Analogues of Capsaicin with Agonist Activity as Novel Analgesic Agents: Structure−Activity Studies. 4. Potent, Orally Active Analgesics

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Structural features of three regions of the capsaicin molecule necessary for agonist properties were delineated by a previously reported modular approach. These *in vitro* agonist effects were shown to correlate with analgesic potency in rodent models. Combination of optimal structural features from each of these regions of the capsaicin molecule have led to highly potent agonists (*e.g.*, **1b**). Evaluation *in vivo* established that **1b** had analgesic properties but poor oral activity, short duration of action, and excitatory side effects which precluded further development of this compound. Preliminary metabolism studies had shown that the phenol moiety of **1b** was rapidly glucuronidated *in vivo*, providing a possible explanation for the poor pharmacokinetic profile. Subsequent specific modification of the phenol group led to compounds **2a**−**j**, which retained *in vitro* potency. The *in vivo* profiles of two representatives of this series, **2a**,**h**, were much improved over the "parent" phenol series, and they are candidates for development as analgesic agents.

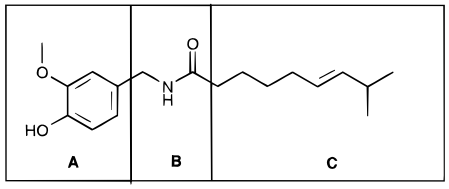
Introduction

Earlier papers in this series have described the potential use of capsaicin-like agonists as analgesic agents.[1−3] These papers described in detail the modular variations of the capsaicin structure which was conveniently broken down into three separate parts: the aromatic "A" region, the amide bond "B" region, and the hydrophobic side-chain "C" region. Each region was varied in turn while holding the others constant. The compounds were evaluated in an *in vitro* screen which provides a measure of capsaicin-like agonism ($^{45}Ca^{2+}$ influx into neonatal rat dorsal root ganglia (DRG) neurons[4]) and which we have established is predictive of analgesia in animal models.[1]

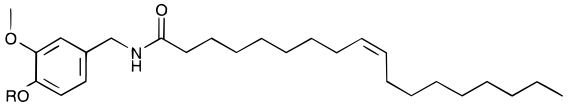
This work has formed the basis of a molecular approach toward more potent capsaicin agonists which are anticipated to be useful as novel analgesic agents. In parallel with our studies, workers at Procter and Gamble Co. have explored structural modifications of the capsaicin molecule. Based on antinociceptive studies, a series of aliphatic vanillylamides were described[5] culminating in the identification of Olvanil (NE 19950, oleyl vanillylamide) as a putative analgesic. The structure−activity relationship (SAR) conclusions from both groups are broadly in concert. More recently Park *et al.*[6] have described capsaicin-like agonists in the patent literature.

Specifically, from our earlier studies, it was established that the natural substitution pattern was optimal in the A region, that a thiourea moiety conferred high potency when incorporated in the B region, and that a hydrophobic unit of limited size, *e.g.*, a substituted aralkyl or aralkenyl substituent, was necessary in the C region. The present paper describes the **combination** of these individual potency-enhancing features in the same molecule with the aim of making compounds with increased *in vitro* potency which might therefore be expected to have enhanced analgesic properties *in vivo*. Realization of this goal and the subsequent refinements of the target structures based on *in vivo* data are described below and have led to compounds with therapeutic potential.

Chart 1



Capsaicin



R = H, Olvanil, oleyl vanillylamide, (NE 19950).

R = $CH_2.CH_2.NH_2$, Oleyl 4-O-aminoethylvanillylamide, (NE 21610).

Chemistry

The synthetic approaches to the target molecules are unexceptional and not discussed here in detail. The skeletal assembly, involving the coupling of an amine component with an isothiocyanate component, is shown in outline in Scheme 1, and the resulting target structures are listed in Table 1. The protecting group strategies (described by X in Scheme 1 and Table 1) varied from compound to compound and are described in detail in the Experimental Section. For the "parent" phenol series **1a**−**i**, the phenolic group was either left unprotected or carried through the isothiocyanate coupling step as the ethoxyethyl acetal which was subsequently removed using dilute acid. For the 2-aminoethyl phenolic ether series **2a**−**j**, the primary amino group was protected as the phthalimide in the coupling step and then removed using hydrazine under standard conditions.

The (*Z*)-ethenyl compound **1d** was made by photochemical isomerization of the *E* compound **1c**. The

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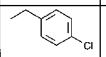
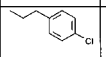
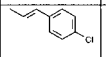
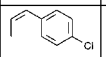
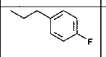
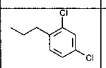
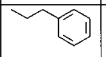
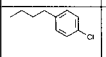
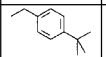
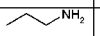
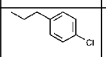
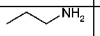
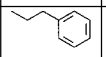
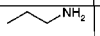
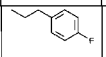
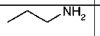
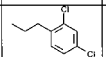
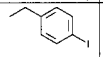
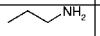
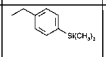
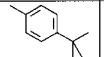
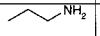
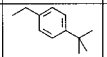
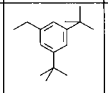
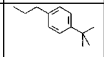
Table 1. Routes of Synthesis (Scheme 2) and $^{45}Ca^{2+}$ Influx Activity of Thioureas

Compound Number	R_1	R_2=	Route	X	Ca^{2+}influx EC_{50} (μM)
Capsaicin					0.3±0.04
1a	H		A	H	0.075±0.021
1b	H		A	H	0.056±0.016
1c	H		A	H	0.41±0.05
1d	H		-	H	0.041±0.003
1e	H		B		0.13±0.08
1f	H		A	H	0.13±0.04
1g	H		A	H	0.48±0.09
1h	H		B	H	0.48±0.09
1i	H		A	H	0.51±0.08
2a			A		0.95±0.13
2b			A		2.72±0.46
2c			A		2.30±0.29
2d			A		0.32±0.15
2e			B		1.34±0.26
2f			B		0.33±0.03
2g			A		1.66±0.19
2h			B		0.17±0.03
2i			B		0.22±0.03
2j			A		0.71±0.12
3			A		5.48±1.29
4			A		7.03±0.59
5			-	-	3.52±0.06
6			A		>100
7			A		53±17
8			-	-	5.13±0.29
9			-	-	6.14±0.47
10			-	-	3.00±0.18
Oleyl vanillylamide					0.17±0.03
Oleyl 4-O-aminoethyl vanillylamide					0.25±0.09

Scheme 1

(methylamino)ethyl side chain of **4** was protected as the FMoc derivative for the coupling step. The protecting group was subsequently removed using piperidine under standard conditions. The (dimethylamino)ethyl phenol ether **5** was prepared from the aminoethyl compound **2a** by treatment with paraformaldehyde and subsequent reduction with sodium cyanoborohydride. The acylated compounds **8**−**10** were made from **2a** using acetyl chloride, ethyl chloroformate, and di-*tert*-butyl dicarbonate, respectively.

Biological Results and Discussion

Combination of the optimal features of each of the three regions of the capsaicin molecule has led to the aralkyl and aralkenyl thioureas **1a**−**i**. As anticipated, evaluation of these compounds in the $^{45}Ca^{2+}$ influx assay has established that **1a**−**i** are capsaicin-like agonists with potencies, in some cases, greater than that of capsaicin itself (see Table 1). Indeed, with the exception of the complex natural product resiniferatoxin and its analogues,[7−9] these compounds are the most potent capsaicin-like agonists reported.

The C region substituents (R_2), exemplified in **1a**−**i**, fall within the molecular size limit ($M_r \leq 55$) for the "hydrophobic substituent of limited size" compatible with high potency which had been proposed earlier.[3] The significant difference in activity between the isomers **1c**,**d** is interesting and may reflect a further manifestation of the shape and size limitation in this region. Further consideration of the SAR establishes the importance of substitution of the aromatic ring (*i.e.*, the unsubstituted analogue **1g** is less potent), but further attempts to rationalize the nature or pattern of substitution seem pointless from these data given the similar activities of these analogues.

The *p*-chlorophenethyl thiourea **1b** was selected for *in vivo* evaluation to assess its potential as an analgesic agent. The potent (*E*)-ethenyl compound **1d** was precluded from consideration because of its limited chemical stability.

From the data shown in Table 2 the analgesic properties of **1b** support the principle established earlier,[1−3] namely, that potent capsaicin-like agonism correlates with analgesic effects in rodent models. This molecule was however precluded from further development as a therapeutic agent because of several unwanted properties. Although **1b** was effective in several rodent analgesia models given subcutaneously ($ED_{50} = 11.8 \pm 3.6$ μmol/kg, 4.0 ± 1.2 mg/kg), oral activity was poor ($ED_{50} = 370 \pm 54$ μmol/kg, 113 ± 16 mg/kg) and the

Table 2. Comparative *in Vivo* Data for Thioureas **1b** and **2a**,**h** and Reference Compounds

compound	tail-flick latency ED_{50} (μmol/kg) sc	tail-flick latency ED_{50} (μmol/kg) po	duration of action (h)[a]	writhing ED_{50} (μmol/kg) sc	writhing ED_{50} (μmol/kg) po	bronchoconstriction threshold dose[b] (μmol/kg iv)
capsaicin	10.3 ± 2.9	962 ± 272		5.72 ± 0.63		0.018 ± 0.003
1b	11.8 ± 3.6	370 ± 54	<2	10.13 ± 1.16		0.025 ± 0.005
oleyl vanillylamide	11.3 ± 1.6	>400		2.10 ± 0.59		>0.100
2a	4.8 ± 1.2	22.87 ± 3.18	5	3.32 ± 0.36	11.45 ± 2.81	0.137 ± 0.037
2h	0.40 ± 0.09	1.45 ± 0.55	>6	0.49 ± 0.04	2.55 ± 0.50	0.062 ± 0.012
oleyl 4-*O*-(aminoethyl)vanillylamide	6.3 ± 1.0	184 ± 45		3.06 ± 0.57		>0.100
morphine	21.61 ± 2.98			4.27 ± 0.16		

[a] At ED_{50} dose. [b] Threshold dose required to induce an increase in P_{ao}.

duration of the response was short (total loss of activity at 2 h after an ED_{50} dose sc). The spectrum of excitatory side effects of **1b** was similar to that of capsaicin.[10] In particular, bronchoconstrictive effects of **1b** (threshold dose 0.025 ± 0.005 μmol/kg, 8.9 ± 1.8 μg/kg, iv, guinea pig), which are a characteristic property of capsaicin,[11] were observed at similar doses to the latter. The excitatory properties of **1b** were also manifest in its pungency and irritant properties which were akin to those of capsaicin itself. Olvanil (oleyl vanillylamide), while clearly less pungent than capsaicin,[5b] suffers from some of the same defects as **1b**, particularly, poor oral activity.

