Nonpeptide Bradykinin B_2 Receptor Antagonists: Conversion of Rodent-Selective Bradyzide Analogues into Potent, Orally-Active Human Bradykinin B_2 Receptor Antagonists[1]

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The 1-(2-nitrophenyl)thiosemicarbazide (TSC) derivative, (*S*)-1-[4-(4-benzhydrylthiosemicarbazido)-3-nitrobenzenesulfonyl]pyrrolidine-2-carboxylic acid {2-[(2-dimethylaminoethyl)methylamino]ethyl}amide (bradyzide; (*S*)-**4**), was recently disclosed as a novel, potent, orally active nonpeptide bradykinin (BK) B_2 receptor antagonist. The compound inhibited the specific binding of [^{3}H]BK to NG108-15 cell membrane preparations (rodent neuroblastoma-glioma) expressing B_2 receptors with a K_i of 0.5 ± 0.2 nM. Compound (*S*)-**4** also demonstrated oral efficacy against Freund's complete adjuvant (FCA)-induced mechanical hyperalgesia in rats with an ED_{50} value of 0.84 μmol/kg. After we optimized the terminal binding determinants projecting from the TSC framework, we found that it was possible to replace the potentially toxicophoric nitro and divalent sulfur moieties with only a 15-fold loss in binding affinity ((*S*)-**14a**). However, bradyzide and its congeners were found to have much lower affinities for cloned human B_2 receptors, expressed in Cos-7 cells. The hitherto synthesized TSC series was screened against the human B_2 receptor, and the dibenzosuberane (DBS) pharmacophore emerged as the key structural requirement for potency. Incorporation of this group resulted in a series of derivatives ((*S*)-**14d**,**e** and **19b**−**d**) with K_i ranges of 10.7−176 nM in NG108-15 cells (expressing the rodent B_2 receptor) and 0.79−253 nM in Cos-7 cells (expressing the human B_2 receptor). There was no evidence of agonist activity with any of the nonpeptides in any of the cell lines tested. In vivo, oral administration of compound **19c** reversed FCA-induced and turpentine-induced mechanical hyperalgesia in rodents with ED_{50} values of 0.027 and 0.32 μmol/kg, respectively. The selectivity profiles of compounds (*S*)-**14f** and (*S*)-**14g** were also assessed to determine the conformational and/or steric preferences of the double-ring arrangement. The affinity of (*S*)-**14g** for the human B_2 receptor suggested that it may be a hydrophobic interaction with the ethane bridge of the DBS moiety that accounts for the increased potency of compounds (*S*)-**14d**,**e** and **19b**,**c** at this receptor, by favoring a binding mode inaccessible to the unsubstituted diphenylmethyl derivative, (*S*)-**4**.

Introduction

Bradykinin (BK; Arg^1-Pro^2-Pro^3-Gly^4-Phe^5-Ser^6-Pro^7-Phe^8-Arg^9) and kallidin (KD; [Lys^0]BK; Lys^1-Arg^2-Pro^3-Pro^4-Gly^5-Phe^6-Ser^7-Pro^8-Phe^9-Arg^{10}) are the two major mammalian representatives of the kinin family of endogenous oligopeptide hormones. These linear peptides are released predominantly by the action of kallikrein enzymes (trypsin-like serine proteases) on large inactive precursor proteins (kininogens).[2] BK itself is produced in plasma from a high molecular weight form of kininogen. The physiological actions of kinins include vasodilation, increased vascular permeability, stimulation of sensory neurones, vascular and bronchial smooth muscle contraction, intestinal ion secretion, release of prostaglandins and cytokines, and the production of nitric oxide.[2−5] Overproduction of kinins under pathophysiological conditions such as tissue trauma or injury is believed to underlie a number of clinically relevant disorders including pain and inflammation, hypotension, asthma, rhinitis, pancreatitis, sepsis, and rheumatoid arthritis.[6−8] BK has also been implicated in the peripheral inflammatory processes associated with Alzheimer's disease[9] and as a growth stimulant for certain solid tumors.[10]

Most of the effects of BK are mediated by the B_2 receptor subtype,[8,11] a constitutive receptor expressed in many tissues.[3] The chronic responses to kinins, on the other hand, are mediated by B_1 receptors,[5,11] which are inducible and recognize the C-terminally truncated

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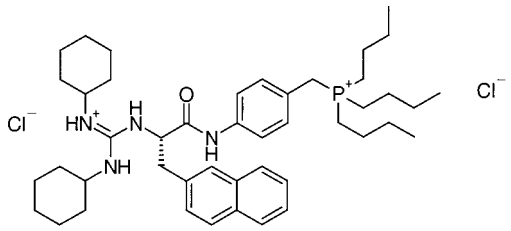
des-Arg active metabolites of BK and KD.[12−14] Both receptor subtypes are members of the G-protein-coupled seven transmembrane receptor superfamily and have been cloned and pharmacologically characterized.[13,15,16] It follows that antagonism of B_2 (and B_1) receptor activation could constitute a novel approach to the treatment of pain[17,18] and the other inflammatory diseases listed above.[6,19−21]

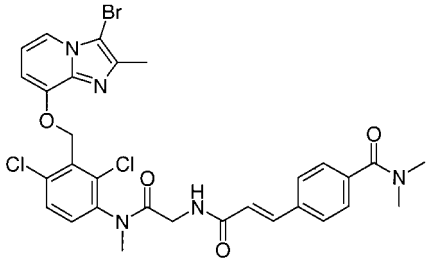
Over the past decade or so, four generations of sequence-related peptide BK receptor antagonists have been described, building on the seminal work of Vavrek and Stewart in the mid-1980s.[22] Between them, NPC 567[23] ([D-Arg^0, Hyp^3, D-Phe^7]BK), HOE 140[24] (icatibant; [D-Arg^0, Hyp^3, Thi^5, D-Tic^7, Oic^8]BK), B-9430[25] ([D-Arg^0, Hyp^3, Igl^5, D-Igl^7, Oic^8]BK), and B-10056[26] ([D-Arg^0, Hyp^3, Igl^5, D-f5f^7, Igl^8]BK) chart the evolution of highly potent and stable peptide antagonists. While these may find medical application in particular instances, agents for oral administration are likely to be nonpeptidic in nature and devoid of the limitations that hinder broader therapeutic use of peptides.

The first competitive nonpeptide B_2 antagonist was published in 1993 by Salvino et al. at Sterling Winthrop.[27] WIN 64338 (**1**, Figure 1) inhibited [^{3}H]BK binding to B_2 receptors in human IMR 90 fetal lung fibroblasts with an K_i of 60 ± 10 nM. In 1997, Fujisawa researchers disclosed a second class of nonpeptide B_2 antagonist, exemplified by FR167344 (**2**) and FR173657 (**3**).[28] These were not only potent (nanomolar IC_{50} values) and selective but also orally active. Subsequent reports, principally from Fournier[29] and Hoechst,[30] have described compounds very much patterned after the Fujisawa model.

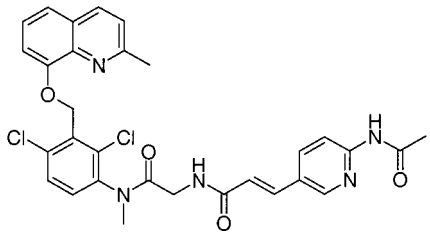
From our own research program, the 1-(2-nitrophenyl)thiosemicarbazide (TSC) derivative, (*S*)-1-[4-(4-benzhydrylthiosemicarbazido)-3-nitrobenzenesulfonyl]-pyrrolidine-2-carboxylic acid {2-[(2-dimethylaminoethyl)-methylamino]ethyl}amide (bradyzide; (*S*)-**4**), was recently disclosed as a novel, potent, orally active nonpeptide BK B_2 receptor antagonist.[31,32] The compound inhibited the specific binding of [^{3}H]BK to NG108-15 cell membrane preparations (rodent neuroblastoma-glioma) expressing B_2 receptors with a K_i of 0.5 ± 0.2 nM. Compound (*S*)-**4** also demonstrated oral efficacy against Freund's complete adjuvant (FCA)-induced mechanical hyperalgesia in rats with an ED_{50} value of 0.84 μmol/kg. While there was no evidence that (*S*)-**4** possessed mutagenic activity, we found that it was possible to replace the undesirable aromatic nitro and divalent sulfur groups and retain nanomolar affinity. However, compound (*S*)-**4** and its analogues were more than 1000-fold less potent at cloned human B_2 receptors, expressed in Cos-7 cells. A reevaluation of our compound library revealed an apparently modest structural feature, which had a significant impact on human BK B_2 receptor affinity once it was incorporated into the full TSC structure. In this paper, we wish to document the structural changes that enabled us to solve the problem of species specificity in this compound series.



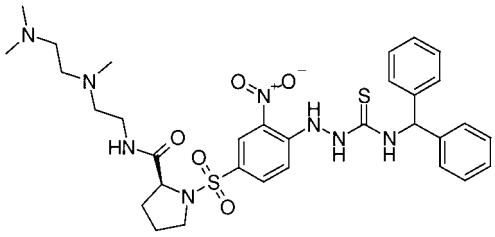
WIN 64338 (**1**)



FR 167344 (**2**)



FR 173657 (**3**)



Bradyzide ((*S*)-**4**)

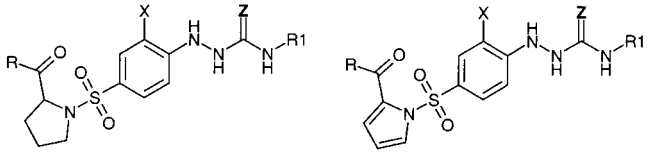
Figure 1. Structures of BK B_2 receptor antagonists WIN 64338 (**1**), FR 167344 (**2**), FR 173657 (**3**), and bradyzide ((*S*)-**4**).

Chemistry

The compounds described herein are presented in Table 1, and the methods for their syntheses are shown in Schemes 1−3. Sulfonylation of (*S*)- or (*R*)-proline ((*S*)-**9a** or (*R*)-**9a**) or (*S*)-prolinamide ((*S*)-**9b**) with commercially available 4-chloro-3-nitrobenzenesulfonyl chloride or 3-chloro-4-fluorobenzenesulfonyl chloride in aqueous sodium carbonate solution gave the sulfonamides **10a**−**c**. This modification of our previously published procedure[31] allowed us to omit an ester deprotection step from the reaction sequence. In an analogous fashion, the corresponding pyrrole derivatives **16a**,**b** were prepared by sulfonylation of pyrrole-2-carboxylic acid (**15**) following deprotonation with 2.2 equiv of *n*-butyllithium in tetrahydrofuran (THF) at −20 °C.

