was stirred for 1 h at ambient temperature and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:1) afforded 114 mg (67%) of the carbamate: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31 (d, 1H, J = 1.8 Hz), 6.99 (d, 1H, J = 8.1 Hz), 6.68 (d, 1H, J = 8.3 Hz), 4.13 (d, 2H, J = 5.5 Hz), 1.43 (s, 9H); LRMS (FAB) *m/z* 301 (M + H$^+$). Anal. ($C_{12}H_{17}BrN_2O_2$) C, H, N.

***tert*-Butyl *N*-3-Bromo-4-[(methylsulfonyl)amino]benzylcarbamate.** The sulfonamide was prepared (81% yield) from the above carbamate by the procedure described for **14a**: ^{1}H NMR (CD_3OD, 300 MHz) δ 7.55 (s, 1H), 7.49 (d, 1H, J = 8.2 Hz), 7.27 (dd, 1H, J = 1.8, 8.3 Hz), 4.18 (s, 2H), 2.98

(s, 3H), 1.44 (s, 9H); LRMS (FAB) *m/z* 379 (M + H$^+$). Anal. ($C_{13}H_{19}BrN_2O_4S$) C, H, N, S.

***N*-(2-Bromo-4-[([4-(*tert*-butyl)benzyl]aminocarbothioyl)-amino]methylphenyl)methanesulfonamide (30a).** To a solution of the above methanesulfonamide (85.5 mg, 0.2 mmol) in MeOH (4 mL) was added *c*-HCl (150 μL). The reaction mixture was refluxed for 2 h and dried on a vacuum pump to give 74 mg (100%) of **29a**, which was directly used for the next step.

The methanesulfonamide **30a** was prepared (90% yield) from **29a** by the procedure described for **17a**, which was directly used for the next step: White solid: mp = 73−76 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.21−7.63 (m, 7H), 6.84 (bs, 1H), 4.72 (s, 2H), 4.59 (s, 2H), 2.97 (s, 3H), 1.26 (s, 9H); LRMS (FAB) *m/z* 484 (M + H$^+$). Anal. ($C_{20}H_{26}BrN_3O_2S_2$) C, H, N, S.

***N*-(4-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)amino]-methyl-2-iodophenyl)methanesulfonamide (30b).** The methanesulfonamide **30b** was prepared (98% yield) from **28b** by the procedure described for compound **30a**. White solid: mp = 75−76 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.75 (s, 1H), 7.47−7.50 (d, 1H, *J* = 8.0 Hz), 7.32−7.35 (d, 2H, *J* = 7.9 Hz), 7.19−7.24 (m, 4H), 6.74 (bs, 1H), 4.68 (s, 2H), 4.57 (s, 2H), 2.97 (s, 3H), 1.26 (s, 9H); LRMS (EI) *m/z* 531 (M$^+$). Anal. ($C_{20}H_{26}IN_3O_2S_2$) C, H, N, S.

1*H*-1,3-Benzimidazole-5-carbaldehyde (32a). To a solution of the acid **31a** (380 mg, 2.3 mmol) in THF (10 mL) at −78 °C was added LAH (180 mg, 4.7 mmol). The reaction mixture was stirred for 10 h at ambient temperature and was quenched by MeOH. The resulting mixture was filtrated and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:MeOH = 10:1) afforded 206 mg (59%) of alcohol.

To a solution of the above alcohol (206 mg, 1.4 mmol) in CH_2Cl_2 (5 mL) were added 4 Å molecular sieve, TPAP (44 mg, 0.1 mmol), and NMO (550 mg, 4.9 mmol). After stirring for 1 h, the reaction mixture was diluted with ether, filtered, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:MeOH = 10:1) afforded 131 mg (65%) of the aldehyde **32a**: ^{1}H NMR (CD_3OD, 300 MHz) δ 10.03 (s, 1H), 8.37 (s, 1H), 8.20 (s, 1H), 7.72−7.87 (m, 2H); LRMS (FAB) *m/z* 147 (M + H$^+$). Anal. ($C_8H_6N_2O$) C, H, N.