The medicinal chemistry goal to develop a therapeutic entity therefore was focused to address these issues, namely, the improvement of the bioavailability and the reduction of the excitatory properties of **1b**. Preliminary metabolism studies on phenol **1b** established that the molecule was rapidly metabolized to the *O*-glucuronide, which was the only detectable component in plasma at all time points after oral dosing in rats and dogs.[12] This marked glucuronidation and thereby probable inactivation of the parent molecule **1b** would explain the low oral bioavailability and the relatively short duration of action of this compound.

It had been established from our earlier studies[1] that, in general, blocking of the phenolic OH group with a variety of substituents removed or drastically reduced agonist activity in capsaicin analogues; however, substitution with the 2-aminoethyl group led to the series of compounds **2a**−**j** in which agonist potency *in vitro* was substantially maintained. Comparison of structural pairs (*e.g.*, **1b** and **2a**, **1e** and **2c**, **1f** and **2d**, and **1g** and **2b**) showed relatively lower potency in the O-substituted analogues over the parent phenols with one clear exception (**1i** and **2h**). Contemporaneously the Procter and Gamble group claimed that this same substituent attached to the phenolic moiety of their aliphatic vanillylamides improved the water solubility, reduced the irritant effects, and retained the antinociceptive properties of these compounds.[13] A representative of this chemical class is the compound oleyl 4-*O*-(aminoethyl)vanillylamide (NE 21610).

With the identification of the 2-aminoethyl substituent as an acceptable replacement for the metabolically labile parent phenolic hydrogen, it was considered important to explore the SAR of this substituent. The results of this structural investigation are shown in Table 1 from the series of analogues of **2a**. It is clear from the *in vitro* data presented in Table 1 that all modifications of the 2-aminoethyl substituent are deleterious. Thus lengthening of the methylene bridge (in **3**) and alkylation (in **4**−**6**) and acylation (in **7**−**10**) of the amino group all lead to less active compounds than the unsubstituted compound **2a**. The lack of activity of the quaternary ammonium compound **6** is particularly noteworthy, possibly implying the existence of an access barrier, *e.g.*, membrane penetration, to such a compound. Further investigation of this hypothesis is in progress.

On the basis of the *in vitro* data compounds **2a**,**h** were evaluated *in vivo* in analgesia models in comparison with morphine as a representative established analgesic agent and oleyl 4-*O*-(aminoethyl)vanillylamide (NE 21610), another capsaicin agonist. These data are shown in Table 2.

The modification of the phenol group described above was undertaken to improve the pharmacokinetic properties of these molecules. It can be seen from the data in Table 2 that this improvement has been achieved. While the potency of the phenol **1b** is comparable with that of **2a** by the subcutaneous route, the oral potency of the latter is much improved. In contrast the same improvement is not observed with oleyl 4-*O*-(aminoethyl)vanillylamide, where, from the tail-flick latency data (see Table 2), it appears that this molecule has only poor oral activity. We have no explanation for this difference which clearly lies elsewhere than *in vitro* potency as oleyl 4-*O*-(aminoethyl)vanillylamide is comparable in the Ca^{2+} influx assay to **2h**. The potency of **2h** is significantly increased by both routes of administration over that of **2a**, perhaps reflecting the greater *in vitro* activity of the former; moreover **2h** is more potent than the reference molecule morphine. Another improvement is illustrated by the increased duration of action of both compounds, **2a**,**h**, over the "parent" phenol **1b**.

Fortuitously this structural change, which has improved the analgesic profile of analogues such as **2a**,**h**, also reduced the excitatory properties of these compounds in comparison to the phenolic compounds. In contrast to **1b**, a clear separation of bronchoconstrictive effects from analgesia was achieved with **2a** and (to a lesser extent) with the more potent compound **2h**. These compounds are also virtually nonpungent in comparison to capsaicin and **1b**. A possible explanation for the reduced excitatory properties of O-substituted compounds such as **2h** over the phenol series, *e.g.*, **1b**, appears to lie in their *rate* of excitation of the sensory neuron.[14]

On the basis of the combination of its antinociceptive properties and its reduced excitatory effects compared with other capsaicin agonists, **2h** has been selected as a clinical development candidate with a novel mode of analgesic action. Mechanistic studies are ongoing to elaborate a molecular explanation for the improved properties of **2h** and congeners which involves the rate

of membrane penetration, and this will be reported in due course.

Experimental Section

General Information. Melting points were determined using a Reichert hot-stage microscope and are uncorrected. Routine NMR spectra were recorded using Hitachi-Perkin Elmer R12B and Varian Gemini 200 machines. High-field spectra were recorded using Varian VX400 400 MHz (University College London Chemistry Department) and Bruker AM360 360 MHz (Sandoz, Basle) instruments. All spectra were recorded using tetramethylsilane (TMS) as an internal standard, and chemical shifts are reported in parts per million (δ) downfield from TMS. Coupling constants are reported in hertz. A Perkin-Elmer 781 machine was used to record IR spectra. Elemental analyses were performed by the Analytical Department of University College London and were within 0.4% of theory unless otherwise indicated. Mass spectra were recorded by the Mass Spectrometry Department of University College London, using a VG 7070F/H spectrometer, and FAB spectra were recorded in Sandoz, Basle, using a VG 70-SE spectrometer. Accurate mass determinations were made by M. Cocksedge and Dr. D. Carter, London School of Pharmacy, using a VG ZAB SE mass spectrometer and FAB ionization.

TLC was performed using Merck Kieselgel 60 F_{254} silica plates or Merck aluminum oxide 60 F_{254} plates, and components were visualized using UV light and iodine vapor. HPLC was performed using a Waters 600 system (μ-Bondapak C-18 column (RP_{18}), using CH_3CN/0.1% aqueous TFA gradients of compositions stated in the text. Compounds were purified by flash column chromatography[15] using Merck Kieselgel 60 (230−400 mesh) unless otherwise indicated. Solvents were HLPC grade and used without further purification. Solvents were dried according to the standard procedures.[16] Test compounds were homogenous by TLC or HPLC unless otherwise stated. Chemical yields were not optimized.

General Procedure for the Synthesis of Vanillyl Thioureas. The thioureas described herein were prepared either by coupling vanillylamine (or a 4-alkyloxy derivative) with the relevant isothiocyanate (method A) or by coupling vanillyl isothiocyanate (or a 4-alkyloxy derivative) with the relevant amine (method B). Both are exemplified below.

CAUTION. *Some of the vanillyl thioureas described below are pungent and are irritant to broken skin and mucous membranes (akin to the effects of capsaicin itself). Appropriate handling precautions should be taken.*

Method A: *N*-(4-Hydroxy-3-methoxybenzyl)-*N′*-(4-chlorobenzyl)thiourea (1a). Vanillylamine hydrochloride (2.5 g, 0.013 mol) was dissolved in DMF (20 mL) with 5 M NaOH (5.2 mL). The mixture was stirred and cooled on ice. 4-Chlorobenzyl isothiocyanate (2.66 g, 0.04 mol) was dissolved in DMF (10 mL) and added dropwise. The mixture was stirred for 18 h and then poured into water (250 mL). The aqueous mixture was extracted with diethyl ether (4 × 100 mL); the combined organic phase was washed with 1 M HCl_{aq} (50 mL), saturated $NaHCO_{3aq}$ (50 mL), and brine (50 mL) and then dried over $MgSO_4$ with stirring. The solution was filtered and the solvent removed *in vacuo* to give an oil. The residue was purified by flash column chromatography (silica, CH_2Cl_2, changing to CH_2Cl_2/MeOH, 50:1) and recrystallized from MeOH to give 1.15 g of a white crystalline solid (38% yield): mp 135−138 °C; TLC (silica, CH_2Cl_2/MeOH, 5:1) R_f 0.59; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 3.74 (3H, s, ArOCH_3), 4.56 (2H, br m, ArCH_2NH), 4.70 (2H, br m, ArCH_2NH), 6.72 (2H, m, benzyl Ar$H_{5,6}$), 6.91 (1H, s, vanillyl ArH_2), 7.35 (4H, m, ArH), 7.92 (2H, br m, thiourea NHs), 8.92 (1H, s, ArOH); MS *m/e* 336 (M^+). Anal. ($C_{16}H_{17}N_2O_2SCl$) C, H, N.

Method B: *N*-(4-Hydroxy-3-methoxybenzyl)-*N′*-(2-(4-fluorophenyl)ethyl)thiourea (1e). 2-(4-Fluorophenyl)ethylamine hydrochloride (1.56 g, 0.0089 mol) was dissolved in dry DMF (10 mL) with triethylamine (0.987 g, 9.75 mL) and stirred. 4-(1-Ethoxyethoxy)-3-methoxybenzyl isothiocyanate (2.37 g, 0.0089 mol) was dissolved in dry DMF (2 mL), added slowly to the reaction mixture, and stirred overnight. The DMF was removed *in vacuo* and the residue partitioned between EtOAc (75 mL) and water (50 mL). The organic phase was washed with water (50 mL) and brine (50 mL) and then dried over Na_2SO_4. After filtration and removal of solvent *in vacuo*, the residue was dissolved in THF (20 mL) and cooled on ice; 1 M HCl_{aq} (5 mL) was added, and the reaction mixture was stirred for 3 h. The THF was removed *in vacuo*; the residue was taken up in EtOAc (50 mL), washed with 1 M HCl_{aq} (25 mL), water (2 × 25 mL), and brine (25 mL), and then dried over Na_2SO_4. The mixture was filtered and the solvent removed *in vacuo*. The residue was purified by recrystallization from MeOH to give 350 mg of an off-white crystalline solid (12% yield): mp 147−150 °C; TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.24; ^{1}H-NMR (DMSO-d_6, 60 MHz) δ 2.85 (2H, m, ArCH_2CH$_2$N), 3.65 (2H, m, ArCH$_2$CH_2N), 3.75 (3H, s, ArOCH_3), 4.55 (2H, d, J = 6.0 Hz, ArCH_2N), 6.7−7.0 (3H, m, ArH), 7.1−7.35 (4H, m, ArH), 7.50 (1H, br m, CH$_2$NH), 7.80 (1H, br m, ArCH$_2$NH); MS *m/e* 334 (M^+). Anal. ($C_{17}H_{19}N_2O_2SF$) C,H,N.

General Procedure for the Synthesis of Isothiocyanates. Unless commercially available, or otherwise indicated, all isothiocyanates mentioned herein were synthesized by the method illustrated by the synthesis of 4-*tert*-butylbenzyl isothiocyanate described below.

4-*tert*-Butylbenzyl Isothiocyanate. 4-*tert*-Butylbenzylamine (2.0 g, 0.0123 mol) was dissolved in EtOAc (20 mL) with triethylamine (2.53 g, 0.025 mol) and added dropwise to a solution of thiophosgene (1.41 g, 0.0123 mol) in EtOAc (50 mL), previously cooled on ice, with stirring. The reaction mixture was allowed to come to room temperature and stirred for 2 h, until complete by TLC. The reaction mixture was washed with water (2 × 50 mL) and brine (50 mL) before drying over Na_2SO_4. The solution was filtered, and the solvent was removed *in vacuo* to leave a brown solid, which was purified by flash column chromatography (silica, cyclohexane/EtOAc, 4:1) to give 1.71 g of a yellow-brown solid (68% yield): TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.61.