Treatment of sulfonamides **10a**−**c** and **16a**,**b** with hydrazine monohydrate at reflux in THF in the presence of triethylamine afforded the phenylhydrazine derivatives **11a**−**c** and **17a**,**b**, respectively. Generally, these were not isolated but coupled in situ with isothiocyanates (**12a**,**c**,**e**,**f**)[33] or isocyanates (**12b**,**d**)[34] to give the corresponding thiosemicarbazides **13a**,**b**,**d**,**f**,**g** and **18a**,**b**

Table 1. Binding Affinities (K_i) of Compounds at the Rat and Human BK B_2 Receptors (B_2R)

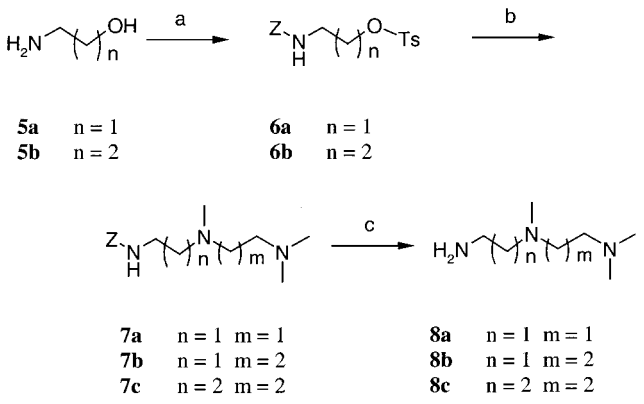


4, 13b, 14a-g **19a-d**

					in vitro K_i (nM)	
compd[a]	R	X	Z	R1[b]	rat B_2R NG108-15 cells[c]	human B_2R Cos-7 cells[d]
(*S*)-**4**	$Me_2N(CH_2)_2N(Me)(CH_2)_2NH$-	NO_2	S	DPM	0.5 ± 0.2[e]	772 ± 144[e]
(*R*)-**4**	$Me_2N(CH_2)_2N(Me)(CH_2)_2NH$-	NO_2	S	DPM	0.6 ± 0.12[e]	1430 ± 500
(*S*)-**13b**	H_2N	NO_2	S	DBS	654 ± 65	299 ± 33
(*S*)-**14a**	$Me_2N(CH_2)_2N(Me)(CH_2)_2NH$-	Cl	O	DPM	7.3 ± 1.8	ND[f]
(*S*)-**14b**	$Me_2N(CH_2)_3N(Me)(CH_2)_2NH$-	NO_2	S	DPM	0.023 ± 0.007	119 ± 30
(*S*)-**14c**	$Me_2N(CH_2)_3N(Me)(CH_2)_3NH$-	NO_2	S	DPM	0.31 ± 0.1	95 ± 10
(*S*)-**14d**	$Me_2N(CH_2)_2N(Me)(CH_2)_2NH$-	NO_2	S	DBS	14.5 ± 2.5	4.67 ± 0.7
(*S*)-**14e**	$Me_2N(CH_2)_3N(Me)(CH_2)_2NH$-	NO_2	O	DBS	10.7 ± 1.6	0.79 ± 0.14
(*S*)-**14f**	$Me_2N(CH_2)_3N(Me)(CH_2)_2NH$-	NO_2	S	FL	ND	645 ± 142
(*S*)-**14g**	$Me_2N(CH_2)_2N(Me)(CH_2)_2NH$-	NO_2	S	DCDPM	5.46 ± 1.7	34.1 ± 6.5
19a	$Me_2N(CH_2)_2N(Me)(CH_2)_2NH$-	NO_2	S	DPM	ND	237 ± 38
19b	$Me_2N(CH_2)_2N(Me)(CH_2)_2NH$-	NO_2	S	DBS	64.5 ± 19.4	1.47 ± 0.34
19c	$Me_2N(CH_2)_3N(Me)(CH_2)_2NH$-	NO_2	O	DBS	176 ± 51	2.79 ± 0.47
19d	$Me_2N(CH_2)_3N(Me)(CH_2)_2NH$-	Cl	O	DBS	ND	253 ± 33

[a] Unless otherwise noted, elemental microanalyses were within ±0.4% of the theoretical values for the formulas given in the Experimental Section. [b] Abbreviations: DPM, diphenylmethyl; DBS, dibenzosuberyl; FL, 9-fluorenyl; DCDPM, 2,2′-dichlorodiphenylmethyl. [c] Concentration required to inhibit specific binding of [^{3}H]BK (1 nM) to B_2 receptors by 50% in NG108-15 cell membranes. See the Experimental Section for more details. [d] Concentration required to inhibit specific binding of [^{3}H]BK (1 nM) by 50% to cloned B_2 receptors expressed in Cos-7 cells. See the Experimental Section for further details. The results are expressed as the mean of at least three determinations ± SEM and calculated on the basis of a one site binding model using LIGAND software (see ref 46). [e] Previously published, see ref 31. [f] ND, not determined.

Scheme 1[a]



5a n = 1 **6a** n = 1
5b n = 2 **6b** n = 2

7a n = 1 m = 1 **8a** n = 1 m = 1
7b n = 1 m = 2 **8b** n = 1 m = 2
7c n = 2 m = 2 **8c** n = 2 m = 2

[a] Reagents: (a) (i) ZOSu, EtOAc, (ii) TsCl, pyridine; (b) *N*,*N*,*N*′-trimethylethylenediamine (for **7a**) or *N*,*N*,*N*′-trimethylpropylenediamine (for **7b**,**c**); (c) HCO_2NH_4, Pd/C, MeOH or H_2, Pd/C, MeOH.

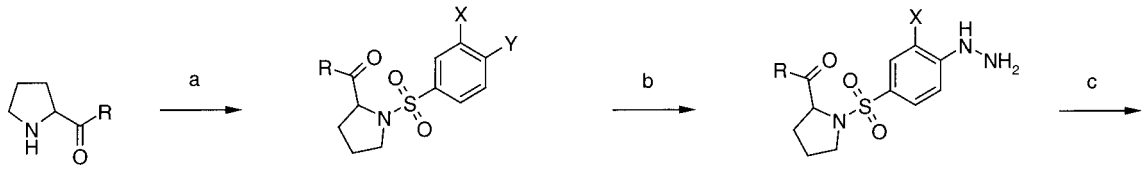
or semicarbazides (*S*)-**13c**,**e** and **18c**,**d**, respectively. In a convergent approach, the final compounds were assembled by mixed anhydride coupling of the carboxylic acids **13a**−**g** and **18a**−**d** with the preformed triamines **8a**−**c**[35,36] using isopropyl- or isobutyl chloroformate in the presence of *N*-methyl morpholine (Schemes 2 and 3). Isopropyl chloroformate was the reagent of choice for the pyrrole series as this was found to minimize attack at the "wrong" carbonyl group of the mixed anhydride species. The triamines **8a**−**c** were synthesized in a two step procedure from tosylated *N*-Z ethanolamine (**6a**)[37] and tosylated *N*-Z propanolamine (**6b**) (Scheme 1).[38] The procedures described in the Experimental Section for the displacement of the tosyl group by the trimethyldiamines and for the hydrogenolysis of the Z group could be used interchangeably.

The desired products were mostly purified by preparative high-performance liquid chromatography (HPLC). Those incorporating the fluorenyl (FL) or diphenylmethyl (DPM) moiety (**4**, (*S*)-**14a**−**c**,**f**,**g** and **19a**) could be isolated as amorphous trifluoroacetate (TFA) salts by concentrating the column fractions under reduced pressure. However, loss of the dibenzosuberyl (DBS) group was experienced under the same conditions. Compounds (*S*)-**13b**, (*S*)-**14d**,**e**, and **19b**−**d** were therefore isolated by organic solvent extraction from the neutralized HPLC fractions, followed by organic solvent extraction in vacuo to afford amorphous solids. The absolute configurations of the final products were assigned by extrapolation from whichever proline enantiomer was employed. Enantiomeric pairs of compounds were not routinely synthesized, but a nuclear magnetic resonance (NMR)-based enantiomeric purity determination on one such pair (data not shown) utilizing the chiral resolving agent (*R*)-(+)-*tert*-butylphenylphosphinothioic acid[39] indicated that the stereochemical integrity of the final compounds was not compromised by the reaction conditions employed.

Results and Discussion

We have recently described a series of novel nonpeptide BK B_2 receptor antagonists based on the TSC framework.[31] These compounds had nanomolar affinities at the rat BK B_2 receptor as measured in a radioligand binding assay in NG108-15 cell membranes using [^{3}H]BK as the radiolabel. In particular, bradyzide ((*S*)-**4**) emerged as the most potent member of this series with a K_i value of 0.5 ± 0.2 nM (Table 1).[32] A stereochemical preference for binding to the rat BK B_2 receptor was not observed, with the (*R*) enantiomer ((*R*)-

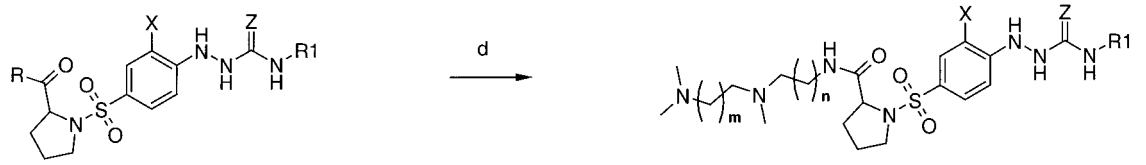
Scheme 2[a]



(S)-**9a** R = OH
(R)-**9a** R = OH
(S)-**9b** R = NH_2

(S)-**10a** R = OH X = NO_2 Y = Cl
(R)-**10a** R = OH X = NO_2 Y = Cl
(S)-**10b** R = NH_2 X = NO_2 Y = Cl
(S)-**10c** R = OH X = Cl Y = F

(S)-**11a** R = OH X = NO_2
(R)-**11a** R = OH X = NO_2
(S)-**11b** R = NH_2 X = NO_2
(S)-**11c** R = OH X = Cl

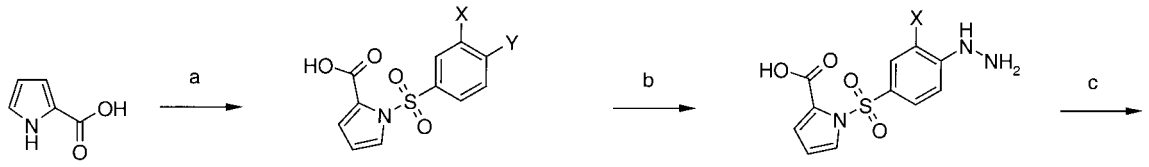


(S)-**13a**	R = OH	X = NO_2	Z = S	R1 = DPM
(R)-**13a**	R = OH	X = NO_2	Z = S	R1 = DPM
(S)-**13b**	R = NH_2	X = NO_2	Z = S	R1 = DBS
(S)-**13c**	R = OH	X = Cl	Z = O	R1 = DPM
(S)-**13d**	R = OH	X = NO_2	Z = S	R1 = DBS
(S)-**13e**	R = OH	X = NO_2	Z = O	R1 = DBS
(S)-**13f**	R = OH	X = NO_2	Z = S	R1 = FL
(S)-**13g**	R = OH	X = NO_2	Z = S	R1 = DCDPM