1*H*-1,3-Benzimidazole-5-carbaldehyde Oxime (33a). To a solution of **32a** (131 mg, 0.9 mmol) in MeOH (3 mL) were added NH_2OH·HCl (75.1 mg, 1.08 mmol) and NaOAc (88.6 mg, 1.08 mmol). The reaction mixture was stirred for 1 h and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc only) afforded 112 mg (77%) of the oxime **33a**: ^{1}H NMR (CD_3OD, 300 MHz) δ 8.20 (d, 2H, *J* = 2.7 Hz), 7.79 (s, 1H), 7.58 (bs, 2H); LRMS (FAB) *m/z* 162 (M + H$^+$). Anal. ($C_8H_7N_3O$) C, H, N.

***N*-(1*H*-1,3-Benzimidazol-5-ylmethyl)-*N*′-[4-(*tert*-butyl)-benzyl]thiourea (35a).** The amine **34a** was prepared (100% yield) from **33a** by the same reduction procedure described for **16a**, which was directly used for the next step.

The thiourea **35a** was prepared (29% yield) from **34a** by the procedure described for **17a**. Yellow solid: mp = 163−164 °C. ^{1}H NMR (acetone-d_6, 300 MHz) δ 8.13 (s, 1H), 7.61 (s, 1H), 7.55 (d, 1H, *J* = 8.0 Hz), 7.20−7.23 (m, 5H), 4.90 (d, 2H, *J* = 8.4 Hz), 4.76 (d, 2H, *J* = 5.6 Hz), 1.27 (s, 9H); LRMS (EI) *m/z* 352 (M$^+$). Anal. ($C_{20}H_{24}N_4S$) C, H, N, S.

***N*-(1*H*-1,2,3-Benzotriazol-5-ylmethyl)-*N*′-[4-(*tert*-butyl)-benzyl]thiourea (35b).** The thiourea **35b** was prepared (20% yield) from **31b** by the procedure described for **35a**. White solid: mp = 106 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ 7.87−7.76 (m, 2H), 7.44 (d, 1H, *J* = 8.6 Hz), 7.30 (d, 2H, *J* = 8.0 Hz), 7.19 (d, 2H, *J* = 7.9 Hz), 4.90 (s, 2H), 4.67 (s, 2H), 1.27 (s, 9H); LRMS (ESI) *m/z* 354.2 (M + H$^+$). Anal. ($C_{19}H_{23}N_5S$) C, H, N, S.

***N*-[4-(*tert*-Butyl)benzyl]-*N*′-(1*H*-indol-5-ylmethyl)thiourea (39a).** To a solution of $AlCl_3$ (126 mg, 0.9 mmol) and LAH (55 mg, 1.5 mmol) in ether (2 mL) at 0 °C was added dropwise a solution of 5-cyanoindole (**37a**, 103 mg, 0.7 mmol) in ether (4 mL). The reaction mixture was stirred for 5 h at ambient temperature and quenched by MeOH. The resulting mixture was basified with 1 N aqueous NaOH and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$, concentrated, and dried on a vacuum pump to give 121 mg (88%) of the amine **38a**, which was directly used for the next step.

The thiourea **39a** was prepared as a white solid (68% yield) from **38a** by the procedure described for compound **17a**: mp = 70−71 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.31 (bs, 1H), 7.46 (s, 1H), 7.18−7.32 (m, 4H), 7.01−7.09 (m, 3H), 6.46 (m, 1H), 6.18 (bs, 1H), 6.07 (bs, 1H), 4.58 (s, 2H), 4.54 (s, 2H), 1.26 (s, 9H); LRMS (EI) *m/z* 351 (M$^+$). Anal. ($C_{21}H_{25}N_3S$) C, H, N, S.

***N*-(1,3-Benzodioxol-5-ylmethyl)-*N*′-[4-(*tert*-butyl)benzyl]-thiourea (39b).** The thiourea **39a** was prepared as a white solid (70% yield) from **37b**, prepared from **36** by analogy with **15a,** by the procedure described for compound **39a**: White solid: mp = 134−135 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40 (d, 2H, *J* = 8.3 Hz), 7.23 (d, 2H, *J* = 8.3 Hz), 6.78−6.70 (m, 3H), 5.98 (s, 2H), 4.60 (s, 2H), 4.57 (s, 2H), 1.35 (s, 9H); LRMS (FAB) *m/z* 357 (M + H$^+$). Anal. ($C_{20}H_{24}N_2O_2S$) C, H, N, S.