***N*-(4-Hydroxy-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)ethyl)thiourea (1b)**: synthesized from vanillylamine hydrochloride and 2-(4-chlorophenyl)ethyl isothiocyanate by method A; purification by recrystallization from aqueous MeOH gave a white crystalline solid, 48% yield; mp 143−144 °C; TLC (silica, CH_2Cl_2/MeOH, 5:1) R_f 0.69; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.82 (2H, t, J = 7.16 Hz, ArCH_2CH$_2$), 3.65 (2H, br m, ArCH$_2$CH_2N), 3.76 (3H, s, ArOCH_3), 4.52 (2H, br m, ArCH_2N), 6.73 (2H, m, ArH), 6.9 (1H, s, ArH), 7.31 (4H, m, ArH), 7.40 (1H, br m, thiourea NH), 7.75 (1H, br m, thiourea NH), 8.90 (1H, s, ArOH); MS *m/e* 350 (M^+). Anal. ($C_{17}H_{19}N_2O_2SCl$) C,H,N.

4-Chlorobenzoyl Nitrile. This compound was prepared from 4-chlorobenzoyl chloride, according to the method of Burger and Hornbaker.[17]

2-(4-Chlorophenyl)-2-hydroxyethylamine. Lithium aluminum hydride (50 g) was suspended in dry diethyl ether (500 mL) and stirred on a salt/ice bath. A solution of 4-chlorobenzoyl nitrile (115.0 g, 0.695 mol) in diethyl ether (500 mL) was added dropwise over 30 min. The mixture was stirred under N_2 at room temperature for 18 h and then refluxed for 6 h. The mixture was cooled to room temperature and then on ice, and wet diethyl ether (500 mL) was added to deactivate the $LiAlH_4$ followed by the slow addition of 5 N $NaOH_{aq}$. The mixture was filtered, the solution was dried over Na_2SO_4 and then filtered, and the solvent was removed *in vacuo*. The residue was purified by flash column chromatography (silica, CH_2Cl_2/MeOH, 10:1) to give 39.0 g of a white crystalline solid (33% yield): TLC (CH_2Cl_2/MeOH/AcOH, 120:90:5) R_f 0.45.

2-(4-Chlorophenyl)-2-chloroethylamine Hydrochloride. 2-(4-Chlorophenyl)-2-hydroxyethylamine (38.2 g, 0.223 mol) was dissolved in chloroform (500 mL). Thionyl chloride (55 mL, 0.765 mol) in chloroform (100 mL) was added slowly with stirring, and a dense beige precipitate formed. The mixture was stirred and refluxed for 3 h and then cooled, and the solvent was removed *in vacuo*. The residue was sonicated in MeOH, filtered, washed with diethyl ether, and then dried to give 25.9 g of an off-white crystalline solid (51% yield): TLC (silica, CH_2Cl_2/MeOH, 5:1) R_f 0.6; ^{1}H-NMR (CD_3OD, 60 MHz)

δ 3.55 (2H, d, J = 7.2 Hz, CHClCH_2NH$_2$), 5.4 (1H, t, J = 7.2 Hz, ArCHClCH$_2$), 7.6 (4H, s, ArH); FABMS *m/e* 190 (MH$^+$).

2-(4-Chlorophenyl)-2-chloroethyl Isothiocyanate. 2-(4-Chlorophenyl)-2-chloroethylamine hydrochloride (25.9 g, 0.114 mol) was suspended in water (300 mL), with a few crystals of phenolphthalein. Thiophosgene (9.2 mL, 0.120 mol) was added in CH_2Cl_2 (200 mL) with stirring followed by the gradual addition of 2 M NaOH$_{aq}$ until the aqueous layer retained a permanent purple coloration. After stirring for 30 min, the layers were partitioned using a separating funnel. The organic phase was washed with saturated NaCl$_{aq}$, dried over Na_2SO_4, and then filtered and the solvent removed *in vacuo*. The residue was purified by flash column chromatography (silica, cyclohexane/EtOAc, 50:1) to give 22.3 g of a yellow crystalline solid (84% yield): TLC (silica, cyclohexane/EtOAc, 50:1) R_f 0.25; ^{1}H-NMR ($CDCl_3$, 60 MHz) δ 3.95 (2H, d, J = 7.0 Hz, CHCH_2NCS), 5.0 (1H, t, J = 7.0 Hz, ArCHClCH$_2$), 7.4 (4H, m, ArH); IR ν 2090 cm^{-1} (NCS stretch).

(*E*)-2-(4-Chlorophenyl)ethenyl Isothiocyanate. 2-(4-Chlorophenyl)-2-chloroethyl isothiocyanate (9.35 g, 0.04 mol) was dissolved in toluene (100 mL) and stirred at 100 °C; triethylamine (4.5 g, 0.045 mol) was added, and the reaction mixture was stirred for 18 h, at which point a further 4.5 g of triethylamine was added. The reaction mixture was stirred at 100 °C for another 18 h. The reaction mixture was cooled to room temperature and filtered, and the solvent was removed *in vacuo*. The residue was partially purified by flash column chromatography (silica, cyclohexane/EtOAc, 50:1); the mixture of cis and trans isomers was then recrystallized from *n*-hexane to give 1.55 g of the pure trans isomer, a white crystalline solid (20% yield): TLC (silica, cyclohexane/EtOAc, 50:1) R_f 0.45; ^{1}H-NMR (acetone-d_6, 200 MHz) δ 6.84 (1H, d, J = 14.13 Hz, ArCH=CHNCS), 7.05 (1H, d, J = 13.96 Hz, ArCH=CHNCS), 7.33 (4H, m, ArH).

***N*-(*E*)-(2-(4-Chlorophenyl)ethenyl)-*N*′-(4-hydroxy-3-methoxybenzyl)thiourea (1c)**: synthesized from vanillylamine hydrochloride and (*E*)-2-(4-chlorophenyl)ethenyl isothiocyanate by method A; purification by recrystallization from EtOAc/cyclohexane gave a white crystalline solid, yield 87%; mp 180−183 °C; TLC (silica, CH_2Cl_2/MeOH, 25:1) R_f 0.32; ^{1}H-NMR (acetone-d_6, 400 MHz) δ 3.8 (3H, s, ArOCH_3), 4.7 (2H, d, J = 5 Hz, ArCH_2NH), 6.1 (1H, d, J = 14.6 Hz, ArCH=CHNH), 6.7−7.4 (7H, m, ArH), 7.6 (2H, br m, ArCH$_2$NH + ArOH), 8.1 (1H, m, ArCH=CHNH), 9.3 (1H, br d, ArCH=CHNH); MS *m/e* 348 (M$^+$). Anal. ($C_{17}H_{17}N_2O_2SCl$) C,H,N.

***N*-(*Z*)-(2-(4-Chlorophenyl)ethenyl)-*N*′-(4-hydroxy-3-methoxybenzyl)thiourea (1d).** A solution of **1c** (300 mg, 0.000 86 mol) in anhydrous THF (120 mL) was prepared, and dry Ar was bubbled through the solution for 40 min. The solution was irradiated for 47 min with a mercury lamp, until no starting material remained. The solvent was removed *in vacuo*, and the residue was purified by flash column chromatography (silica, cyclohexane/EtOAc, 1:1) and recrystallization from EtOAc/cyclohexane to give 170 mg of a white crystalline solid (57% yield): mp 143 °C; TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.28; ^{1}H-NMR (acetone-d_6, 400 MHz) δ 3.82 (3H, s, ArOCH_3), 4.70 (2H, br s, ArCH_2N), 5.61 (1H, d, J = 9.6 Hz, ArCH=CHNH), 6.75−6.85 (2H, m, ArH), 7.01 (1H, d, J = 1.6 Hz, ArH), 7.33 (4H, m, ArH), 7.55 (1H, m, ArCH=CHNH), 7.58 (1H, s, ArOH), 7.85 (1H, br m, thiourea NH), 8.85 (1H, br m, CH=CHNH); MS *m/e* 348 (M$^+$). Anal. ($C_{17}H_{17}N_2O_2SCl$) C,H,N.

4-(1-Ethoxyethoxy)-3-methoxybenzyl isothiocyanate: from 4-(1-ethoxyethoxy)-3-methoxybenzylamine,[12] to give a brown oil, used without further purification after workup, crude yield 100%; TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.46.

2-(2,4-Dichlorophenyl)ethyl isothiocyanate: synthesized from 2-(2,4-dichlorophenyl)ethylamine, to give a brown oil, which was used without further purification after workup, crude yield 98%; TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.7.

***N*-(2-(2,4-Dichlorophenyl)ethyl)-*N*′-(4-hydroxy-3-methoxybenzyl)thiourea (1f)**: synthesized from vanillylamine hydrochloride and 2-(2,4-dichlorophenyl)ethyl isothiocyanate by method A; purification by recrystallization from EtOAc/petroleum ether gave a white crystalline solid, yield 33%; mp 136−138 °C; TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.28; ^{1}H-NMR (DMSO-d_6, 60 MHz) δ 2.95 (2H, m, NHCH$_2$CH_2Ar), 3.65 (2H, m, NHCH_2CH$_2$Ar), 3.75 (3H, s, ArOCH_3), 4.55 (2H, d, J = 6.0 Hz, ArCH_2NH), 6.75−7.0 (3H, m, ArH), 7.1−7.9 (5H, m, 3 × ArH + 2 × NH), 8.85 (1H, s, ArH); MS *m/e* 384 (M$^+$). Anal. ($C_{17}H_{18}N_2O_2SCl$) C,H,N.

2-Phenylethyl isothiocyanate: synthesized from 2-phenylethylamine, to give an orange oil, which was used without further purification after workup, crude yield 97%.

***N*-(4-Hydroxy-3-methoxybenzyl)-*N*′-(2-phenylethyl)thiourea (1g)**: synthesized from 2-phenylethyl isothiocyanate and vanillylamine hydrochloride by method A; purification by recrystallization from EtOAc/petroleum ether gave a white crystalline solid, yield 32%; mp 105−107 °C; ^{1}H-NMR (DMSO-d_6, 60 MHz) δ 2.80 (2H, t, J = 7 Hz, ArCH_2CH$_2$), 3.70 (2H, m, ArCH$_2$CH_2N), 3.75 (3H, s, ArOCH$_3$), 4.55 (2H, d, J = 6 Hz, ArCH_2N), 6.75−6.95 (3H, m, ArH), 7.2−7.4 (5H, m, ArH), 7.40 (1H, br t, thiourea NH), 7.75 (1H, br t, thiourea NH), 8.80 (1H, s, ArOH); MS *m/e* 316 (M$^+$). Anal. ($C_{17}H_{20}N_2O_2S$) C,H,N.