(S)-**4**	m = 1	n = 1	X = NO_2	Z = S	R1 = DPM
(R)-**4**	m = 1	n = 1	X = NO_2	Z = S	R1 = DPM
(S)-**14a**	m = 1	n = 1	X = Cl	Z = O	R1 = DPM
(S)-**14b**	m = 2	n = 1	X = NO_2	Z = S	R1 = DPM
(S)-**14c**	m = 2	n = 2	X = NO_2	Z = S	R1 = DPM
(S)-**14d**	m = 1	n = 1	X = NO_2	Z = S	R1 = DBS
(S)-**14e**	m = 2	n = 1	X = NO_2	Z = O	R1 = DBS
(S)-**14f**	m = 2	n = 1	X = NO_2	Z = S	R1 = FL
(S)-**14g**	m = 1	n = 1	X = NO_2	Z = S	R1 = DCDPM

[a] Reagents: (a) Na_2CO_3, H_2O, 4-chloro-3-nitrobenzenesulfonyl chloride (for **10a**,**b**) or 3-chloro-4-fluorobenzenesulfonyl chloride (for **10c**); (b) $H_2NNH_2 \cdot H_2O$, Et_3N, THF, reflux; (c) R1NCS (R1 = DPM, **12a**; DBS, **12c**; FL, **12e**; DCDPM, **12f**), THF, room temperature or R1NCO (R1 = DPM, **12b**; DBS, **12d**), THF, room temperature; (d) *N*-methyl morpholine, $ClCO_2iBu$, THF, −20 °C, **8a** (for **4**, (*S*)-**14a**,**d**,**g**), **8b** (for (*S*)-**14b**,**e**,**f**) or **8c** (for (*S*)-**14c**). See Table 1 footnotes for **12a**−**f** abbreviations.

Scheme 3[a]



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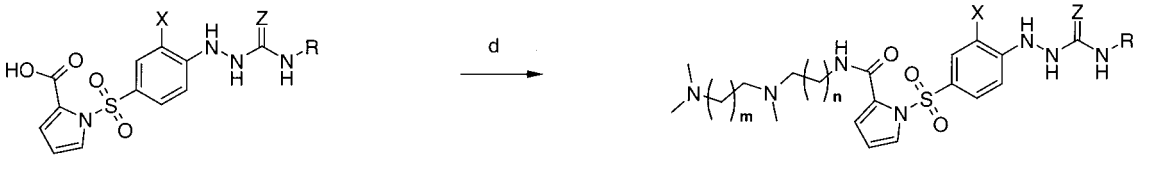
16a X = NO_2 Y = Cl
16b X = Cl Y = F

17a X = NO_2
17b X = Cl



18a	X = NO_2	Z = S	R = DPM
18b	X = NO_2	Z = S	R = DBS
18c	X = NO_2	Z = O	R = DBS
18d	X = Cl	Z = O	R = DBS

19a	m = 1	n = 1	X = NO_2	Z = S	R = DPM
19b	m = 1	n = 1	X = NO_2	Z = S	R = DBS
19c	m = 2	n = 1	X = NO_2	Z = O	R = DBS
19d	m = 2	n = 1	X = Cl	Z = O	R = DBS

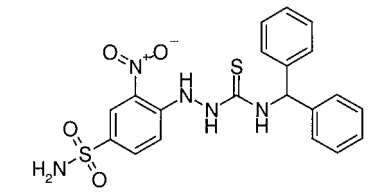
[a] Reagents: (a) ^{n}BuLi, THF, −20 °C, Ar, 4-chloro-3-nitrobenzenesulfonyl chloride (for **16a**) or 3-chloro-4-fluorobenzenesulfonyl chloride (for **16b**); (b) $H_2NNH_2 \cdot H_2O$, Et_3N, THF, reflux; (c) RNCS (R = DPM, **12a**; DBS, **12c**), THF, reflux or RNCO (R = DBS, **12d**), THF, room temperature, 65 h; (d) *N*-methyl morpholine, $ClCO_2iPr$, THF, −20 °C, **8a** (for **19a**,**b**) or **8b** (for **19c**,**d**). See Table 1 footnotes for **12a**,**c**,**d** abbreviations.

4) eliciting a similar subnanomolar K_i value (0.6 ± 0.12 nM). Compound (*S*)-**4** also demonstrated oral efficacy against FCA-induced mechanical hyperalgesia in rats with an ED_{50} value of 0.84 μmol/kg and a duration of

action in excess of 4 h. As a potential development candidate, the presence of aromatic nitro and divalent sulfur groups in (*S*)-**4** was of obvious concern, despite the absence of clastogenicity and/or aneugenicity in tests to assess its mutagenic potential. When we optimized the groups appended to the sulfonamide and thiosemicarbazide terminals of the TSC core, the nitro and sulfur moieties could be replaced by chloro and oxygen, respectively; this was a combined alteration that was not previously tolerated in compounds with lower receptor affinities. The resulting compound, (*S*)-**14a**, was ca. 15-fold less potent at the rat B_2 receptor but still displayed a respectable K_i value of 7.3 ± 1.8 nM. However, these compounds were found to have much lower affinities for cloned human B_2 receptors, expressed in Cos-7 cells, despite the high sequence homology between the species (ca. 80% identical at the amino acid level). The K_i values of (*S*)-**4** and (*R*)-**4** for human B_2 receptors were ca. 1500- and 2400-fold higher, respectively, than those recorded against rat B_2 receptors, with only a marginal preference for the (*S*) stereochemistry. While the affinity of (*S*)-**14a** in Cos-7 cells was not determined, our structure−activity relationship (SAR) experience of the series suggested that the compound was very unlikely to manifest any useful activity at human receptors. To address the disparity between rat and human B_2 receptor affinities, the ca. 400 TSC compounds synthesized to date were rescreened against the human B_2 receptor in order to identify structural elements responsible for any improved binding characteristics at this subtype. Table 1 shows a comparison of the binding affinities of a core set of compounds for the rodent and human B_2 receptor.

Extending the terminal dimethylamino group by increasing the length of the alkyl chain between the basic nitrogen atoms from ethylene ((*S*)-**4**) to propylene ((*S*)-**14b**) was seen to afford a 6.5-fold increase in binding affinity in the human Cos-7 cell system. Potency at the rat receptor was increased ca. 22-fold, and with a K_i value of 0.023 nM, (*S*)-**14b** approaches the potency levels of many peptidic antagonists. Although further extension of the remaining ethylene linker resulted in little additional benefit ((*S*)-**14c**), an overall 8-fold improvement in B_2 binding affinity could be achieved at the human receptor by manipulating the proline amide side chain in this way. This result suggested that the rat and human receptors prefer interaction with a more basic chain, as an ethylene-to-propylene increase in the separation of basic nitrogen centers is known to raise their pK_a values.[40] In a separate observation, removal of the C-2 stereogenic center by replacement of the pyrrolidine ring of (*S*)-**4** with a pyrrole ring (**19a**) led to a 3-fold increase in affinity at the human B_2 receptor. Given the low eudismic ratio observed with the enantiomers of **4**, such a replacement would have been expected to maintain potency at the very least.

By far the most important result of our rescreening exercise was that revealed by (*S*)-**13b**, a compound with an unsubstituted prolinamide terminus and whose DPM pharmacophore is formally adopted into the tricyclic DBS moiety. This latter structural feature was identified as the key element responsible for human B_2 receptor affinity (K_i = 299 nM; the DPM analogue was inactive) and for the 2-fold selectivity of (*S*)-**13b** for



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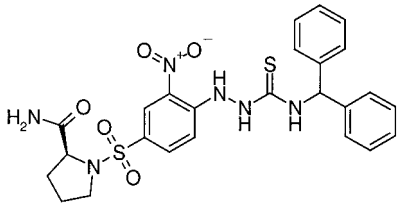

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Figure 2. Potent rat BK B_2 receptor antagonists.

human B_2 receptors over the rat counterparts (K_i = 654 nM). When the DBS moiety was incorporated into full TSC structures containing the basic chain of (*S*)-**4** and **19a**, a ca. 160-fold increase in affinity at the human B_2 receptor was achieved ((*S*)-**14d**, K_i = 4.7 nM; **19b**, K_i = 1.5 nM). In addition, affinity at the rat receptor was maintained at a level below 20 nM for the pyrrolidine (*S*)-**14d** and just above 60 nM for the pyrrole **19b**. A 160-fold increase in affinity corresponds to a free energy of interaction of 3.1 kcal/mol, which may reflect a moderately strong ionic interaction with the receptor, an interaction in which the basic chain could participate. This result represents a divergence in human and rat B_2 binding SAR. In NG108-15 cells, low molecular weight compounds such as **20** and **21** (the aforementioned DPM analogue of (*S*)-**13b**; Figure 2) still exhibited high potencies (<10 nM) at the rat receptor as long as the DPM motif was in place.[31] In contrast, the human receptor appears to be more discriminating with respect to the ligands it accommodates, requiring the binding energy contributions of both the DBS group and the basic chain for full recognition. Interestingly, the need for an extended molecular framework incorporating an additional pharmacophore was also observed by Fujisawa researchers in their efforts to overcome the species difference between guinea pig and man.[41]

In the DBS series, we also examined the influence of the basic chain and the five-membered ring on receptor affinity. Increasing the length of the alkyl chain between the basic nitrogen atoms, from ethylene ((*S*)-**14d** and **19b**) to propylene ((*S*)-**14e** and **19c**, respectively), resulted in a ca. 6-fold increase in human receptor affinity for the pyrrolidine and a 2-fold decrease for the pyrrole, with overall K_i values in a narrow range (0.8−4.7 nM). The K_i values of these compounds at the rat receptor were both higher and spread over a wider range (10.7−176 nM). It should be noted that (*S*)-**14e** and **19c** incorporate a semicarbazide backbone, and in general, the isosteric replacement of sulfur by oxygen was very well-tolerated in the DBS series, leading to compounds of very similar potencies at the human B_2 receptor. (*S*)-**14e** was confirmed as a functional antagonist at rodent and human B_2 receptors. It inhibited the increase in $^{45}Ca^{2+}$ efflux from NG108-15 cells caused by activation of the rat B_2 receptor with an IC_{50} value of 105 ± 8 nM

($n = 3$). It inhibited B_2 receptor-mediated contractions of rat uterus smooth muscle with an IC_{50} value of 2.4 ± 0.7 nM ($n = 3$). The IC_{50} values for antagonism of the $^{45}Ca^{2+}$ efflux actions of BK at the human receptor lie between 17 ± 3 nM ($n = 3$; human WI38 fibroblasts) and 91 ± 9 nM ($n = 3$; human SK-N-SH neuroblastoma cells). In vivo, (*S*)-**14e** reversed FCA-induced and turpentine-induced mechanical hyperalgesia in rats with ED_{50} values of 0.043 μmol/kg (0.03 mg/kg) and 0.11 μmol/kg (0.08 mg/kg), respectively, by oral administration, achieving maximum reversals of ca. 70%. However, the dose−response curves for (*S*)-**14e** were bell-shaped in both models of hyperalgesia, with a decrease in response to the compound when the doses were increased beyond 1 μmol/kg po in the FCA model and beyond 0.5 μmol/kg po in the turpentine model. This loss of activity at higher doses did not appear to be the result of a prohyperalgesic effect, as (*S*)-**14e** had no effect in naïve animals up to 5 μmol/kg po. Nor was this a consequence of poor solubility, as (*S*)-**14e** had a solubility of >6.2 mg/mL in pH 7.4 buffered saline solution.