***N*-(2-Fluoro-4-iodophenyl)acetamide (41b).** To a solution of 2-fluoro-4-iodoaniline (**40**, 120 mg, 0.5 mmol) in CH_2Cl_2 (1.7 mL) at 0 °C were added dropwise pyridine (0.08 mL, 1.0 mmol) and acetyl chloride (55 μL, 0.8 mmol). The reaction mixture was stirred for 2 h at ambient temperature diluted with CH_2Cl_2. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:3) afforded 113 mg (80%) of **41b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.08 (d, 1H, *J* = 8.0 Hz), 7.39−7.44 (m, 2H), 2.19 (t, 3H, *J* = 2.3 Hz); LRMS (FAB) *m/z* 279 (M + H$^+$). Anal. (C_8H_7FINO) C, H, N.

Ethyl 2-(2-Fluoro-4-iodoanilino)acetate (41h). To a solution of **40** (100 mg, 0.4 mmol) in EtOH (10 mL) at 0 °C were added *c*-HCl (0.1 mL) and ethyl glyoxylate (0.17 mL, 0.8 mmol). The reaction mixture was stirred for 30 min at ambient temperature, and sodium cyanoborohydride (55.6 mg, 0.8 mmol) was added. The resulting reaction mixture was extracted with EtOAc, and the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:10) afforded 133 mg (98%) of **41h**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28−7.23 (m, 2H), 6.31 (t, 1H, *J* = 8.8 Hz), 4.23 (q, 2H, *J* = 7.1 Hz), 3.88 (s, 2H), 1.27 (t, 3H, *J* = 7.1 Hz); LRMS (FAB) *m/z* 323 (M + H$^+$). Anal. ($C_{10}H_{11}FINO_2$) C, H, N.

4-Amino-3-fluorobenzenecarbonitrile (42a). The nitrile **42a** was prepared (83% yield) from **41a** by the procedure described for **15a**, which was directly used for the next step. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.50 (t, 1H, *J* = 8.0 Hz), 7.39 (d, 1H, *J* = 8.6 Hz), 7.32 (dd, 1H, *J* = 2.0, 10.6 Hz), 2.20 (s, 3H); LRMS (FAB) *m/z* 137 (M + H$^+$). Anal. ($C_7H_5FN_2$) C, H, N.

***N*-(4-Amino-3-fluorobenzyl)-*N*′-[4-(*tert*-butyl)benzyl]-thiourea (44a).** The benzylamine **43a** was prepared (100% yield) from **42a** by the procedure described for **16a**, which was directly used for the next step.

The thiourea **44a** was prepared as a white solid (60% yield) from **43a** by the procedure described for **17a**: mp = 127−128 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.08−7.26 (m, 4H), 6.64−6.83 (m, 3H), 4.56 (s, 2H), 4.47 (s, 2H), 1.20 (s, 9H); LRMS (EI) *m/z* 345 (M$^+$). Anal. ($C_{19}H_{24}FN_3S$) C, H, N, S.

***N*-(4-{[({[4-(*tert*-Butyl)benzyl]amino}carbothionyl)-amino]methyl}-2-fluorophenyl)acetamide (44b).** The thiourea **44b** was prepared (79% yield) from **41b** by the procedure described for **44a**. Pale yellow solid: mp = 92−93 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.94 (t, 1H, *J* = 8.1 Hz), 7.44 (bs, 1H), 7.34 (d, 2H, *J* = 8.1 Hz), 7.18 (d, 2H, *J* = 8.3 Hz), 6.94 (d, 1H, *J* = 11.3 Hz), 6.85 (d, 1H, *J* = 8.6 Hz), 4.60 (s, 2H), 4.56 (s, 2H), 1.27 (s, 9H); LRMS (ESI) *m/z* 388.3 (M + H$^+$). Anal. ($C_{21}H_{26}FN_3OS$) C, H, N, S.

***N*-(4-{[({[4-(*tert*-Butyl)benzyl]amino}carbothionyl)-amino]methyl}-2-fluorophenyl)-2,2,2-trifluoroacetamide (44c).** The thiourea **44c** was prepared (92% yield) from

40 by the procedure described for **44b**. Pale yellow solid: mp = 59−60 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.14 (t, 1H, J = 8.1 Hz), 8.04 (bs, 1H), 7.35 (d, 2H, J = 8.1 Hz), 7.20 (d, 2H, J = 8.2 Hz), 7.03 (t, 2H, J = 11.4 Hz), 4.71 (s, 2H), 4.54 (s, 2H), 1.28 (s, 9H); LRMS (EI) *m/z* 441 (M^+). Anal. ($C_{21}H_{23}F_4N_3OS$) C, H, N, S.