3-(4-Chlorophenyl)propyl Methyloxime. Methoxylamine hydrochloride (4.8 g, 0.0575 mol) and sodium acetate (4.80 g, 0.0575 mol) were suspended in MeOH (40 mL) and added to a solution of 3-(4-chlorophenyl)propanal (3.0 g, 0.0178 mol) in MeOH (30 mL). The reaction mixture was refluxed for 90 min and cooled and the solvent removed *in vacuo*. The residue was partitioned between CH_2Cl_2 and water; the organic phase was washed with brine, dried over $MgSO_4$, and filtered and the solvent removed *in vacuo*. The residue was purified by flash column chromatography (silica, cyclohexane/EtOAc, 30:1) to give 1.50 g of a colorless oil (43% yield): TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.60; ^{1}H-NMR ($CDCl_3$, 60 MHz) δ 2.80 (4H, m, ArCH_2CH_2CH), 3.80 (3H, s, NOCH_3), 6.70 (1H, m, CH$_2$CH=NOCH$_3$), 7.20 (4H, m, ArH); MS *m/e* 197 (M$^+$).

3-(4-Chlorophenyl)propylamine. 3-(4-Chlorophenyl)propyl methyloxime (1.71 g, 0.0087 mol) was dissolved in dry THF (10 mL) and stirred under Ar on ice; 1 M borane−THF complex in THF (4.5 mL) was added slowly to the oxime solution, and the reaction mixture was refluxed for 24 h. Another 4.5 mL of 1 M borane−THF solution was added and the reaction mixture refluxed for a further 4 h and then cooled on ice. MeOH (60 mL) and 5 M NaOH$_{aq}$ (14 mL) were slowly added, and the reaction mixture was refluxed for 2 h. The solvent was removed *in vacuo*, and the residue was partitioned between water and EtOAc. The organic phase was dried over $MgSO_4$ and filtered, and the solvent was removed *in vacuo* to give 1.45 g (98%) of a pale brown oil, which was used without purification/characterization: TLC (silica, CH_2Cl_2/MeOH/AcOH, 120:90:5) R_f 0.45 (ninhydrin positive).

Vanillyl Isothiocyanate. Vanillylamine hydrochloride (1.89 g, 0.010 mol) was dissolved in water (20 mL) with CH_2Cl_2 (20 mL) and calcium carbonate (3.0 g, 0.030 mol) and stirred. Thiophosgene (0.9 mL, 0.012 mol) was added slowly in CH_2Cl_2 (10 mL) over 90 min. The reaction mixture was stirred at room temperature for 24 h and then filtered, and the organic phase was purified by flash column chromatography (silica, cyclohexane/EtOAc, 4:1) to give 1.1 g of a dark brown oil (56% yield): TLC (silica, cyclohexane/EtOAc, 4:1) R_f 0.43.

***N*-(3-(4-Chlorophenyl)propyl)-*N*′-(4-hydroxy-3-methoxybenzyl)thiourea (1h)**: synthesized from vanillyl isothiocyanate and 3-(4-chlorophenyl)propylamine by method B in dry THF; purification by flash column chromatography (silica, CH_2Cl_2/MeOH, 50:1) and recrystallization from EtOAc/*n*-hexane gave a white crystalline solid, yield 44%; mp 109−111 °C; TLC (silica, CH_2Cl_2/MeOH, 25:1) R_f 0.45; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.60 (2H, t, J = 7.58 Hz, ArCH_2CH$_2$), 3.43 (2H, br m, CH$_2$CH_2NH), 3.88 (3H, s, ArOCH_3), 4.47 (2H, br m, ArCH_2N), 5.63 (1H, s, ArOH), 5.73 (1H, br m, thiourea NH), 6.00 (1H, br m, thiourea NH), 6.80 (3H, m, vanillyl ArH), 7.15 (4H, m, ArH): MS *m/e* 364 (M$^+$). Anal. ($C_{18}H_{21}N_2O_2SCl$) C,H,N.

***N*-(4-*tert*-Butylbenzyl)-*N*′-(4-hydroxy-3-methoxybenzyl)thiourea (1i)**: synthesized from vanillylamine hydrochloride and 4-*tert*-butylbenzyl isothiocyanate by method A; purification by flash column chromatography (silica, cyclohexane/EtOAc, 3:1) gave a pure white foamed solid (50% yield), which was

recrystallized from EtOAc/*n*-hexane to give a white crystalline solid, yield 21%; mp 110−111 °C; TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.30; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.30 (9H, s, *tert*-butyl), 3.84 (3H, s, ArOCH_3), 4.51 (2H, d, J = 5.12 Hz, ArCH_2NH), 4.58 (2H, d, J = 4.76 Hz, ArCH_2NH), 5.61 (1H, s, ArO*H*), 6.02 (2H, br m, thiourea NHs), 6.73−6.86 (3H, m, vanillyl ArH), 7.26 (4H, m, ArH); MS *m/e* 358 (M^+). Anal. ($C_{20}H_{26}N_2O_2S$) C,H,N.

Boc-vanillylamine. Vanillylamine hydrochloride (18.0 g, 0.095 mol) and triethylamine (10.6 g, 0.11 mol) were dissolved in water (250 mL). Di-*tert*-butyl dicarbonate (20.5 g, 0.095 mol) in dioxane (200 mL) was added with stirring over a period of 15 min and the resulting mixture stirred overnight at room temperature. The dioxane was removed *in vacuo* and the aqueous residue extracted with $CHCl_3$ (3 × 150 mL). The combined extracts were dried over $MgSO_4$ and filtered and the solvent removed *in vacuo* to leave a brown oil which was purified by flash column chromatography (silica, cyclohexane/EtOAc, 5:2) to give 21.5 g of a colorless oil (89% yield) which crystallized on standing: TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.5.

Boc-4-(2-bromoethoxy)-3-methoxybenzylamine. Boc-vanillylamine (21.0 g, 0.083 mol), 1,2-dibromoethane (250 mL), 40% aqueous KOH (66 mL), and 40% aqueous tetrabutylammonium hydroxide (6.6 mL) were combined and heated at 50 °C for 3 h with rapid stirring. The mixture was cooled, diluted with CH_2Cl_2 (200 mL), and washed with water (3 × 200 mL), and the combined aqueous washings were extracted once with CH_2Cl_2 (600 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and filtered, and the solvent was removed *in vacuo* to leave 23.0 g of a white solid (77% yield) which was used without further purification: TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.6.

Boc-4-(2-phthalimidoethoxy)-3-methoxybenzylamine. Boc-4-(2-bromoethoxy)-3-methoxybenzylamine (23.0 g, 0.064 mol) and potassium phthalimide (11.8 g, 0.064 mol) were suspended in dry DMF (500 mL). The resulting suspension was heated at 50 °C for 2 h with rapid stirring (after 30 min the mixture became homogeneous). The mixture was then cooled and the DMF removed under high vacuum. The resulting solid residue was purified by flash column chromatography (silica, cyclohexane/EtOAc, 1:1) to give 26.5 g of a white solid (97% yield): TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.45.

(4-(2-Phthalimidoethoxy)-3-methoxybenzyl)ammonium Trifluoroacetate. Boc-4-(2-phthalimidoethoxy)-3-methoxybenzylamine (26.5 g, 0.062 mol) was dissolved in CH_2Cl_2 (200 mL), and trifluoroacetic acid (15 mL) was added dropwise with stirring. On completion of the addition, the mixture was stirred for a further 2 h at room temperature until the reaction was complete by TLC (silica, cyclohexane/EtOAc, 1:1). The solvent was removed *in vacuo*, and the resulting colorless oil solidified on standing to give 24.5 g of a white crystalline solid (90% yield): ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 3.63 (3H, s, ArOCH_3), 3.95 (4H, m, OCH_2CH$_2$N + ArCH_2N), 4.23 (2H, t, J = 5.9 Hz, OCH$_2$CH_2N), 6.95−7.10 (3H, m, ArH), 7.89 (4H, m, ArH).

General Procedure for the Synthesis of Aminoethoxy Analogues. All deprotections of phthalimidoethoxy compounds to give the desired aminoethoxy compounds were carried out by the method exemplified by the synthesis of compound **2a**.

***N*-(4-(2-Aminoethoxy)-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)ethyl)thiourea (2a).** A suspension of **7** (2.0 g, 0.0038 mol) in ethanol (10 mL) was heated to 60 °C, until the solution became homogeneous. Hydrazine monohydrate (0.95 mL, 980 mg, 0.0195 mol) was added and the mixture heated for 90 min. After 15 min, a white precipitate was formed, and a small amount of ethanol was added to keep the mixture mobile. The reaction mixture was cooled and transferred to a separating funnel, and methyl *tert*-butyl ether (50 mL) and 0.5 M $NaOH_{aq}$ (50 mL) were added. The organic layer was extracted, washed with brine, dried over Na_2SO_4, and filtered and the solvent removed *in vacuo*. The residue was purified by flash column chromatography (silica, CH_2Cl_2/MeOH, 10:1, changing to MeOH). The product fractions were evaporated and dried *in vacuo* to give 1.35 g of a glassy solid (90% yield). The hydrochloride salt was prepared by dissolving **2a** in boiling 1 M HCl_{aq}, cooling to room temperature, and filtering. The filtrate was recrystallized from EtOH/water and dried *in vacuo* over P_2O_5 to give 1.40 g of a colorless crystalline solid (78% overall yield): mp 83−84 °C; TLC (silica, $CHCl_3$/Et_3N/MeOH, 93:2:5) R_f 0.3, (silica, nBuOH/AcOH/H_2O, 4:1:1) R_f 0.3; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.80 (2H, t, J = 6.8 Hz, ArCH_2CH$_2$N), 3.00 (2H, t, J = 5.2 Hz, OCH$_2$CH_2N), 3.62 (2H, m, ArCH$_2$CH_2N), 3.80 (3H, s, ArOCH_3), 4.00 (2H, t, J = 5.2 Hz, OCH_2CH$_2$N), 4.57 (2H, br s, ArCH_2N), 6.78−6.95 (3H, m, ArH), 7.30 (4H, m, ArH), 7.67 (1H, br s, NH), 7.98 (1H, br s, NH); FABMS *m/e* 394 (MH^+). Anal. ($C_{19}H_{24}N_3O_2SCl$·HCl·2.5H_2O) C,H,N,O,S,Cl.

***N*-(4-(2-Phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(2-phenylethyl)thiourea**: synthesized from 4-(2-phthalimidoethoxy)-3-methoxybenzylamine trifluoroacetate and 2-phenylethyl isothiocyanate by method A in EtOAc with the addition of triethylamine, which gave the product in 54% yield; TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.2.