Changing from the pyrrolidine ring to the pyrrole ring had negligible in vitro effects at the human B_2 receptor ((*S*)-**14d** vs **19b** and (*S*)-**14e** vs **19c**) but resulted in a ca. 4- and 16-fold reduction in affinity at the rat receptor, respectively. Compound **19c** inhibited the increase in $^{45}Ca^{2+}$ efflux from NG108-15 cells caused by activation of the rat B_2 receptor with a similar IC_{50} value to that exhibited by (*S*)-**14e** (141 ± 15 nM, $n = 3$), and its inhibition of B_2 receptor-mediated contractions of rat uterus smooth muscle was also of similar magnitude (IC_{50} 1.9 ± 0.4 nM, $n = 3$). However, despite a lower solubility (0.4 mg/mL at pH 7.4), **19c** displayed a marked improvement in in vivo activity compared to the corresponding pyrrolidine, (*S*)-**14e**, with no fall off in activity at higher doses. Compound **19c** reversed FCA-induced and turpentine-induced mechanical hyperalgesia in rats with ED_{50} values of 0.027 μmol/kg (0.02 mg/kg) and 0.32 μmol/kg (0.22 mg/kg), respectively, by oral administration and with maximum reversals of ca. 67%. Following administration of **19c** at 1 μmol/kg po, withdrawal thresholds were significantly raised for at least 4 h. Despite its long duration of action as an antihyperalgesic agent, preliminary data suggested that its antagonism of the hypotensive response to BK, when given iv, is relatively short-lasting. Compound **19c** may therefore have a short plasma half-life, a pharmacokinetic trait it appears to share with (*S*)-**4**.[31] The aforementioned difference in rat and human B_2 receptor binding SAR was also evident upon attempting to remove the nitro group from **19c**. Replacement of this moiety with a chloro group led to a much more rapid decrease in binding affinity at the human receptor (91-fold, **19d** vs **19c**) than previously observed in the rat system (ca. 15-fold, (*S*)-**14a** vs (*S*)-**4**).

To elucidate the steric requirements of the key DBS pharmacophore for human B_2 receptor affinity, we synthesized a number of structurally related analogues. Modifications to the tricyclic ring system, such as deletion of one or both of the lateral aromatic rings or transposing them to other fused positions around the central seven-membered ring to afford isomeric tricyclic systems, were all detrimental to activity (data not shown). However, receptor preference for a benzhydryl-based system was conditional upon the satisfaction of other steric parameters.[42] For example, adoption of a coplanar tricyclic arrangement, as afforded by the FL derivative (*S*)-**14f** resulted in a drastic 816-fold reduction in affinity ((*S*)-**14f** vs (*S*)-**14e**), suggesting that the tricyclic system must adopt a bent configuration in which the lateral aromatic rings have at least a limited ability to twist in relation to one another. Interestingly, the 2,2′-dichlorodiphenylmethyl (DCDPM) analogue (*S*)-**14g** exhibited a K_i value of 34 nM at the human receptor, a ca. 23-fold improvement in affinity compared to that of the unsubstituted DPM derivative, (*S*)-**4**. This increase in affinity corresponds to 1.9 kcal/mol, which is low for a full ionic interaction and may represent a simple hydrogen bond. Substituents other than chloro were also investigated but were found to be inferior, with potency decreasing in the order 2,2′-dichloro (34 nM) > 2,2′-dimethyl (50 nM) > 2,2′-dimethoxy (92 nM) > 2-chloro (147 nM) ≫ 2-methyl (4100 nM). It is intriguing that there is only a ca. 7-fold difference in human B_2 affinity between (*S*)-**14g** and (*S*)-**14d**, when one would expect the aromatic rings of the DCDPM and DBS groups to have markedly different angles of torsion. If (*S*)-**14g** and (*S*)-**14d** make similar binding contacts within the human receptor, as their affinities imply, then it may be that the binding modes selected by these ligands depend on the nature of the hydrophobic substituent at the N(4) position of the TSC core. We explored the possible influence of the hydrophobic region further by generating pharmacophore hypotheses using the CATALYST[43] software program. The best-computed pharmacophore model to rationalize the observed activities of (*S*)-**14d** (DBS), (*S*)-**4** (DPM), and (*S*)-**14f** (FL) is shown in Figure 3. In this paradigm, the two carbon bridging fragment of the DBS moiety (shown in red) enhances affinity through an aliphatic hydrophobic interaction in addition to the aromatic interactions afforded by the two phenyl rings. This may favor a binding mode that maximizes the binding contacts that (*S*)-**14d** makes in the human receptor. The DPM group (green) adopts a slightly different conformation with respect to the phenyl groups and of course does not possess the aliphatic bridge. The FL group (grey) is unable to occupy the required pharmacophores to any great extent. Also shown in Figure 3 is the corresponding overlay, suggested by CATALYST, for the DCDPM group (in black), which is able to map reasonably well with the DBS group. In this case, one of the ortho-chloro atoms is also able to act as the important aliphatic hydrophobe, and the CATALYST program correctly predicted the higher binding affinity of the DCDPM-containing analogue (*S*)-**14g**, compared to that of the DPM analogue (*S*)-**4** (see Experimental Section for more details). Thus, it may be that (*S*)-**4** adopts a distinctly different binding mode in the human receptor to that of (*S*)-**14d**, which prevents it from exercising the additional interactions it clearly enjoys in the rat receptor environment.

Conclusion

Potent, orally active rat BK B_2 receptor antagonists based on the TSC framework and exemplified by bradyzide ((*S*)-**4**) were found to be much less potent at cloned human B_2 receptors. We restored affinity at human receptors by replacing the DPM moiety of (*S*)-**4**

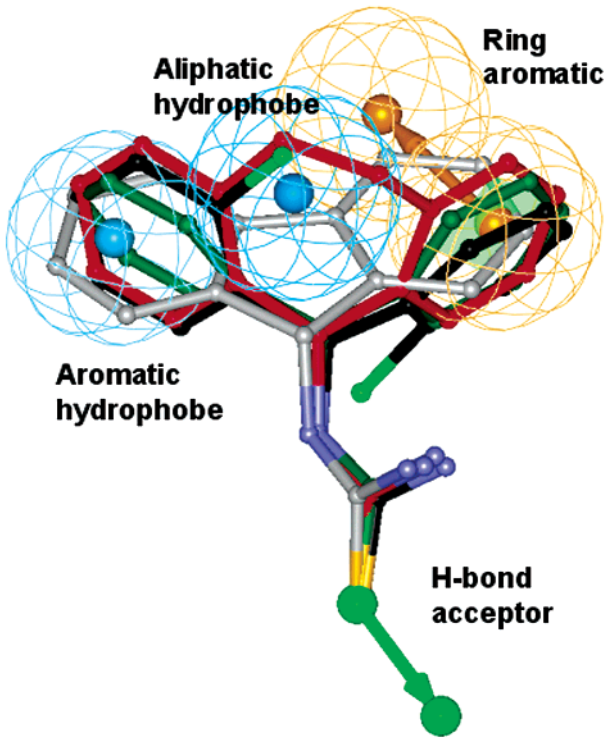

Figure 3. CATALYST-generated pharmacophore model of human BK B_2 receptor antagonists. The DBS group (red) is able to map all four pharmacophoric features generated in the CATALYST hypothesis, whereas the DPM group (green) lacks the aliphatic hydrophobe between the two aromatic interactions and the FL group (grey) can only partially fit the aromatic region. The DCDPM group (black) is able to overlay with the DBS group reasonably well, with one of the ortho-chloro atoms mimicking one of the methylene groups in the DBS bridge. CATALYST correctly predicted the DCDPM group to have higher affinity at human BK B_2 receptors than the DPM group.

with the DBS group, a modification revealed upon screening our TSC compound collection against the human receptor. Activity could be further tuned at the sulfonamide terminus by manipulating the basic chain and the five-membered heterocyclic ring, and this led to compound **19c**. Compound **19c** is a potent and selective[44] inhibitor of human BK B_2 receptors. The IC_{50} values for antagonism of the actions of BK at the human receptor lie between 12 ± 2 nM (SK-N-SH cells) and 55 ± 9 nM (WI38 cells). Furthermore, after oral administration, the compound is highly potent in alleviating the hyperalgesia associated with inflammation in two models of persistent inflammatory hyperalgesia. As such, this compound represents a novel prototype for the design of agents to treat the many painful inflammatory conditions in which BK is an important mediator. Further studies aimed at replacing the nitro group and retaining human B_2 receptor affinity will be reported in a subsequent paper.[45]

Experimental Section

(A) Chemistry. Commercial chemicals and solvents were of reagent grade and used without further purification. The following abbreviations are used for reagents and solvents: DCM, dichloromethane; DMF, dimethyl formamide; DMSO, dimethyl sulfoxide; EtOAc, ethyl acetate; Et_2O, diethyl ether; EtOH, ethanol; MeOH, methanol; and TFA, trifluoroacetic acid. Merck silica gel (Kieselgel 60) was used for analytical thin-layer chromatography (TLC; F_{254} plates) and flash column chromatography (230−400 mesh). Preparative reverse phase HPLC was performed on a Waters Delta Prep 3000 liquid chromatograph using a C18 bonded silica gel column (Dynamax-300 Å, 12 μm, 41.4 mm × 250 mm; Rainin Instrument Co.). The mobile phase contained acetonitrile (10−100%, v/v) in water containing 0.1% (v/v) TFA. Gradient elution at a flow rate of 70 mL/min was employed; peak detection was at 254 nm. Chemical yields are not optimized. Melting points (mp) were determined on a Reichert Thermovar hot stage melting point apparatus and are uncorrected. Optical rotations were determined with an AA-100 polarimeter (Optical Activity Ltd., U.K.). Infrared (IR) spectra were obtained on a Bruker IFS 66 infrared spectrophotometer using KBr disks, unless otherwise noted, and are reported in cm^{-1}. Proton NMR (^1H NMR) spectra were recorded on a Varian Gemini-200 spectrometer (200 MHz), Bruker AM-360 spectrometer (360 MHz), or on a Bruker Avance DPX 400 spectrometer (400 MHz) and are referenced to residual solvent signals: $CDCl_3$ (δ 7.26) or DMSO-d_6 (δ 2.49). Chemical shifts are reported in δ units (parts per million) downfield from tetramethylsilane and are assigned as singlets (s), doublets (d), doublet of doublets (dd), triplets (t), quartet (q), quintet (quin), multiplets (m), broad signals (br), or very broad signals (v br). Coupling constants (J) are reported in Hertz (Hz). Mass spectra (MS) were measured on Finnigan MAT 8430 (for EI) or VG 70-SE (for fast atom bombardment (FAB)) mass spectrometers. The parent ion is given followed by peaks corresponding to major fragment losses with relative intensities in parentheses. Elemental analysis data for final compounds were obtained from Novartis Pharma AG, Basel, and were within ±0.4% of the theoretical values for the formulas given, unless otherwise noted.