Methyl *N*-(4-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)-amino]methyl-2-fluorophenyl)carbamate (44d). The thiourea **44d** was prepared as (62% yield) from **40** by the procedure described for **44b**. White solid: mp = 149−150 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.97 (t, 1H), 7.35 (d, 2H, J = 8.3 Hz), 7.18 (d, 2H, J = 7.8 Hz), 6.95 (d, 2H, J = 8.0 Hz), 6.82 (s, 1H), 4.62 (s, 2H), 4.46 (s, 2H), 3.76 (s, 3H), 1.26 (s, 9H); LRMS (FAB) *m/z* 404 ($M + H^+$). Anal. ($C_{21}H_{26}FN_3O_2S$) C, H, N, S.

Ethyl *N*-(4-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)-amino]methyl-2-fluorophenyl)carbamate (44e). The thiourea **44e** was prepared as a pale yellow oil (47% yield) from **40** by the procedure described for **44b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.95 (s, 1H), 7.33 (d, 2H, J = 8.0 Hz), 7.17 (d, 2H, J = 8.0 Hz), 6.94 (d, 2H), 6.77 (s, 1H), 4.60 (s, 2H), 4.55 (s, 2H), 4.19 (q, 2H, J = 7.2 Hz), 1.27 (m, 12H); LRMS (FAB) *m/z* 418 ($M + H^+$). Anal. ($C_{22}H_{28}FN_3O_2S$) C, H, N, S.

***N*-(4-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)amino]-methyl-2-fluorophenyl)-2-methoxyacetamide (44f).** The thiourea **44f** was prepared (35% yield) from **40** by the procedure described for **44b**.White solid: mp = 59−60 °C. 1H NMR ($CDCl_3$, 300 MHz) δ 8.49 (s, 1H), 8.07 (t, 1H, J = 8.0 Hz), 7.36 (d, 2H, J = 8.0 Hz), 7.23 (d, 2H, J = 8.0 Hz), 7.03 (d, 1H, J = 11.2 Hz), 6.93 (d, 1H, J = 8.3 Hz), 6.66 (bs, 1H), 4.67 (s, 2H), 4.62 (s, 2H), 3.49 (s, 3H), 1.32 (s, 9H); LRMS (FAB) *m/z* 418 ($M + H^+$). Anal. ($C_{22}H_{28}FN_3O_2S$) C, H, N, S.

Methyl 2-(4-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)-amino]methyl-2-fluoroanilino)acetate (44g). The thiourea **44g** was prepared (56% yield) from **41g** by the procedure described for **44b**. White solid: mp = 131−132 °C. 1H NMR ($CDCl_3$, 300 MHz) δ 7.39 (d, 2H, J = 8.3 Hz), 7.23 (d, 2H, J = 8.3 Hz), 6.93 (s, 1H), 6.90 (s, 1H), 6.52 (t, 1H, J = 8.4 Hz), 6.36 (s, 1H), 4.60 (s, 2H), 4.53 (s, 2H), 3.83 (s, 2H), 3.74 (s, 3H), 1.34 (s, 9H); LRMS (FAB) *m/z* 418 ($M + H^+$). Anal. ($C_{22}H_{28}FN_3O_2S$) C, H, N, S.

Ethyl 2-(4-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)-amino]methyl-2-fluoroanilino)acetate (44h). The thiourea 44h was prepared (34% yield) from **41h** by the procedure described for **44b**. White solid: mp = 112 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31 (d, 2H, J = 8.5 Hz), 7.15 (d, 2H, J = 8.3 Hz), 6.86 (s, 1H), 6.83 (s, 1H), 6.46 (t, 1H, J = 8.4 Hz), 6.10 (d, 1H), 4.53 (s, 2H), 4.48 (s, 2H), 4.20 (q, 2H, J = 7.1 Hz), 3.75 (s, 2H), 1.27 (m, 12H); LRMS (FAB) *m/z* 432 ($M + H^+$). Anal. ($C_{23}H_{30}FN_3O_2S$) C, H, N, S.