***N*-(4-(2-Aminoethoxy)-3-methoxybenzyl)-*N′*-(2-phenylethyl)thiourea (2b)**: synthesized from *N*-(4-(2-phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(2-phenylethyl)thiourea, which gave a pale yellow glassy solid, 93% yield; TLC (silica, MeOH) R_f 0.1; HPLC reverse phase C-18 (CH_3CN/0.1% TFA_{aq} gradient 10−100%) t_R = 8.9 min (>98% purity); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.86 (2H, t, J = 7.0 Hz, ArCH_2CH$_2$N), 3.08 (2H, t, J = 5.0 Hz, OCH$_2$CH_2N), 3.16 (2H, br m, NH_2), 3.71 (2H, t, J = 7.0 Hz, ArCH$_2$CH_2N), 3.77 (3H, s, ArOCH_3), 4.00 (2H, t, J = 5.0 Hz, OCH_2CH$_2$N), 4.44 (2H, d, J = 4.2 Hz, ArCH_2N), 6.09 (1H, br m, NH), 6.43 (1H, br m, NH), 6.68−6.79 (3H, m, ArH), 7.11−7.31 (5H, m, ArH); HRMS ($C_{21}H_{29}N_3O_2ClS$) calcd 422.1669, found 422.1662.

***N*-(4-(2-Phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(2-(4-fluorophenyl)ethyl)thiourea**: synthesized from 4-(2-phthalimidoethoxy)-3-methoxybenzylamine trifluoroacetate and 2-(4-fluorophenyl)ethyl isothiocyanate by method A in EtOAc with the addition of triethylamine, which gave the product in 47% yield; TLC (silica, cyclohexane/EtOAc, 1:2) R_f 0.3.

***N*-(4-(2-Aminoethoxy)-3-methoxybenzyl)-*N′*-(2-(4-fluorophenyl)ethyl)thiourea (2c)**: synthesized from *N*-(4-(2-phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(2-phenylethyl)thiourea, to give a pale yellow glassy solid, 42% yield; TLC (MeOH) R_f 0.2; HPLC reverse phase C-18 (CH_3CN/0.1% TFA_{aq} gradient 10−100%) t_R = 8.7 min (>98% purity); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.53 (2H, br m, NH_2), 2.99 (2H, t, J = 7.2 Hz, ArCH_2CH$_2$N), 3.11 (2H, t, J = 5.2 Hz, OCH$_2$CH_2N), 3.75 (2H, br m, ArCH$_2$CH_2N), 3.84 (3H, s, ArOCH_3), 4.03 (2H, t, J = 5.2 Hz, OCH_2CH$_2$N), 4.45 (2H, br m, ArCH_2N), 5.82 (1H, br m, NH), 6.18 (1H, br m, NH), 6.82, 7.17, 7.35 (7H, m, ArH); HRMS ($C_{19}H_{25}N_3O_2FS$) calcd 378.1652, found 378.1650.

***N*-(4-(2-Phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(2-(2,4-dichlorophenyl)ethyl)thiourea**: synthesized from 4-(2-phthalimidoethoxy)-3-methoxybenzylamine trifluoroacetate and 2-(2,4-dichlorophenylethyl isothiocyanate by method A in EtOAc with the addition of triethylamine, which gave the product in 44% yield; TLC (silica, cyclohexane/ethyl acetate, 1:1) R_f 0.35.

***N*-(4-(2-Aminoethoxy)-3-methoxybenzyl)-*N′*-(2-(2,4-dichlorophenyl)ethyl)thiourea (2d)**: synthesized from *N*-(4-(2-phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(2-(2,4-dichlorophenyl)ethyl)thiourea, to give a pale yellow glassy solid, 35% yield; TLC (silica, MeOH) R_f 0.15; HPLC reverse phase C-18 (CH_3-CN/0.1% TFA_{aq} gradient 10−100%) t_R = 9.3 min (>98% purity); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 1.54 (2H, br m, NH_2), 2.84 (2H, t, J = 7.2 Hz, ArCH_2CH$_2$N), 3.10 (2H, t, J = 5.4 Hz, OCH$_2$CH_2N), 3.72 (2H, br m, ArCH$_2$CH_2N), 3.83 (3H, s, ArOCH_3), 4.02 (2H, t, J = 5.4 Hz, OCH_2CH$_2$N), 4.42 (2H, br m, NH), 5.72 (1H, br m, NH), 6.20 (1H, br m, NH), 6.32−7.13 (6H, m, ArH); HRMS ($C_{19}H_{24}N_3O_2Cl_2S$) calcd 428.0966, found 428.0962.

4-(2-Phthalimidoethoxy)-3-methoxybenzyl isothiocyanate: prepared by the reaction of 4-(2-phthalimidoethoxy)-3-methoxybenzylamine trifluoroacetate (5.0 g, 0.011 mol) with thiophosgene, as described for 2-(4-chlorophenyl)-2-chloroethyl isothiocyanate, and purified by flash column chromatography

(silica, cyclohexane/ethyl acetate, 50:1) to give 2.8 g of a yellow oil (67% yield) which was used immediately.

***N*-(4-(2-Phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(4-iodobenzyl)thiourea**: synthesized from 4-(2-phthalimidoethoxy)-3-methoxybenzyl isothiocyanate and 4-iodobenzylamine by method B, in anhydrous THF; purification by flash column chromatography (silica, cyclohexane/ethyl acetate, 2:1) gave 1.6 g of the product (78% yield); TLC (silica, cyclohexane/ethyl acetate, 1:1) R_f 0.25; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ 3.54 (3H, s, ArOCH_3), 3.93 (2H, t, J = 6 Hz, OCH$_2$CH_2N), 4.17 (2H, t, J = 6 Hz, OCH_2CH$_2$N), 4.50−4.67 (4H, br d, 2 × CH_2-Ar), 6.70−6.96 (3H, m, ArH), 7.07 (2H, d, J = 9 Hz, I-ArH), 7.67 (2H, d, J = 9 Hz, I-ArH), 7.80−7.98 (5H, br s, ArH).

***N*-(4-(2-Aminoethoxy)-3-methoxybenzyl)-*N′*-(4-iodobenzyl)thiourea (2e)**: synthesized from *N*-(4-(2-phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(4-iodobenzyl)thiourea, to give a yellow glassy solid (13% yield); TLC (silica, MeOH) R_f 0.2; HPLC reverse phase C-18 (CH$_3$CN/0.1% TFA$_{aq}$ gradient 10−100%) t_R = 9.8 min (>98% purity); ^{1}H-NMR (CD$_3$OD, 200 MHz) δ 3.15 (2H, t, J = 6 Hz, OCH$_2$CH_2NH$_2$), 3.81 (3H, s, ArOCH_3), 4.10 (2H, t, J = 6 Hz, OCH_2CH$_2$N), 4.77 and 4.80 (4H, 2 × br s, 2 × CH_2Ar), 6.79−7.02 (3H, m, ArH), 7.05 (2H, d, J = 9 Hz, I-ArH), 7.63 (2H, d, J = 9 Hz, I-ArH); HRMS (C$_{18}$H$_{23}$N$_3$O$_2$IS) calcd 472.0556, found 472.0550.

4-(Trimethylsilyl)toluene. 4-Chlorotoluene (4.0 g, 0.032 mol), trimethylsilyl chloride (84 mL), and Mg dust (840 mg, 0.045 mol) were suspended in anhydrous THF, and the resultant mixture was refluxed for 18 h, after which time all the magnesium had been consumed. The solution was cooled to room temperature and poured into water (150 mL), and the product was extracted into diethyl ether. The organic layer was dried over MgSO$_4$ and filtered, and the solvent was removed *in vacuo*. The product was purified by vacuum distillation (0.1 mmHg, bp 28 °C) to give 2.9 g of a colorless oil (55% yield).

4-(Trimethylsilyl)benzyl Bromide. 4-(Trimethylsilyl)-toluene (2.0 g, 0.012 mol), *N*-bromosuccinimide (2.2 g, 0.012 mol), and a catalytic amount of dibenzoyl peroxide were combined in 60 mL of dry carbon tetrachloride and stirred under nitrogen. The mixture was refluxed for 6 h, until conversion was complete by ^{1}H-NMR, and then poured into water. The organic phase was dried over MgSO$_4$ and filtered, and the solvent was removed *in vacuo*. Purification by flash column chromatography (silica, cyclohexane) gave 1.6 g of a pale yellow oil (55% yield): TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.8; ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 0.28 (9H, s, ArSi(CH_3)$_3$), 4.50 (2H, s, ArCH_2Br), 7.35−7.55 (4H, m, ArH).

(4-(Trimethylsilyl)benzyl)phthalimide: synthesized from 4-(trimethylsilyl)benzyl bromide, as described for Boc-4-(2-phthalimidoethoxy)-3-methoxybenzylamine; purification by flash column chromatography (silica, cyclohexane/EtOAc, 10:1) gave a white crystalline solid (77% yield); TLC (silica, cyclohexane/EtOAc, 10:1) R_f 0.22; ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 0.24 (9H, s, ArSi(CH_3)$_3$), 4.85 (2H, s, ArCH_2N), 7.47 (4H, s, ArH), 7.70 (2H, m, ArH), 7.85 (2H, m, ArH).

4-(Trimethylsilyl)benzylamine: synthesized from (4-(trimethylsilyl)benzyl)phthalimide, as described for **2a**; purification by flash column chromatography (silica, CH$_2$Cl$_2$/MeOH, 10:1) gave a white solid (77% yield); TLC (silica, CH$_2$Cl$_2$/MeOH, 10:1) R_f 0.2; ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 0.24 (9H, s, ArSi(CH_3)$_3$), 3.80 (2H, br s, ArCH$_2$NH_2), 3.90 (2H, s, ArCH_2NH_2), 7.37 (2H, m, ArH), 7.52 (2H, m, ArH).

***N*-(4-(2-Phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(4-(trimethylsilyl)benzyl)thiourea**: synthesized from 4-(2-phthalimidoethoxy)-3-methoxybenzyl isothiocyanate and 4-(trimethylsilyl)benzylamine by method B, 97% yield; used without purification; TLC (silica, cyclohexane/ethyl acetate, 1:1) R_f 0.35; ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 0.26 (9H, s, Si(CH_3)$_3$), 3.67 (3H, s, ArOCH_3), 4.06 (2H, t, J = 6 Hz, OCH$_2$CH_2N), 4.24 (2H, t, J = 6 Hz, OCH_2CH$_2$N), 4.54 and 4.66 (2 × 2H, d, J = 7 Hz, 2 × ArCH_2NH), 6.26 (2H, br m, 2 × NH), 6.69−6.88 (3H, m, ArH), 7.27 (2H, d, J = 8 Hz, ArH), 7.50 (2H, d, J = 8 Hz, ArH), 7.70−7.90 (4H, m, ArH).