General Procedure for Synthesis of Triamines 8a−c. *N*-(2-Aminoethyl)-*N*,*N*′,*N*′-trimethyl-1,2-ethanediamine (8a).[35] **Step 1.** A solution of toluene-4-sulfonic acid 2-benzyloxycarbonylaminoethyl ester (**6a**)[37] (46.79 g, 133.9 mmol) in *N*,*N*,*N*′-trimethylethylenediamine (180 mL) was stirred at room temperature for 48 h. Excess diamine was removed by evaporation under reduced pressure. The residue was diluted with EtOAc (400 mL) and washed with 1 M NaOH solution (2 × 90 mL). The combined aqueous washings were reextracted with EtOAc (2 × 100 mL), and the combined organic layers were washed with brine (2 × 70 mL), dried over $MgSO_4$, and evaporated under reduced pressure to afford the Z-protected triamine **7a** as a straw-colored syrup (34.81 g, 93%), which was used without further purification.

Step 2. A solution of the Z-protected triamine **7a** (34.78 g, 125 mmol) in anhydrous MeOH (50 mL) was added to a suspension of 10% Pd/C catalyst (4 g) in anhydrous MeOH (200 mL) under an argon atmosphere, followed by solid ammonium formate (8.64 g, 137 mmol). After it was stirred at room temperature for 24 h, the reaction mixture was filtered through Celite and the filtrate was evaporated under reduced pressure. A portion of the product (4.16 g) was purified by distillation under reduced pressure to yield **8a** as a colorless oil (2.85 g, 68.5%): bp 120 °C (7 mmHg) [literature[35] bp 38−43 °C (0.2 mm Hg)].

***N*-(2-Aminoethyl)-*N*,*N*′,*N*′-trimethyl-1,3-propanediamine (8b).**[36] **Step 1.** A solution of **6a** (15 g, 42.9 mmol) in *N*,*N*,*N*′-trimethyl-1,3-propanediamine (25 mL) was stirred at 140 °C for 4 h. After it was cooled to room temperature, excess diamine was removed by evaporation under reduced pressure. The residue was diluted with EtOAc (250 mL) and washed with 1 M NaOH solution (2 × 100 mL). The combined aqueous washings were reextracted with EtOAc (2 × 100 mL), and the combined organic layers were washed with brine (100 mL), dried over $MgSO_4$, and evaporated under reduced pressure to afford the Z-protected triamine **7b** as a straw-colored syrup (10.2 g, 81%), which was used without further purification.

Step 2. A solution of the Z-protected triamine **7b** (10 g, 34.1 mmol) in anhydrous MeOH (75 mL) was stirred with 10% Pd/C catalyst (0.5 g) under an atmosphere of hydrogen for 24 h. The catalyst was removed by filtration through Celite, and the filtrate was evaporated under reduced pressure to afford the

title compound as a syrup (5.0 g, 93%), which was used without further purification.

***N*-(3-Aminopropyl)-*N*,*N*′,*N*′-trimethyl-1,3-propanediamine (8c).**[36] The starting tosylated *N*-Z propanolamine **6b**[38] (4.0 g, 11.0 mmol) was converted to the title compound (1.6 g, 84% over 2 steps) as described for **8a**. The syrup **8c** was used without further purification.

Diphenylmethyl Isothiocyanate (12a).[33,34] A mixture of bromodiphenylmethane (4.94 g, 20.0 mmol) and 18-crown-6 (0.15 g, 0.57 mmol) in 1,2-dichlorobenzene (10 mL) was heated to 180 °C with stirring, whereupon potassium thiocyanate (2.5 g, 25.0 mmol) was added. The reaction was followed on TLC and was complete after stirring at reflux for 30 min. After it was cooled to room temperature, the mixture was washed with water (2 × 25 mL) and brine (25 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The resulting syrup was purified by chromatography on silica gel (initial eluent 100% hexane, final eluent hexane:Et_2O 50:1) to afford **12a** (3.03 g, 67%) as a white crystalline solid; mp 54−57 °C [literature[33a] mp 59 °C (EtOH)]. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38−7.29 (10H, m), 5.97 (1H, s). IR (KBr): ν_{max} 2103 (N═C═S) cm^{-1}. MS (EI): *m*/*z* 167 (M^+ − NCS; 100%).

Diphenylmethyl Isocyanate (12b).[34] A stirred solution of aminodiphenylmethane (0.92 g, 5 mmol) in anhydrous toluene (25 mL) under a nitrogen atmosphere was treated dropwise at room temperature with a ∼20% solution of phosgene in toluene (10.4 mL), whereupon a white suspension was formed. The reaction mixture was heated to reflux, and after a further 10 min, a colorless solution was observed. After it was cooled to room temperature, the solvent was removed under reduced pressure to afford **12b** as an oil (1.0 g, ∼100%), which was dried under high vacuum and used without characterization or purification.

5-Isothiocyanato-10,11-dihydro-5*H*-dibenzo[a,d]cycloheptene (12c). Step 1. A stirred solution of dibenzosuberone (30 g, 144 mmol) in pyridine (30 mL) and EtOH (150 mL) was treated with hydroxylamine hydrochloride (30.02 g, 432 mmol), and the mixture was heated at 100 °C for 6 days under a nitrogen atmosphere. The solvent was removed under reduced pressure, and the residue was partitioned between EtOAc (200 mL) and water (200 mL). The aqueous layer was reextracted with EtOAc (50 mL). The organic phases were combined and dried over $MgSO_4$, and the solvent was removed under reduced pressure to afford the oxime intermediate as a pale yellow solid. This was recrystallized from EtOAc−cyclohexane to afford pure dibenzosuberone oxime (29.19 g, 91%) as a creamy-white crystalline solid.

Step 2. A stirred suspension of dibenzosuberone oxime (20 g, 89.58 mmol) in EtOH (90 mL) and concentrated ammonia solution (450 mL) was treated with ammonium acetate (3.45 g, 44.76 mmol) followed by portionwise addition of zinc powder (29.28 g, 447.9 mmol). The mixture was heated to 50 °C, and once effervescence had ceased to be vigorous, the mixture was refluxed overnight. After it was cooled to room temperature, the mixture was diluted with EtOAc (400 mL), stirred for 30 min, and filtered. The two phase filtrate was transferred to a separating funnel, and the phases were separated. The aqueous phase was reextracted with EtOAc (100 mL). The combined organic extracts were dried over $MgSO_4$, and the solvent was removed under reduced pressure to afford 5-aminodibenzosuberane as a pale yellow solid. The solid was purified by chromatography on silica gel (EtOAc:cyclohexane, 1:9) to afford pure 5-aminodibenzosuberane (17.95 g, 96%) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.44−7.3 (2H, m), 7.2−7.0 (6H, m), 5.42 (1H, s), 3.5−3.28 (2H, m), 3.28−3.05 (2H, m), 1.8 (2H, s). IR (KBr): ν_{max} 3361, 3303, 2853, 1600, 1580, 1476, 1454, 1351 cm^{-1}. MS (EI): *m*/*z* 209 (M^+; 5%), 208 (16%), 192 (100%).

Step 3. A stirred solution of thiophosgene (1.6 g, 14.3 mmol) in EtOAc (200 mL) at −20 °C was treated with triethylamine (2.9 g, 28.6 mmol) followed by a solution of 5-aminodibenzosuberane (3.0 g, 14.3 mmol) in EtOAc (100 mL). The reaction was followed on TLC (Et_2O:hexane 1:4) and was complete after stirring for 10 min. The mixture was washed with water (2 × 200 mL) and brine (100 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to afford **12c** as a solid (3.3 g, 92%), which could be used without further purification. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.5−7.35 (2H, m), 7.33−7.1 (6H, m), 6.34 (1H, s), 3.45−3.1 (4H, m). IR (KBr): ν_{max} 2096 (N═C═S) cm^{-1}. MS (EI): *m*/*z* 193 (M^+ − NCS; 100%).

5-Isocyanato-10,11-dihydro-5*H*-dibenzo[a,d]cycloheptene (12d). A stirred solution of 5-chlorodibenzosuberane (2.0 g, 8.5 mmol) in anhydrous DMF (20 mL) was treated at room temperature with sodium cyanate (0.57 g, 8.75 mmol) in one portion. After 6.5 h, the solvent was evaporated under reduced pressure, and the residue was taken up in EtOAc (20 mL) and stored in a refrigerator overnight. The mixture was filtered, and the clear filtrate was evaporated under reduced pressure to afford **12d** (2.2 g, ∼100%) as a straw-colored syrup, which slowly solidified to a pale yellow wax upon storage in a refrigerator. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.6−7.5 (2H, m), 7.4−7.15 (6H, m), 6.29 (1H, s), 3.48−3.17 (4H, m).

9-Isothiocyanato-9*H*-fluorene (12e).[33a] 9*H*-Fluoren-9-ylamine (4.5 g, 24.8 mmol) was converted to the title compound (1.8 g, 88%) as described for **12c**. The solid compound **12e** was used without further purification. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.8−7.6 (4H, m), 7.55−7.3 (4H, m), 5.75 (1H, s). IR (KBr): ν_{max} 2099 (N═C═S) cm^{-1}. MS (EI): *m*/*z* 223 (M^+; 15%), 165 (M^+ − NCS; 100%).