2-(4-[([4-(*tert*-Butyl)benzyl]aminocarbothioyl)amino]-methyl-2-fluoroanilino)acetic Acid (44i). To a solution of thiourea **44g** (55 mg, 0.1 mmol) in THF/H_2O (1:1, 2 mL) was added LiOH−H_2O (16 mg, 0.66 mmol). The reaction mixture was stirred for 24 h at ambient temperature, acidified with 1 N aqueous HCl, and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was dried on a vacuum pump to give 50 mg (95%) of the acid **44i** as a white solid: mp = 163 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ 7.34 (d, 2H, J = 8.5 Hz), 7.18 (d, 2H, J = 8.3 Hz), 6.94−6.88 (m, 2H), 6.56 (t, 1H, J = 8.5 Hz), 4.65 (s, 2H), 4.55 (s, 2H), 3.70 (s, 2H), 1.28 (s, 9H); LRMS (ESI) *m/z* 426.1 ($M + Na^+$), 442.1 ($M + K^+$) Anal. ($C_{21}H_{26}FN_3O_2S$) C, H, N, S.

Ethyl *N*-([4-(*tert*-Butyl)benzyl]aminocarbothioyl)carbamate (46). To a solution of ethyl isothiocyanate (0.27 mL, 2.3 mmol) in CH_2Cl_2 (5 mL) was added 4-*tert*-butylbenzylamine **45** (0.64 mL, 3.4 mmol) dropwise. The reaction mixture was stirred for 3 h at ambient temperature and diluted with CH_2Cl_2. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:10) afforded 610 mg (91%) of the thiourea **46**: ^{1}H NMR (CD_3OD, 300 MHz) δ 7.39 (d, 2H, J = 6.2 Hz), 7.27 (d, 2H, J = 8.6 Hz), 4.79 (s, 2H), 4.22 (q, 2H, J = 7.1 Hz), 1.31 (s, 9H), 1.30 (t, 3H, J = 7.1 Hz); LRMS (FAB) *m/z* 295 ($M + H^+$). Anal. ($C_{15}H_{22}N_2O_2S$) C, H, N, S.

Ethyl N-[[4-(*tert*-Butyl)benzyl]amino(3-fluoro-4-[(methylsulfonyl)amino]benzylamino)methylene]carbamate (47a). To a solution of thiourea **46** (300 mg, 1.0 mmol) and EDCI (240 mg, 1.8 mmol) in DMF (5.0 mL) was added Et_3N (2.9 mL, 20.0 mmol). The reaction mixture was stirred for 30 min at ambient temperature, and a solution of amine **8** (260 mg, 1.0 mmol) and (2.9 mL, 20.0 mmol) in DMF (11 mL) was added dropwise using a cannula. The resulting mixture was stirred for 24 h and acidified with 2 N aqueous HCl. The resulting mixture was extracted with EtOAc, and the combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:1) afforded 210 mg (43%) of the guanidine **47a** as a white solid: mp = 174−175 °C. ^{1}H NMR (CD_3OD, 300 MHz) δ 7.39−7.36 (m, 3H), 7.20−7.03 (m, 4H), 4.47 (s, 2H), 4.43 (s, 2H), 4.07 (q, 2H, J = 7.1 Hz), 2.96 (s, 3H), 1.30 (s, 9H), 1.23 (t, 3H, J = 7.1 Hz); LRMS (ESI) 479.1 ($M + H^+$). Anal. ($C_{23}H_{31}FN_4O_4S$) C, H, N, S.