***N*-(4-(2-Aminoethoxy)-3-methoxybenzyl)-*N′*-(4-(trimethylsilyl)benzyl)thiourea (2f)**: synthesized from *N*-(4-(2-phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(4-(trimethylsilyl)-benzyl)thiourea, to give a yellow glassy solid (58% yield); TLC (silica, MeOH) R_f 0.1; HPLC reverse phase C-18 (CH$_3$CN/0.1% TFA$_{aq}$ gradient 10−100%) t_R = 10.9 min (>98% purity); ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 0.26 (9H, s, Si(CH_3)$_3$), 3.08 (2H, t, J = 6 Hz, OCH$_2$CH_2N), 3.80 (3H, s, ArOCH_3), 4.00 (2H, t, J = 6 Hz, OCH_2CH$_2$N), 4.56 and 4.66 (2 × 2H, 2 × br d, J = 7 Hz, 2 × ArCH_2NH), 4.80 (~2H, br s, NH$_2$), 6.43 (2H, br s, 2 × NH), 6.72−6.83 (3H, m, ArH), 7.25 (2H, d, J = 8 Hz, ArH), 7.50 (2H, d, J = 8 Hz, ArH); HRMS (C$_{21}$H$_{32}$N$_3$O$_2$SSi) calcd 418.1985, found 418.1980.

***N*-(4-(2-Phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(4-*tert*-butylphenyl)thiourea**: synthesized from (4-(2-phthalimidoethoxy)-3-methoxybenzyl)ammonium trifluoroacetate and 4-*tert*-butylphenyl isothiocyanate by method A; purification by flash column chromatography (silica, CHCl$_3$) gave 1.11 g of a white powdered solid (41% yield); TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.38; ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 1.26 (9H, s, *tert*-butyl), 3.70 (3H, s, ArOCH_3), 4.08 (2H, t, J = 5.6 Hz, OCH$_2$CH_2N), 4.25 (2H, t, J = 5.6 Hz, OCH_2CH$_2$N), 4.75 (2H, d, J = 5.3 Hz, ArCH_2NH), 6.17 (1H, br t, NH), 6.80 (3H, m, ArH), 7.24 (4H, m, ArH), 7.76 (4H, m, ArH).

***N*-(4-(2-Aminoethoxy)-3-methoxybenzyl)-*N′*-(4-*tert*-butylphenyl)thiourea (2g)**: synthesized from *N*-(4-(2-phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(4-*tert*-butylphenyl)thiourea; purification by flash column chromatography (silica, CH$_2$Cl$_2$/MeOH, 10:1) gave a colorless glassy solid (48% yield); TLC (silica, CH$_2$Cl$_2$/MeOH, 10:1) R_f 0.15; HPLC reverse phase C-18 (CH$_3$CN/0.1% TFA$_{aq}$ gradient 10−100%) t_R = 9.9 min (>98% purity); ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 1.30 (9H, s, *tert*-butyl), 2.32 (2H, br m, NH$_2$), 3.12 (2H, m, OCH$_2$CH_2N), 3.85 (3H, s, ArOCH_3), 4.03 (2H, t, J = 5.5 Hz, OCH_2CH$_2$), 4.82 (2H, d, J = 5.5 Hz, ArCH_2NH), 6.31 (1H, br t, NH), 6.86 (3H, m, ArH), 7.30 (4H, m, ArH), 7.85 (1H, br m, NH); HRMS (C$_{21}$H$_{30}$N$_3$O$_2$S) calcd 388.2059, found 388.2055.

***N*-(4-(2-Phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(4-*tert*-butylbenzyl)thiourea**: synthesized from 4-(2-phthalimidoethoxy)-3-methoxybenzyl isothiocyanate and 4-*tert*-butylbenzylamine by method B, 97% yield; used directly without further purification; TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.3; ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 1.26 (9H, s, *tert*-butyl), 3.62 (3H, s, ArOCH_3), 4.03 (2H, t, J = 5.6 Hz, OCH$_2$CH_2N), 4.19 (2H, t, J = 5.6 Hz, OCH_2CH$_2$), 4.50 and 4.57 (2 × 2H, 2 × br d, $J \approx$ 5 Hz, 2 × ArCH_2NH), 6.19 (2H, br s, 2NH), 6.64−6.85 (3H, m, ArH), 7.12−7.34 (4H, m, ArH), 7.65−7.87 (4H, m, ArH).

***N*-(4-(2-Aminoethoxy)-3-methoxybenzyl)-*N′*-(4-*tert*-butylbenzyl)thiourea (2h). 2h** was synthesized from *N*-(4-(2-phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(4-*tert*-butylbenzyl)-thiourea, to give a colorless glassy solid (66% yield). The hydrochloride salt was formed by the addition of aqueous HCl to a solution of **2h** in MeOH; the salt was filtered, recrystallized from EtOH/water, and dried *in vacuo* at 75 °C for 48 h, to give a colorless crystalline solid (46% overall yield): TLC (silica, MeOH) R_f 0.2; mp 125−130 °C; ^{1}H-NMR (CDCl$_3$, 400 MHz) δ 1.30 (9H, s, *tert*-butyl), 1.54 (2H, br s, NH$_2$), 3.08 (2H, t, J = 5 Hz, OCH$_2$CH_2N), 3.80 (2H, s, ArOCH_3), 4.00 (2H, t, J = 5 Hz, OCH_2CH$_2$N), 4.54 (2H, br s, ArCH_2N), 4.59 (2H, br s, ArCH_2N), 6.25 (2H, br s, 2 × NH), 6.76 (3H, m, ArH), 7.26 (4H, m, ArH); MS *m/e* 401 (M$^+$). Anal. (C$_{22}$H$_{31}$N$_3$O$_2$S·HCl·H$_2$O) C,H,N,O,S,Cl.

3,5-Di-*tert*-butylbenzoyl Chloride. 3,5-Di-*tert*-butylbenzoic acid (500 mg, 0.002 mol) was suspended in thionyl chloride and refluxed for 3 h. The thionyl chloride was removed *in vacuo*, and the crude product was used without purification.

3,5-Di-*tert*-butylbenzyl Alcohol. 3,5-Di-*tert*-butylbenzoyl chloride (200 mg, 0.8 mmol) was dissolved in anhydrous THF (5 mL), added dropwise to a stirred suspension of LiAlH$_4$ (100 mg, 0.0024 mol) in anhydrous THF (5 mL) under nitrogen, and stirred at room temperature for 3 h. 'Wet' THF was then cautiously added to decompose the excess LiAlH$_4$; the mixture was filtered, the supernatant was dried over MgSO$_4$ and filtered, and the solvent was removed *in vacuo*, to leave a colorless oil, which was used without further purification: ^{1}H-NMR (CDCl$_3$, 200 MHz) δ 1.31 (18H, s, 2 × tBu), 4.65 (2H, br s, ArCH_2OH), 7.22 (2H, m, ArH), 7.38 (1H, m, ArH).

3,5-Di-*tert*-butylbenzaldehyde. 3,5-Di-*tert*-butylbenzyl alcohol (200 mg, 0.9 mmol) and MnO_2 (400 mg, 0.0044 mol) were suspended in chloroform and refluxed for 5 h. After cooling and filtration, the solvent was removed to leave a colorless crystalline solid which was used without further purification: TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.65; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.38 (18H, s, 2 × tBu), 7.72 (3H, s, ArH), 10.00 (1H, s, ArC*H*O).

3,5-Di-*tert*-butylbenzyl methylaldoxime: synthesized from 3,5-di-*tert*-butylbenzaldehyde as described for 3-(4-chlorophenyl)propyl methyloxime to give an off-white solid (87% yield); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.31 (18H, s, 2 × tBu), 3.95 (3H, s, NOCH_3), 7.41 (3H, m, ArH), 8.06 (1H, s, ArC*H*=N).

3,5-Di-*tert*-butylbenzylamine: synthesized from 3,5-di-*tert*-butylbenzyl methyloxime as described for 3-(4-chlorophenyl)propylamine; purification by flash column chromatography (silica, cyclohexane/EtOAc, 1:10) gave 850 mg of a colorless oil (70% yield); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.31 (18H, s, 2 × tBu), 1.75 (2H, br s, NH_2), 3.85 (2H, s, ArCH_2N), 7.20 (3H, m, ArH).

***N*-(4-(2-Phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(3,5-di-*tert*-butylbenzyl)thiourea**: synthesized from 4-(2-phthalimidoethoxy)-3-methoxybenzyl isothiocyanate and 3,5-di-*tert*-butylbenzylamine by method B, in EtOAc (75% yield); TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.16; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.28 (18H, s, 2 × tBu), 3.65 (3H, s, ArOCH_3), 4.15 (4H, m, OCH_2CH_2N), 4.55 (4H, br s, 2 × ArCH_2N), 6.10 (2H, 2 × br s, 2 × NH), 6.75 (3H, m, ArH), 7.10 (2H, m, ArH), 7.35 (1H, m, ArH), 7.75 (4H, m, ArH).

***N*-(4-(2-Aminoethoxy)-3-methoxybenzyl)-*N′*-(3,5-di-*tert*-butylbenzyl)thiourea (2i)**: synthesized from *N*-(4-(2-phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(3,5-di-*tert*-butylbenzyl)-thiourea; purification by flash column chromatography (silica, CH_2Cl_2/MeOH, 10:1, changing to MeOH) gave a colorless glassy solid (33% yield); TLC (silica, MeOH) R_f 0.15; HPLC reverse phase C-18 (CH_3CN/0.1% TFA_{aq} gradient 10−100%) t_R = 9.8 min (>98% purity); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.28 (18H, s, 2 × *t*Bu), 2.62 (2H, br, NH_2), 3.04 (2H, t, J = 5 Hz, OCH_2CH_2N), 3.72 (3H, s, ArOCH_3), 3.95 (2H, t, J = 5 Hz, OCH_2CH_2N), 4.55 (4H, br s, 2 × ArCH_2N), 6.35 (2H, 2 × br s, 2 × NH), 6.75 (3H, m, ArH), 7.10 (2H, m, ArH), 7.30 (1H, m, ArH): HRMS ($C_{26}H_{40}N_3O_2S$) calcd 458.2841, found 458.2847.

2-(4-*tert*-Butylphenyl)ethyl isothiocyanate: synthesized from 2-(4-*tert*-butylphenyl)ethylamine; purification by flash column chromatography (silica, cyclohexane) gave a yellow oil (80% yield).

***N*-(4-(2-Phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(2-(4-*tert*-butylphenyl)ethyl)thiourea**: synthesized from 4-(2-phthalimidoethoxy)-3-methoxybenzyl isothiocyanate and 2-(4-*tert*-butylphenyl)ethylamine by method B; purification by flash column chromatography (silica, cyclohexane/EtOAc, 1:1) gave a white solid (38% yield).