Bis-(2-chlorophenyl)methyl Isothiocyanate (12f). Step 1. A stirred solution of 2,2′-dichlorobenzophenone (3.06 g, 12.19 mmol) in anhydrous pyridine (25 mL) was treated with hydroxylamine hydrochloride (3.39 g, 48.74 mmol), and the mixture was heated at 100 °C overnight. The reaction was followed on TLC (EtOAc:hexane 1:5), and upon completion, the solvent was removed under reduced pressure. The residue was diluted with EtOAc (150 mL) and washed sequentially with 2 M HCl solution (2 × 50 mL), water (50 mL), and brine (50 mL). The washings were combined and reextracted with EtOAc (50 mL). The organic phases were combined and dried over $MgSO_4$, and the solvent was removed under reduced pressure to afford the oxime intermediate (3.2 g, ∼100%) as a white solid. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.62 (1H, br s), 7.55−7.2 (8H, m). IR (KBr): ν_{max} 3224 (N−OH) cm^{-1}. MS (EI): *m*/*z* 265 (M^+; 47%), 248 (53%), 230 (100%).

Step 2. A stirred suspension of the oxime (3.0 g, 11.27 mmol) in EtOH (11 mL) and concentrated ammonia solution (55 mL) was treated with ammonium acetate (0.45 g, 5.86 mmol) and zinc powder (3.97 g, 60.73 mmol). The mixture was heated at reflux for 4 h. After it was cooled to room temperature, the mixture was filtered and the solid residue was washed with 10 M NaOH solution (50 mL) and EtOAc (50 mL). The two phase filtrate was transferred to a separating funnel and washed with EtOAc (2 × 100 mL). The combined organic extracts were washed with brine (2 × 30 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to afford bis-(2-chlorophenyl)methylamine (2.8 g, 99%) as a pale yellow oil, which was used without further purification. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.45−7.1 (8H, m), 5.87 (1H, s), 1.8 (2H, s). IR (film): ν_{max} 3400−3300 (NH_2) cm^{-1}. MS (EI): *m*/*z* 252 (M^+; 4%), 235 (17%), 140 (100%).

Step 3. A stirred solution of bis-(2-chlorophenyl)methylamine (1.0 g, 3.97 mmol) in anhydrous EtOAc (40 mL) at 0 °C was treated with triethylamine (1.11 mL, 7.93 mmol) followed by thiophosgene (0.3 mL, 3.97 mmol) whereupon the reaction mixture immediately became opaque. After 3.5 h, the mixture was diluted with EtOAc (100 mL) and washed with water (2 × 30 mL) and brine (30 mL). The combined washings were reextracted with EtOAc (30 mL), and the combined organic phases were dried over $MgSO_4$. The solvent was removed under reduced pressure to afford **12f** (1.12 g, 96%) as a brown syrup, which slowly solidified upon standing. Compound **12f** was used without further purification. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.5−7.2 (8H, m), 6.8 (1H, s). IR (KBr): ν_{max} 2048 (N═C═S) cm^{-1}. MS (EI): *m*/*z* 235 (M^+ − NCS; 75%), 199 (55%), 165 (100%).

General Procedure for Preparation of Pyrrolidines 4, (*S*)-14a−g. (*S*)-1-[4-(4-Benzhydryl-thiosemicarbazido)-3-nitrobenzenesulfonyl]pyrrolidine-2-carboxylic Acid {**2-**

[(2-Dimethylaminoethyl)methylamino]ethyl}amide [(*S*)-4]. Step 1. A stirred solution of (*S*)-proline ((*S*)-**9a**) (7.5 g, 65.1 mmol) in water (100 mL) at 0 °C was treated with Na_2CO_3 (13.8 g, 130.2 mmol), followed by 4-chloro-3-nitrobenzenesulfonyl chloride (16.7 g, 65.1 mmol), which was added in portions. The reaction was followed on TLC (EtOAc:hexane 1:3) and was complete after stirring for 4 h. The mixture was treated with 2 M HCl solution [CAUTION: vigorous effervescence] until acidic (pH 2). The mixture was washed with EtOAc (3 × 100 mL), and the combined organic phases were washed with water (2 × 100 mL) and brine (100 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure, and the resulting yellow oil solidified on standing at ambient temperature to afford (*S*)-**10a** (19.4 g, 89%), which could be used without further purification. ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.5 (1H, d, J = 2.6 Hz), 8.13 (1H, dd, J = 7.7, 2.6 Hz), 8.0 (1H, d, J = 7.7 Hz), 4.3 (1H, dd, J = 10.2, 4.6 Hz), 3.5−3.2 (2H, m), 2.2−1.6 (4H, m). IR (KBr): ν_{max} 3500−2500, 1712, 1536, 1352 cm^{-1}. MS (FAB): m/z 337 (MH^+; 18%), 335 (MH^+; 44%), 291 ($M^+ - CO_2H$; 36%), 289 ($M^+ - CO_2H$; 100%).

Step 2. A stirred solution of (*S*)-**10a** (1.0 g, 2.99 mmol) in anhydrous THF (25 mL) was treated at room temperature with triethylamine (0.61 g, 5.98 mmol), followed by hydrazine monohydrate (0.15 g, 2.99 mmol). The reaction mixture was heated at reflux for 8 h, whereupon TLC analysis indicated complete reaction. On cooling, **12a** (0.81 g, 3.6 mmol) was added and the reaction mixture was stirred overnight at room temperature. The mixture was poured into water (600 mL), and 2 M HCl solution was added until a pH of 2 was achieved. The resulting precipitate was isolated by filtration and dried in vacuo to afford (*S*)-**13a** (1.38 g, 81%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 10.0 (2H, s,), 9.1 (1H, d, J = 10.2 Hz), 8.42 (1H, d, J = 1.7 Hz), 8.04 (1H, dd, J = 10.2, 1.7 Hz), 7.44−7.06 (11H, m), 7.0 (1H, d, J = 10.2 Hz), 4.18 (1H, dd, J = 7.5, 5.4 Hz), 3.38 (1H, m), 3.2 (1H, m), 2.1−1.75 (3H, m), 1.66 (1H, m). IR (KBr): ν_{max} 3650−2900, 3346, 3290, 1749, 1613, 1524, 1493, 1342, 1161 cm^{-1}. MS (FAB): m/z 556 (MH^+; 29%), 167 (100%).

Step 3. A stirred solution of (*S*)-**13a** (1.0 g, 1.8 mmol) in anhydrous THF (50 mL) at −20 °C was treated with *N*-methyl morpholine (0.18 g, 1.8 mmol) and isobutyl chloroformate (0.25 g, 1.8 mmol). After it was stirred for 1 h at −20 °C, the reaction mixture was treated dropwise with a solution of **8a** (0.26 g, 1.8 mmol) in anhydrous THF (25 mL). After a further 1.5 h period, TLC analysis (DCM:MeOH:TFA 20:2:0.5) indicated a complete disappearance of (*S*)-**13a** and formation of a more polar compound. The solvent was removed under reduced pressure, and the residue was purified by preparative HPLC. All fractions containing pure product were combined, and the solvent was removed under reduced pressure to afford the title compound as an amorphous orange solid, which was dried under high vacuum (0.8 g, 49%); $[\alpha]_D^{22}$ = −43° (c = 0.47, MeOH). ^{1}H NMR ((*S*)-**4**·TFA, 360 MHz, DMSO-d_6 at 120 °C): δ 9.62 (1H, br s), 8.67 (1H, v br s), 8.42 (1H, d, J = 3 Hz), 7.92 (1H, dd, J = 9, 3 Hz), 7.65 (1H, br t), 7.33−7.13 (12H, m), 6.85 (1H, br s), 4.12 (1H, dd, J = 9, 6 Hz), 3.41 (1H, m), 3.35−3.22 (3H, m), 3.2−3.1 (2H, t, J = 7.2 Hz), 2.93−2.83 (2H, t, J = 7.2 Hz), 2.78 (6H, s), 2.75−2.67 (2H, t, J = 7.2 Hz), 2.44 (3H, s), 1.92−1.77 (3H, m), 1.66 (1H, m). IR (KBr): ν_{max} 3337, 1676, 1615, 1522, 1346, 1202, 1163, 1131 cm^{-1}. MS (FAB): m/z 683 (MH^+; 55%), 241 (17%), 167 (100%). Anal. ($C_{32}H_{42}N_8O_5S_2$·2.5TFA·0.5H_2O) C, H, N, O, S, F.

The following compounds were prepared by the general procedure described above for the preparation of (*S*)-**4**, except that the isolation procedure in step 4 was used for compounds (*S*)-**13b** and (*S*)-**14d**,**e** after HPLC purification.

Step 4. After preparative HPLC purification, all fractions containing pure product were combined, and acetonitrile solvent was removed under reduced pressure. The pH of the solution was adjusted to 8 with saturated aqueous $NaHCO_3$ solution. The aqueous solution was washed with EtOAc (3 × 50 mL), and the combined organic extracts were washed with brine (50 mL) and dried over Na_2SO_4. After it was filtered, the solvent was removed under reduced pressure to afford the final product as an amorphous solid.

(*R*)-1-[4-(4-Benzhydryl-thiosemicarbazido)-3-nitrobenzenesulfonyl]pyrrolidine-2-carboxylic Acid {2-[(2-Dimethylaminoethyl)methylamino]ethyl}amide [(*R*)-4]. All spectral data for (*R*)-**4** were identical with those described for (*S*)-**4**; $[\alpha]_D^{22}$ = +48.8° (c = 0.33, MeOH). Anal. ($C_{32}H_{42}N_8O_5S_2$·3.24TFA) C, H, N.

(*S*)-1-(4-[4-(10,11-Dihydro-5*H*-dibenzo[a,d]cyclohepten-5-yl)thiosemicarbazido]-3-nitrobenzenesulfonyl)pyrrolidine-2-carboxylic Acid Amide [(*S*)-13b]. $[\alpha]_D^{22}$ = −44.7° (c = 0.51, MeOH); mp 155−159 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.12 (1H, s), 8.58 (1H, d, J = 2 Hz), 7.77 (1H, dd, J = 8.9, 1.9 Hz), 7.45 (1H, d, J = 9 Hz), 7.39−7.38 (3H, apparent d), 7.13−7.05 (7H, m), 6.66 (1H, d, J = 7.8 Hz), 6.57 (1H, br s), 5.52 (1H, br s), 3.97 (1H, dd, J = 8.7, 2.7 Hz), 3.49 (1H, m), 3.12−3.06 (3H, m), 2.98−2.95 (2H, m), 2.15 (1H, m), 1.8 (1H, m), 1.68−1.60 (2H, m). IR (KBr): ν_{max} 3463, 3330, 1680, 1614, 1569, 1518, 1344, 1160 cm^{-1}. MS (FAB): m/z 581 (MH^+; 5%), 193 (100%). Anal. ($C_{27}H_{28}N_6O_5S_2$·0.125H_2O) C, H, N.