***N*-[4-([[4-(*tert*-Butyl)benzyl]amino(cyanoimino)methyl]-aminomethyl)-2-fluorophenyl]methanesulfonamide (47b).** To a solution of Na (20 mg, 0.9 mmol) in EtOH (3 mL) was added a solution of cyanamide (41 mg, 7.0 mmol) in EtOH (2 mL) dropwise. The reaction mixture was stirred for 2 h at ambient temperature, concentrated in vacuo, and then added to a solution of amine **8** (250 mg, 1.0 mmol), EDCI (280 mg, 1.5 mmol), and Et_3N (0.23 mL, 1.7 mmol) in DMF (5 mL) dropwise. The resulting mixture was stirred for 24 h at ambient temperature and was acidified with 2 N aqueous HCl. The mixture was extracted with EtOAc, and the combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:1) afforded 130 mg (30%) of the cyanoguanidine **47b** as white solid: mp = 190−191 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.47 (t, 1H, J = 8.1 Hz), 7.38 (d, 2H, J = 8.3 Hz), 7.20 (d, 2H, J = 8.3 Hz), 6.94−6.89 (m, 2H), 5.86 (bs, 1H), 5.49 (bs, 1H), 4.35 (s, 2H), 4.33 (s, 2H), 3.00 (s, 3H), 1.29 (s, 9H); LRMS (ESI) 454.2 ($M + Na^+$). Anal. ($C_{21}H_{26}FN_5O_2S$) C, H, N, S.

***N*-[4-([[4-(*tert*-Butyl)benzyl]amino(methoxyimino)methyl]aminomethyl)-2-fluorophenyl]methanesulfonamide (47c).** To a solution of **4** (100 mg, 0.2 mmol), HgO (56 mg, 0.3 mmol), and NH_2OMe (20 mg, 0.4 mmol) in DMF (3 mL) was added triethylamine (0.10 mL, 0.83 mmol) dropwise. The reaction mixture was stirred for 2 h at ambient temperature, quenched by an addition of saturated aqueous $NaHCO_3$, and extracted with EtOAc. The combined organic layers were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash column chromatography (EtOAc:*n*-hexane = 1:1 → 2:1) afforded 84 mg (80%) of the guanidine **47c** as a yellow oil: ^{1}H NMR (CD_3OD, 300 MHz) δ 7.35−7.32 (m, 3H), 7.16−7.00 (m, 4H), 4.21 (bs, 4H), 3.57 (s, 3H), 2.94 (s, 3H), 1.30 (s, 9H); LRMS (EI) 436 (M^+). Anal. ($C_{21}H_{29}FN_4O_3S$) C, H, N, S.

45Ca^{2+} Uptake-Assays. Culture of DRG Neurons. DRG neurons were prepared from neonatal Sprague−Dawley rats. DRGs of all spinal levels were dissected aseptically and collected. Ganglia were incubated sequentially for 30 min at 37 °C in 200 U/mL collagenase and 2.5 mg/mL trypsin. The digestion was halted by an addition of an equal volume of DME/F12 medium supplemented with 10% horse serum. The ganglia were then triturated through a fire-polished Pasteur pipet, filtered through nylon membrane, and spun down. Dissociated cells were plated onto Terasaki plates previously coated with 10 μg/mL poly-D-ornithine at a density of 1500−1700 neurons/well. The cells were then cultured for 3 days in DME/F12 medium containing 1.2 g/L sodium bicarbonate, 15 mM HEPES, 50 mg/L gentamycin, and 10% horse serum, diluted 1:1 with identical medium conditioned by C6 glioma cells (2 days on a confluent monolayer), in a humidified atmosphere at 37 °C containing 5% CO_2. Medium was supplemented with 200 ng/mL nerve growth factor. Cytosine arabi-

noside (100 μM) was added for the first 2 days to kill dividing nonneuronal cells.

Uptake Assays. Terasaki plates containing DRG neurons grown for 3 days were equilibrated with four washes of HEPES (10 mM, pH 7.4)-buffered calcium- and magnesium-free Hank's balanced salt solution. The solution in each well was removed from the individual wells. For antagonistic studies, medium (10 μL) containing 10 μCi/ml $^{45}Ca^{2+}$ and 0.5 M capsaicin together with the test concentration of the compound was added to each well. The neurons were incubated at room temperature for 10 min, and then the Terasaki plates were washed six times in HEPES (10 mM, pH 7.4)-buffered calcium- and magnesium-free Hank's balanced salt solution and dried in an oven. Sodium dodecyl sulfate (0.3%, 10 μL) was then added to dissolve the cells and extract the $^{45}Ca^{2+}$. The contents of each well were transferred to scintillation vials and counted in 3 mL of Aquasol-2 scintillant. Antagonistic activities of test compounds were given as IC_{50} (the concentration of the compound necessary to reduce the response to 0.5 μM capsaicin by 50%). The IC_{50} values were estimated at least three replicates at each concentrated. Each compound was tested at least in two independent experiments.

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Supporting Information Available: Elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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