***N*-(4-(2-Aminoethoxy)-3-methoxybenzyl)-*N′*-(2-(4-*tert*-butylphenyl)ethyl)thiourea (2j)**: synthesized from *N*-(4-(2-phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(2-(4-*tert*-butylphenyl)ethyl)thiourea; purification by flash column chromatography (silica, CH_2Cl_2/MeOH, 10:1, changing to MeOH) gave a colorless glassy solid (56% yield); TLC (silica, MeOH) R_f 0.1; HPLC reverse phase C-18 (CH_3CN/0.1% TFA_{aq} gradient 10−100%) t_R = 9.3 min (>98% purity); ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.30 (9H, s, *tert*-butyl), 2.84 (2H, t, J = 7 Hz, ArCH_2CH_2N), 3.07 (2H, br m, OCH$_2$CH_2N), 3.70 (2H, br m, ArCH$_2$CH_2N), 3.82 (3H, s, ArOCH_3), 4.00 (2H, t, J = 5 Hz, OCH_2CH$_2$N), 4.48 (2H, br m, ArCH_2NH), 5.90 (1H, br m, NH), 6.15 (1H, br m, NH), 6.70−6.85 (3H, m, ArH), 7.12−7.34 (4H, m, ArH); HRMS ($C_{23}H_{34}N_3O_2S$) calcd 416.2372, found 416.2378.

Boc-4-(3-bromopropoxy)-3-methoxybenzylamine: prepared as described for Boc-4-(2-bromoethoxy)-3-methoxybenzylamine from Boc-vanillylamine (2.0 g, 0.008 mol) and 1,3-dibromopropane (50 mL) to give a colorless oil, yield 2.91 g (97%); TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.63; ^{1}H-NMR ($CDCl_3$, 60 MHz) δ 1.46 (9H, s, *tert*-butyl), 2.35 (2H, m, OCH$_2$CH_2CH$_2$Br), 3.65 (2H, m, OCH$_2$CH$_2$CH_2Br), 3.85 (3H, s, ArOCH_3), 4.05−4.30 (4H, m, ArCH_2N, OCH_2CH$_2$CH$_2$Br), 4.82 (1H, br m, NH), 6.85 (3H, s, ArH).

Boc-4-(3-phthalimidopropoxy)-3-methoxybenzylamine: prepared as described for Boc-4-(2-phthalimidoethoxy)-3-methoxybenzylamine from Boc-4-(3-bromopropoxy)-3-methoxybenzylamine (2.80 g, 0.0075 mol); purified by flash column chromatography (silica, cyclohexane/EtOAc, 2:1) to give 2.80 g of a white solid (85% yield); TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.44.

(4-(3-Phthalimidopropoxy)-3-methoxybenzyl)ammonium trifluoroacetate: prepared as described for (4-(3-phthalimidoethoxy)-3-methoxybenzyl)ammonium trifluoroacetate, from Boc-4-(3-phthalimidopropoxy)-3-methoxybenzylamine; used without purification or characterization.

***N*-(4-(3-Phthalimidopropoxy)-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)ethyl)thiourea**: synthesized from (4-(3-phthalimidopropoxy)-3-methoxybenzyl)ammonium trifluoroacetate and 2-(4-chlorophenyl)ethyl isothiocyanate by method A; purification by flash column chromatography (silica, cyclohexane/EtOAc, 1:1) gave a white powdered solid (85% yield), TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.29.

***N*-(4-(3-Aminopropoxy)-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)ethyl)thiourea (3)**: synthesized from *N*-(4-(3-phthalimidopropoxy)-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)-ethyl)thiourea; purification by flash column chromatography (silica, CH_2Cl_2/MeOH, 10:1, changing to MeOH) gave a colorless glassy solid (77% yield); TLC (silica, CH_2Cl_2/MeOH/AcOH, 90:9:1) R_f 0.17; HPLC reverse phase C-18 (CH_3CN/0.1% TFA_{aq} gradient 10−100%) t_R = 8.9 min (>98% purity); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 1.76 (2H, m, OCH$_2$CH_2CH$_2$N), 2.67 (2H, t, J = 6.63 Hz, OCH$_2$CH$_2$CH_2N), 2.82 (2H, t, J = 7.30 Hz, ArCH_2CH$_2$N), 3.62 (2H, br m, ArCH$_2$CH_2N), 3.72 (3H, s, ArOCH_3), 3.98 (2H, t, J = 6.35 Hz, OCH_2CH$_2$CH$_2$N), 4.54 (2H, br m, ArCH_2N), 6.78 (1H, m, ArH), 6.90 (2H, m, ArH), 7.30 (4H, m, ArH), 7.63 (1H, br m, NH), 7.98 (1H, br m, NH); HRMS ($C_{20}H_{27}N_3O_2SCl$) calcd 408.1513, found 408.1520.

***N*-Boc-3-methoxy-4-(2-(methylammonio)ethoxy)benzylamine Hydrobromide.** Boc-4-(2-bromoethoxy)-3-methoxybenzylamine (2.0 g, 0.0056 mol) was dissolved in a 33% solution of methylamine in EtOH (250 mL) and refluxed under a positive pressure of argon for 6 h. Solvent and excess methylamine were removed *in vacuo*. Purification by flash column chromatography ($CHCl_3$/MeOH, 3:2) gave 1.65 g of a colorless glassy solid (76% yield): TLC (silica, CH_2Cl_2/MeOH/AcOH, 120:90:5) R_f 0.65; ^{1}H-NMR (CD_3OD, 60 MHz) δ 1.45 (9H, s, tBu), 2.80 (3H, s, NCH_3), 3.35 (2H, m, CH$_2$CH_2NCH$_3$), 3.90 (3H, s, ArOCH_3), 4.1−4.4 (4H, m, ArCH_2N, OCH_2CH$_2$), 6.45 (3H, m, ArH).

Boc-4-(2-(*N*-((9-fluorenylmethyloxy)carbonyl)-*N*-methylamino)ethoxy)-3-methoxybenzylamine. Boc-3-methoxy-4-(2-(methylammonio)ethoxy)benzylamine hydrobromide (1.65 g, 0.0042 mol) and Fmoc-O-Su (1.57 g, 0.0046 mol) were dissolved in 75 mL of dioxane (plus a few drops of water) with triethylamine (650 μL, 470 mg, 0.0047 mol), and the solution was stirred at room temperature for 6 h. The solvents were removed *in vacuo*, and the residue was partitioned between EtOAc and water. The organic phase was washed with brine and dried over anhydrous Na_2SO_4. The mixture was filtered and the solvent removed *in vacuo*. The product was purified by flash column chromatography (silica, $CHCl_3$) to give 2.1 g of a colorless oil (93% yield): TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.37; ^{1}H-NMR ($CDCl_3$, 60 MHz) δ 1.45 (9H, s, tBu), 3.05 (3H, s, NCH_3), 3.70 (2H, m, CH$_2$CH_2NCH$_3$), 3.80 (3H, s, ArOCH_3), 4.1−4.6 (6H, m, fluorenylCH_2CH, ArCH_2N, OCH_2-CH$_2$), 4.85 (1H, m, C*H*), 6.80 (3H, m, ArH), 7.2−7.9 (8H, m, fluoreneArH).

(4-(2-(*N*-((9-Fluorenylmethyloxy)carbonyl)-*N*-methylamino)ethoxy)-3-methoxybenzyl)ammonium trifluoroacetate: prepared as described for (4-(2-phthalimidoethoxy)-3-methoxybenzyl)ammonium trifluoroacetate, from Boc-4-(2-(*N*-((9-fluorenylmethyloxy)carbonyl)-*N*-methylamino)ethoxy)-3-methoxybenzylamine (2.0 g, 0.0038 mol), to give 2.05 g of a colorless glassy solid (100% yield); ^{1}H-NMR (CD_3OD, 60 MHz) δ 2.95 (3H, s, NCH_3), 3.20−4.60 (12H, m, 4 × CH$_2$, ArOCH_3, CH), 6.70−7.9 (11H, m, ArH).

***N*-(4-(2-(*N*-((9-Fluorenylmethyloxy)carbonyl)-*N*-methylamino)ethoxy)-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)-ethyl)thiourea**: synthesized from (4-(2-(*N*-((9-fluorenylme-

thyloxy)carbonyl)-*N*-methylamino)ethoxy)-3-methoxybenzyl)ammonium trifluoroacetate and 2-(4-chlorophenyl)ethyl isothiocyanate by method A; purification by flash column chromatography (silica, cyclohexane/EtOAc, 1:1) gave a colorless glassy solid (96% yield); TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.23.

***N*-(3-Methoxy-4-(2-(methylamino)ethoxy)benzyl-*N′*-(2-(4-chlorophenyl)ethyl)thiourea (4).** *N*-(4-(2-(*N*-((9-Fluorenylmethyloxy)carbonyl)-*N*-methylamino)ethoxy)-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)ethyl)thiourea (1.90 g, 0.003 mol) was dissolved in CH_2Cl_2 (50 mL) with piperidine (50 mL) and stirred for 30 min at room temperature. The solution was diluted with CH_2Cl_2 and washed with water and brine, and the solvent was removed *in vacuo*. Purification by flash column chromatography (silica, CH_2Cl_2/MeOH, 5:1) gave 300 mg of a colorless glassy solid (24% yield): TLC (silica, CH_2Cl_2/MeOH, 5:1) R_f 0.35; HPLC reverse phase C-18 (CH_3CN/0.1% TFA_{aq} gradient 10−100%) t_R = 9.1 min (>98% purity); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.62 (3H, s, NCH_3), 2.80 (2H, t, J = 7.33 Hz, ArCH_2CH$_2$NH), 3.26 (2H, br m, OCH$_2$CH_2NH), 3.60 (2H, br m, ArCH$_2$CH_2NH), 3.76 (3H, s, ArOCH_3), 4.21 (2H, t, J = 5.1 Hz, OCH_2CH$_2$NH), 4.60 (2H, br m, ArCH_2NH), 6.80 (1H, m, ArH), 7.00 (2H, m, ArH), 7.30 (4H, m, ArH), 7.80 (1H, t, J = 5.2 Hz, NH), 8.07 (1H, t, J = 5.6 Hz, NH); HRMS ($C_{20}H_{27}N_3O_2SCl$) calcd 408.1513, found 408.1508.

***N*-(4-(2-(*N*,*N*-Dimethylamino)ethoxy)-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)ethyl)thiourea (5).** The free base of compound **2a** (250 mg, 0.000 63 mol) was dissolved in 15 mL of MeOH with paraformaldehyde (160 mg, 0.0053 mol) and refluxed for 3 h. After cooling to room temperature, sodium cyanoborohydride (130 mg, 0.002 mol) was added, and the reaction mixture was stirred at room temperature for 30 min. Water (10 mL) was added, and the solvent was removed *in vacuo*. The product was extracted into CH_2Cl_2, the solvent removed *in vacuo*, and the product purified by flash column chromatography (silica, CH_2Cl_2/MeOH, 10:1) to give 80 mg of a colorless glassy solid (30% yield): TLC (silica, CH_2Cl_2/MeOH, 10:1) R_f 0.17; HPLC reverse phase C-18 (CH_3CN/0.1% TFA_{aq} gradient 10−100%) t_R = 9.1 min (>98% purity); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.23 (6H, s, N(CH_3)$_2$), 2.62 (2H, t, J = 5.97 Hz, OCH$_2$CH_2N), 2.82 (2H, t, J = 7.18 Hz, ArCH_2CH$_2$N), 3.63 (2H, br m, ArCH$_2$CH_2N), 3.75 (3H, s, ArOCH_3), 4.02 (2H, t, J = 5.93 Hz, OCH_2CH$_2$N), 4.57 (2H, br m, ArCH_2N), 6.78 (1H, m, ArH), 6.92 (2H, m, ArH), 7.30 (4H, m, ArH), 7.44 (1H, br m, NH), 7.80 (1H, br m, NH); HRMS ($C_{21}H_{29}N_3O_2SCl$) calcd 422.1669, found 422.1662.