(*S*)-1-[4-(4-Benzhydryl-semicarbazido)-3-chlorobenzenesulfonyl]pyrrolidine-2-carboxylic Acid {2-[(2-Dimethylaminoethyl)methylamino]ethyl}amide [(*S*)-14a]. $[\alpha]_D^{22}$ = −51.4° (c = 0.55, MeOH). ^{1}H NMR ((*S*)-**14a**·TFA, 400 MHz, DMSO-d_6): δ 8.28 (2H, s and br s), 8.15 (1H, s), 7.69 (1H, d, J = 2 Hz), 7.63 (1H, dd, J = 8.7, 1.8 Hz), 7.46 (1H, d, J = 8.4 Hz), 7.4−7.15 (10H, m), 6.94 (1H, d, J = 8.7 Hz), 5.98 (1H, d, J = 8.4 Hz), 3.95 (1H, dd, J = 7.8, 3.8 Hz), 3.5−3.35 (6H, m), 3.2−3.05 (2H, m), 2.84 (6H, s), 2.8 (2H, br s), 2.5 (3H, s), 1.78−1.75 (3H, m), 1.53 (1H, m). IR (KBr): ν_{max} 3362, 1678, 1594, 1539, 1496, 1344, 1160 cm^{-1}. MS (FAB): m/z 658 (M^+; 28%), 656 (M^+; 58%), 167 (100%). Anal. ($C_{32}H_{42}ClN_7O_4S$·3.5TFA·H_2O) C, H, N.

(*S*)-1-[4-(4-Benzhydryl-thiosemicarbazido)-3-nitrobenzenesulfonyl]pyrrolidine-2-carboxylic Acid {2-[(3-Dimethylaminopropyl)methylamino]ethyl}amide [(*S*)-14b]. ^{1}H NMR ((*S*)-**14b**·TFA, 200 MHz, DMSO-d_6): δ 10.01 (1H, s), 9.98 (1H, v br s), 9.09 (1H, d, J = 9 Hz), 8.43 (1H, d, J = 3 Hz), 8.40 (1H, br t), 8.04 (1H, dd, J = 9, 3 Hz), 7.40−7.08 (11H, m), 6.99 (1H, d, J = 9 Hz), 4.03 (1H, m), 3.6−3.36 (4H, m), 3.34−2.98 (6H, m), 2.86 (3H, s), 2.81 (6H, s), 2.06 (2H, br quin), 1.94−1.67 (3H, m), 1.57 (1H, m). IR (KBr): ν_{max} 3372, 3033, 1677, 1616, 1523, 1347, 1203, 1164 cm^{-1}. MS (FAB): m/z 697 (MH^+; 63%), 257 (25%), 167 (100%). Anal. ($C_{33}H_{44}N_8O_5S_2$·2.5TFA·2H_2O) C, H, N.

(*S*)-1-[4-(4-Benzhydryl-thiosemicarbazido)-3-nitrobenzenesulfonyl]pyrrolidine-2-carboxylic Acid {3-[(3-Dimethylaminopropyl)methylamino]propyl}amide [(*S*)-14c]. $[\alpha]_D^{22}$ = −37.3° (c = 0.89, MeOH). ^{1}H NMR ((*S*)-**14c**·TFA, 200 MHz, $CDCl_3$): δ 9.59 (1H, s), 9.52 (1H, br s), 8.59 (2H, br s and d, J = 3 Hz), 8.18 (1H, br t), 7.9 (1H, dd, J = 9, 3 Hz), 7.4−7.15 (11H, m), 6.85 (1H, d, J = 9 Hz), 4.0 (1H, m), 3.7−3.0 (10 H, m), 2.8 (9H, s), 2.35 (2H, br quin), 2.2−1.8 (5H, m), 1.69 (1H, m). IR (KBr): ν_{max} 3400, 3250−2700, 1664, 1613, 1493, 1436, 1383, 1339, 1295, 1239, 1196, 1153 cm^{-1}. MS (FAB): m/z 711 (MH^+; 18%), 274 (100%), 167 (59%). Anal. ($C_{34}H_{46}N_8O_5S_2$·2.5TFA·H_2O) C, H, N, O, S, F.

(*S*)-1-(4-[4-(10,11-Dihydro-5*H*-dibenzo[a,d]cyclohepten-5-yl)thiosemicarbazido]-3-nitrobenzenesulfonyl)pyrrolidine-2-carboxylic Acid {2-[(2-Dimethylaminoethyl)-methylamino]ethyl}amide [(*S*)-14d]. ^{1}H NMR (360 MHz, DMSO-d_6 at 120 °C): δ 8.32 (1H, d, J = 3 Hz), 7.55 (1H, dd, J = 9, 3 Hz), 7.47 (1H, d, J = 9 Hz), 7.45−7.05 (8H, m), 6.77 (1H, br s), 4.05 (1H, m), 3.4−3.1 (8H, m), 2.55−2.4 (4H, m), 2.28 (3H, s), 2.25 (6H, s), 2.22 (2H, m), 1.9−1.72 (3H, m), 1.72−1.6 (1H, m). IR (KBr): ν_{max} 3400, 2943, 1675, 1614, 1522, 1496, 1341, 1296, 1240, 1201, 1155 cm^{-1}. MS (FAB): m/z 709 (MH^+; 37%), 193 (100%). Anal. ($C_{34}H_{44}N_8O_5S_2$·0.75TFA) C, H, N.

(*S*)-1-(4-[4-(10,11-Dihydro-5*H*-dibenzo[a,d]cyclohepten-5-yl)semicarbazido]-3-nitrobenzenesulfonyl)pyrrolidine-2-carboxylic Acid {2-[(3-Dimethylaminopropyl)methylamino]ethyl}amide [(*S*)-14e]. $[\alpha]_D^{22}$ = −7.5° (c = 1.1, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.2 (1H, br s), 8.58 (1H, d, J = 2 Hz), 7.8 (1H, dd, J = 9, 2.1 Hz), 7.45−7.3 (4H, m), 7.25−7.1 (7H, m), 6.45 (1H, d, J = 7.2 Hz), 6.13 (1H, d, J = 7.4 Hz), 4.11 (1H, m), 3.49 (1H, m), 3.39 (1H, m), 3.32−3.0

(6H, m), 2.8 (2H, m), 2.54 (6H, s), 2.52−2.43 (4H, m), 2.22 (3H, s), 2.15 (1H, m), 1.9−1.6 (5H, m). IR (KBr): ν_{max} 3363, 2947, 1672, 1615, 1525, 1348, 1202, 1162 cm^{-1}. MS (FAB): *m/z* 707 (MH$^+$; 49%), 193 (100%). Anal. ($C_{35}H_{46}N_8O_6S$·0.5TFA·1.5H_2O) C, H, N, S, F.

(*S*)-1-(4-[4-(9*H*-Fluoren-9-yl)thiosemicarbazido]-3-nitrobenzenesulfonyl)pyrrolidine-2-carboxylic Acid {2-[(3-Dimethylaminopropyl)methylamino]ethyl}amide [(*S*)-14f]. $[\alpha]_D^{22} = -53.1°$ (*c* = 0.52, MeOH). ^{1}H NMR ((*S*)-**14f**·TFA, 360 MHz, DMSO-d_6): δ 10.2 (1H, s), 9.96 (1H, s), 8.87 (1H, d, *J* = 9 Hz), 8.41 (1H, br t), 8.29 (1H, d, *J* = 3 Hz), 8.1 (1H, dd, *J* = 9, 3 Hz), 7.81 (2H, d, *J* = 9 Hz), 7.53−7.42 (2H, m), 7.38 (2H, t, *J* = 7.2 Hz), 7.33−7.18 (2H, m), 7.22 (1H, d, *J* = 9 Hz), 6.94 (1H, d, *J* = 9 Hz), 3.95 (1H, dd, *J* = 9, 3 Hz), 3.56−3.35 (3H, m), 3.35−2.96 (7H, m), 2.87 (3H, s), 2.82 (6H, s), 2.15−1.92 (2H, m), 1.87−1.58 (3H, m), 1.56−1.4 (1H, m). IR (KBr): ν_{max} 3320, 1677, 1615, 1525, 1347, 1202, 1164 cm^{-1}. MS (FAB): *m/z* 695 (M$^+$ − 1; 72%), 257 (59%), 165 (100%). Anal. ($C_{33}H_{42}N_8O_5S_2$·2.7TFA·1.3H_2O) C, H, N, O, S, F.

(*S*)-1-(4-{4-[Bis-(2-chloro-phenyl)methyl]thiosemicarbazido}-3-nitrobenzenesulfonyl)pyrrolidine-2-carboxylic Acid {2-[(2-Dimethylaminoethyl)methylamino]ethyl}amide [(*S*)-14g]. $[\alpha]_D^{22} = -42.8°$ (*c* = 0.49, MeOH). ^{1}H NMR ((*S*)-**14g**·TFA, 400 MHz, DMSO-d_6): δ 9.97 (2H, s), 8.89 (1H, v br s), 8.38 (1H, d, *J* = 2.1 Hz), 8.32 (1H, v br t), 8.05 (1H, dd, *J* = 9.1, 2.1 Hz), 7.43 (2H, d, *J* = 7.8 Hz), 7.34−7.26 (4H, m), 7.18−7.15 (2H, m), 7.02 (2H, v br s), 4.02 (1H, dd, *J* = 7.9, 3.4 Hz), 3.5−3.05 (8H, m), 2.84 (6H, s), 2.78 (2H, br m), 2.5 (3H, s), 1.84−1.75 (3H, m), 1.55 (1H, m). IR (KBr): ν_{max} 3380, 3250−2700, 1670, 1614, 1552, 1496, 1340, 1296, 1240, 1195, 1154 cm^{-1}. MS (FAB): *m/z* 755 (5%), 753 (MH$^+$; 14%), 751 (MH$^+$; 19%), 237 (18%), 235 (24%). Anal. ($C_{32}H_{40}Cl_2N_8O_5S_2$·2.9TFA·0.9$H_2O$) C, H, N, O, Cl, F.

General Procedure for Preparation of Pyrroles 19a−d. 1-[4-(4-Benzhydryl-thiosemicarbazido)-3-nitrobenzenesulfonyl]-1*H*-pyrrole-2-carboxylic Acid {2-[(2-Dimethylaminoethyl)methylamino]ethyl}amide (19a). Step 1. A stirred solution of pyrrole-2-carboxylic acid (**15**) (2.5 g, 22.5 mmol) in anhydrous THF (100 mL) at −20 °C under an argon atmosphere was treated with a solution of *n*-BuLi (19.8 mL, 49.5 mmol, 2.5 M solution in hexanes). After 1 h, the mixture was recooled to −20 °C and treated with a solution of 4-chloro-3-nitrobenzenesulfonyl chloride (7.2 g, 28.1 mmol) in anhydrous THF (5 mL) and allowed to warm to ambient temperature. After it was stirred for 20 h, the mixture was diluted with EtOAc (100 mL) and washed with 2 M HCl solution (2 × 50 mL) followed by brine (50 mL). The EtOAc solution was dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was dissolved in hot 2-propanol-water (1:1, 100 mL), and the stirred solution was treated with activated charcoal (1.0 g). After 5 min, the mixture was filtered and allowed to cool to ambient temperature. The resulting precipitate was collected by filtration and dried under high vacuum to give **16a** as a crystalline solid (4.1 g, 55%). ^{1}H NMR (200 MHz, DMSO-d_6): δ 8.74 (1H, d, *J* = 2.2 Hz), 8.26 (1H, dd, *J* = 8.5, 2.3 Hz), 8.08 (1H, d, *J* = 8.5 Hz), 7.86 (1H, dd, *J* = 3.2, 1.75 Hz), 7.12 (1H, dd, *J* = 3.7, 1.83 Hz), 6.51 (1H, apparent t, *J* = 3.5 Hz). IR (KBr): ν_{max} 3350−2500, 1708, 1680, 1540, 1444, 1379, 1359, 1267, 1190, 1150 cm^{-1}. MS (FAB): *m/z* 331 (MH$^+$; 50%), 313 (100%).