Boc-4-(2-(trimethylammonio)ethoxy)-3-methoxybenzylamine Bromide. Boc-4-(2-bromoethoxy)-3-methoxybenzylamine (1.0 g, 0.0027 mol) was dissolved in a saturated solution of trimethylamine in MeOH, and the solution was refluxed under a positive pressure of N_2 for 18 h, until the reaction was complete by TLC. Solvent was removed *in vacuo* to give 1.15 g of a colorless oil (100% yield); this was used without purification: TLC (silica, CH_2Cl_2/MeOH/AcOH, 120:90:5) R_f 0.30.

(4-(2-(Trimethylammonio)ethoxy)-3-methoxybenzyl)-ammonium ditrifluoroacetate: prepared as described for (4-(3-phthalimidoethoxy)-3-methoxybenzyl)ammonium trifluoroacetate, from Boc-4-(2-(trimethylammonio)ethoxy)-3-methoxybenzylamine bromide; used without purification or characterization.

***N*-(4-(2-(Trimethylammonio)ethoxy)-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)ethyl)thiourea trifluoroacetate (6)**: synthesized from (4-(2-(trimethylammonio)ethoxy)-3-methoxybenzyl)ammonium ditrifluoroacetate and 2-(4-chlorophenyl)ethyl isothiocyanate by method A; purification by preparative HPLC (reverse phase C-18, silica, gradient 90:10 acetonitrile/0.1% TFA_{aq}, changing to 100% acetonitrile) gave 280 mg of a colorless glassy solid (19% yield); TLC (silica, CH_2Cl_2/MeOH/AcOH, 120:90:5) R_f 0.2; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 2.80 (2H, t, J = 7.30 Hz, ArCH_2CH$_2$N), 3.20 (9H, s, N(CH_3)$_2$), 3.60 (2H, br m, ArCH$_2$CH_2N), 3.75 (3H, s, ArOCH_3), 3.78 (2H, t, J = 4.81 Hz, ArOCH_2CH$_2$N), 4.39 (2H, br m, ArOCH$_2$CH_2N(CH$_3$)$_2$), 4.59 (2H, br m, ArCH_2N), 6.82 (1H, m, ArH), 7.02 (2H, m, ArH), 7.30 (4H, m, ArH), 8.13 (1H, br m, NH), 8.38 (1H, br m, NH); MS *m/e* 436 (M^+). Anal. ($C_{20}H_{30}N_3O_2SCl\cdot CF_3CO_2H\cdot H_2O$) C,H,N,O,F.

***N*-(4-(2-Phthalimidoethoxy)-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)ethyl)thiourea (7)**: synthesized from 4-(2-phthalimidoethoxy)-3-methoxybenzylamine trifluoroacetate and 2-(4-chlorophenyl)ethyl isothiocyanate by method A, in EtOAc with triethylamine; purification by flash column chromatography (silica, cyclohexane/EtOAc, 1:1) gave a pale yellow crystalline solid in 69% yield; mp 59−61 °C; TLC (silica, cyclohexane/EtOAc, 1:2) R_f 0.4; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.84 (2H, t, J = 7.0 Hz, ArCH_2CH$_2$N), 3.62 (5H, m, ArCH$_2$CH_2N, ArOCH_3), 4.11 (2H, t, J = 5.7 Hz, OCH_2CH$_2$N), 4.27 (2H, t, J = 5.7 Hz, OCH$_2$CH_2N), 4.39 (2H, d, J = 5.5 Hz, ArCH_2N), 5.62 (1H, br s, NH), 6.03 (1H, br s, NH), 6.70−6.85 (3H, m, ArH), 7.15 (4H, m, ArH), 7.80 (4H, m, ArH): MS *m/e* 523 (M^+). Anal. ($C_{27}H_{26}N_3O_4SCl$) C,H,N.

***N*-(4-(2-Acetamidoethoxy)-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)ethyl)thiourea (8).** Compound **2a** (400 mg, 0.001 mol) was dissolved in anhydrous CH_2Cl_2 (30 mL) with triethylamine (0.160 mL, 116 mg, 0.0012 mol) and acetyl chloride (80 mg, 0.001 mol) and stirred at room temperature under an atmosphere of nitrogen for 2.5 h, until the reaction was complete by TLC. The solution was diluted to 150 mL with CH_2Cl_2 and washed with water and brine. The solvent was removed *in vacuo*. The residue was purified by flash column chromatography (silica, CH_2Cl_2/MeOH, 20:1) and recrystallized from EtOAc/*n*-hexane to give 250 mg of a white crystalline solid (56% yield): mp 121−122 °C; TLC (silica, CH_2Cl_2/MeOH, 10:1) R_f 0.30; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ 1.82 (3H, s, CH_3), 2.80 (2H, t, J = 7.2 Hz, ArCH_2CH$_2$NH), 3.33 (2H, br m, OCH$_2$CH_2NH), 3.62 (2H, br m, ArCH$_2$CH_2NH), 3.72 (3H, s, ArOCH_3), 3.93 (2H, t, J = 5.9 Hz, OCH_2CH$_2$NH), 4.55 (2H, br m, ArCH_2NH), 6.75 (1H, m, ArH), 6.92 (2H, m, ArH), 7.30 (4H, m, ArH), 7.45 (1H, br m, NH), 7.80 (1H, br m, NH), 8.10 (1H, br m, NH); FABMS *m/e* 436 (MH^+). Anal. ($C_{21}H_{26}N_3O_3SCl$) C,H,N.

***N*-(4-(2-((Ethoxycarbonyl)amino)ethoxy)-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)ethyl)thiourea (9)**: prepared as described for compound **8** from the free base of compound **2a** (300 mg, 0.000 76 mol) and ethyl chloroformate (0.075 mL, 86 mg, 0.0008 mol); purification by flash column chromatography (silica, cyclohexane/EtOAc, 1:1) and recrystallization from EtOAc/*n*-hexane gave 140 mg of a white crystalline solid (40% yield); mp 73−74 °C; TLC (silica, CH_2Cl_2/MeOH, 20:1) R_f 0.46; ^{1}H-NMR (CD_3OD, 400 MHz) δ 1.23 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.86 (2H, t, J = 7.08 Hz, ArCH_2CH$_2$NH), 3.46 (2H, t, J = 5.55 Hz, OCH$_2$CH_2N), 3.72 (2H, br m, ArCH$_2$CH_2NH), 3.83 (3H, s, ArOCH_3), 4.02 (2H, t, J = 5.55 Hz, OCH_2CH$_2$), 4.08 (2H, q, J = 6.9 Hz, OC(=O)CH_2CH$_3$)), 4.58 (2H, br m, ArCH_2NH), 6.80−7.00 (3H, m, ArH), 7.20 (4H, m, ArH); FABMS *m/e* 466 (MH^+). Anal. ($C_{22}H_{28}N_3O_4SCl$) C,H,N.

***N*-(4-(2-(Boc-amino)ethoxy)-3-methoxybenzyl)-*N′*-(2-(4-chlorophenyl)ethyl)thiourea (10)**: prepared as described for compound **8** from the free base of compound **2a** (300 mg, 0.000 76 mol) and di-*tert*-butyl dicarbonate (340 mg, 0.0015 mol); purification by flash column chromatography (silica, cyclohexane/EtOAc, 1:1) and recrystallization from EtOAc/*n*-hexane gave 105 mg of a white crystalline solid (28% yield); mp 117 °C; TLC (silica, cyclohexane/EtOAc, 1:1) R_f 0.21; ^{1}H-NMR (CD_3OD, 400 MHz) δ 1.42 (9H, s, *tert*-butyl), 2.86 (2H, t, J = 7.14 Hz, ArCH_2CH$_2$N), 3.41 (2H, t, J = 5.55 Hz, OCH$_2$CH_2N), 3.70 (2H, br m, ArCH$_2$CH_2N), 3.83 (3H, s, ArOCH_3), 4.00 (2H, t, J = 5.57 Hz, OCH_2CH$_2$), 4.58 (2H, br m, ArCH_2NH), 6.79−6.96 (3H, m, ArH), 7.20 (4H, m, ArH); FABMS *m/e* 494 (MH^+). Anal. ($C_{24}H_{32}N_3O_4SCl$) C,H,N.

Biology. The *in vitro* assay ($^{45}Ca^{2+}$ influx into neonatal rat DRG neurons) and the mouse tail-flick *in vivo* antinociceptive assay have been described in part 1 of this series.[1]

Mouse Writhing Antinociceptive Assay. Female mice (CD-1, Charles River, weight 20g) were maintained in a controlled lighting environment (12 h on/12 h off) and fasted overnight prior to testing. Animals received an intraperitoneal injection of 0.3 mL of an acetic acid solution (200 mM), and 5 min later the number of abdominal constrictions was counted in the subsequent 5 min period. Animals received drug or vehicle (10 animals/group) subcutaneously or orally 55 min

prior to administration of acetic acid. Compounds were considered to have interesting antinociceptive properties if they produced a significant increase in threshold ($p < 0.05$).

The percentage analgesic effect was calculated as (mean number of writhes (drug))/(mean number of writhes (vehicle)) $\times$ 100. A logistic function (ORIGIN software) was fitted to these data, and an ED_{50} value was calculated, with standard error, as the dose required to produce a 50% reduction in the number of writhes.

Bronchoconstriction. Capsaicin is known to cause an acute bronchoconstrictor response when administered as an intravenous injection in anesthetized guinea pigs.[10] Dunkin−Hartley guinea pigs (400−450 g, $n = 4$ animals/treatment group) were anesthetized with urethane (2 g/kg of body weight) and placed on a heated blanket to maintain body temperature at 37 °C. The carotid artery was cannulated for blood pressure measurements and a jugular cannula inserted for administration of drugs. The trachea was cannulated and the animal artifically ventilated at a frequency of 60 strokes/min and a tidal volume of 10 mL/kg of body weight. Airway opening pressure (P_{ao}) was measured with a differential pressure transducer (Farnell Electronics), attached to a side arm of the tracheal cannula, as an index of changes in tracheobronchial resistance to airflow. The threshold dose required to induce an increase in P_{ao} was determined following iv administration.

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Supporting Information Available: Analytical HPLC elution solvent gradient (1 page). Ordering information is given on any current masthead page.

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