Step 2. A stirred solution of **16a** (4.1 g, 12.4 mmol) in anhydrous THF (70 mL) was treated at room temperature with triethylamine (2.76 g, 27.3 mmol), followed by hydrazine monohydrate (0.68 g, 13.6 mmol). The reaction mixture was heated at 70 °C for 6 h and then allowed to cool to ambient temperature. On cooling, **12a** (3.35 g, 14.9 mmol) was added and the reaction mixture was heated at reflux for 4 h. The mixture was poured into water (500 mL), and 2 M HCl solution was added until a pH of 2 was achieved. The resulting precipitate was isolated by filtration and dried in vacuo to afford **18a** (5.3 g, 78%). IR (KBr): ν_{max} 3400−2650, 1703, 1613, 1518, 1494, 1449, 1369, 1345, 1277, 1181, 1144 cm^{-1}. MS (FAB): *m/z* 552 (MH$^+$; 21%), 167 (100%).

Step 3. A stirred solution of **18a** (0.4 g, 0.73 mmol) in anhydrous DMF (7 mL) and anhydrous THF (13 mL) was treated with *N*-methyl morpholine (0.09 g, 0.87 mmol). The reaction mixture was cooled to −20 °C and treated with a 1 M solution of isopropyl chloroformate in toluene (0.87 mL, 0.87 mmol). After it was stirred for 45 min at −20 °C, the reaction mixture was treated dropwise with a solution of **8a** (0.13 g, 0.87 mmol) in anhydrous THF (10 mL). After 24 h, the solvent was removed under reduced pressure and the residue was purified by preparative HPLC. All fractions containing pure product were combined, and the solvent was removed under reduced pressure to afford **19a** as an amorphous orange solid, which was dried under high vacuum (0.47 g, 63%). ^{1}H NMR (**19a**·TFA, 400 MHz, DMSO-d_6): δ 10.01 (1H, s), 9.91 (1H, v br s), 9.01 (1H, v br d, *J* = 6.4 Hz), 8.76 (1H, d, *J* = 2.2 Hz), 8.46 (1H, v br t), 8.10 (1H, dd, *J* = 9.1, 2 Hz), 7.54 (1H, apparent s), 7.2−7.1 (6H, br s), 7.1−7.0 (4H, br s), 6.97 (1H, d, *J* = 9.3 Hz), 6.84 (1H, v br s), 6.64 (1H, apparent s), 6.30 (1H, t, *J* = 3.4 Hz), 3.7−3.2 (6H, m), 2.71 (6H, s), 2.6−2.45 (2H, m), 2.41 (3H, s). IR (KBr): ν_{max} 3416, 3200−2700, 1650, 1613, 1555, 1494, 1435, 1363, 1299, 1242, 1172, 1150 cm^{-1}. MS (FAB): *m/z* 679 (MH$^+$; 100%). Anal. ($C_{32}H_{38}N_8O_5S_2$·3TFA·2H_2O) C, H, N, F.

Compounds **19b−d** were prepared following the general procedure described above for the preparation of **19a**, except that after HPLC purification the procedure in step 4 was used to isolate these DBS-containing compounds.

Step 4. After preparative HPLC purification, all fractions containing pure product were combined and acetonitrile solvent was removed under reduced pressure. The pH of the solution was adjusted to 8 with saturated aqueous $NaHCO_3$ solution. The aqueous solution was washed with EtOAc (3 × 50 mL), and the combined organic extracts were washed with brine (50 mL) and dried over Na_2SO_4. After filtration, the solvent was removed under reduced pressure to afford the final product as an amorphous solid.

1-(4-[4-(10,11-Dihydro-5*H*-dibenzo[a,d]cyclohepten-5-yl)thiosemicarbazido]-3-nitrobenzenesulfonyl)-1*H*-pyrrole-2-carboxylic Acid {2-[(2-Dimethylaminoethyl)methylamino]ethyl}amide (19b). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.61 (1H, br s), 8.26 (1H, br t), 7.65 (1H, br d), 7.57 (1H, br s), 7.53 (1H, br s), 7.46 (2H, br m), 7.15−7.1 (6H, br s), 6.72 (1H, v br s), 6.60 (1H, br s), 6.47 (1H, v br s), 6.31 (1H, t, *J* = 3.3 Hz), 3.35−3.05 (6H, m), 2.7−2.42 (6H, m), 2.40 (6H, br s), 2.24 (3H, s). IR (KBr): ν_{max} 3400, 3220−2680, 1660, 1614, 1555, 1523, 1492, 1426, 1360, 1299, 1173, 1151 cm^{-1}. MS (FAB): *m/z* 705 (MH$^+$; 80%), 346 (100%). Anal. ($C_{34}H_{40}N_8O_5S_2$·0.2EtOAc·H_2O) C, H, N.

1-(4-[4-(10,11-Dihydro-5*H*-dibenzo[a,d]cyclohepten-5-yl)semicarbazido]-3-nitrobenzenesulfonyl)-1*H*-pyrrole-2-carboxylic Acid {2-[(3-Dimethylaminopropyl)methylamino]ethyl}amide (19c). ^{1}H NMR (360 MHz, DMSO-d_6 at 120 °C): δ 8.70 (1H, d, *J* = 3 Hz), 8.0 (1H, dd, *J* = 9, 3 Hz), 7.75 (1H, br t), 7.45 (1H, m), 7.37 (1H, d, *J* = 9 Hz), 7.34 (2H, d, *J* = 7.2 Hz), 7.23 (1H, d, *J* = 9 Hz), 7.18−7.05 (8H, m), 6.63 (1H, m), 6.3 (1H, t, *J* = 3 Hz), 6.22 (1H, d, *J* = 9 Hz), 3.26 (2H, apparent q), 3.23−3.1 (4H, m), 2.50 (2H, t, *J* = 7.2 Hz), 2.40 (2H, t, *J* = 7.2 Hz), 2.34 (2H, t, *J* = 7.2 Hz), 2.23 (3H, s), 2.20 (6H, s), 1.55 (2H, quin, *J* = 7.2 Hz). IR (KBr): ν_{max} 3350, 1668, 1614, 1558, 1527, 1373, 1360, 1278, 1179, 1151 cm^{-1}. MS (FAB): *m/z* 703 (MH$^+$; 72%), 193 (100%). Anal. ($C_{35}H_{42}N_8O_6S$·0.2TFA) C, H, N, F.

1-(3-Chloro-4-[4-(10,11-dihydro-5*H*-dibenzo[a,d]cyclohepten-5-yl)semicarbazido]benzenesulfonyl)-1*H*-pyrrole-2-carboxylic Acid {2-[(3-Dimethylaminopropyl)methylamino]ethyl}amide (19d). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.48 (1H, s), 8.28 (1H, br t, *J* = 5.4 Hz), 8.2 (1H, s), 8.09 (1H, d, *J* = 2 Hz), 7.88 (1H, dd, *J* = 9, 2 Hz), 7.64 (1H, d, *J* = 8.4 Hz), 7.55 (1H, m), 7.40−7.38 (2H, m), 7.22−7.19 (6H, m), 6.91 (1H, d, *J* = 8.7 Hz), 6.63 (1H, m), 6.35−6.31 (2H, m), 3.45−3.15 (6H, m), 2.48 (2H, t, *J* = 6.7 Hz), 2.39 (2H, t, *J* = 7.1 Hz), 2.25 (2H, t, *J* = 7.1 Hz), 2.23 (3H, s), 2.14 (6H, s), 1.57 (2H, quin, *J* = 7.1 Hz). IR (KBr): ν_{max} 3373, 3000−2750, 1666, 1591, 1554, 1524, 1460, 1452, 1370, 1152 cm^{-1}. MS (FAB): *m/z* 692 (MH$^+$; 100%), 253 (40%), 193 (72%). Anal.

($C_{35}H_{42}ClN_7O_4S$·0.3TFA·0.75H_2O) C, H, N; F, calcd, 2.3; found, 1.8.

Molecular Modeling. The modeling calculations described herein were performed using the CATALYST[43] software package (version 4.6) running on a Silicon Graphics Octane workstation (IRIX64 6.5 operating system). The models were built starting from a generic monosubstituted thiourea structure, R−$NHCSNH_2$, in accordance with CATALYST standard bond lengths and valence angles, where R = DBS, DPM, FL, and DCDPM. Conformers were generated using the standard CATALYST method ("Best Quality" mode, a maximum of 250 conformers within a 20 kcal/mol energy range).

In the calculation of the pharmacophore model, the chemical features selected for hypothesis generation were hydrogen bond donors, hydrogen bond acceptors, hydrophobic aliphatic regions, hydrophobic aromatic regions, and aromatic ring interactions, as defined in the CATALYST software. Hydrophobic aromatic regions are placed at the centroids of the aromatic rings. Hydrogen bond acceptors are characterized by electronic lone pairs of nitrogen, oxygen, or sulfur atoms. Pharmacophore hypotheses were generated using the standard CATALYST method, employing the human B_2 receptor binding affinities of the full structures (*S*)-**14d** (DBS), (*S*)-**4** (DPM), and (*S*)-**14f** (FL). Subsequently, CATALYST was asked to map the DCDPM group (present in (*S*)-**14g**) to the best hypothesis, resulting in an estimate of predicted activity (K_i = 19 nM) close to that found experimentally (K_i = 34 nM).

(B) Biology. Receptor Binding Assays. Standard binding assays were performed using the membrane preparations described in our previously published procedures.[32] NG108-15 cell membranes were thawed, homogenized, and diluted with binding buffer (composition: 25 mM potassium phosphate, pH 6.5; 1 mM ethyleneglycolbis(aminoethyl ether)-tetraacetate (EGTA); 0.1 mM bacitracin; 54 mg/mL chymostatin; and 2 mg/mL bovine serum albumin (BSA)) to give a membrane protein concentration of about 0.3 mg/mL. The assay mixture comprised 100 μL of [^{3}H]BK (specific activity 65 Ci/mmol; Amersham Corp.), 50 μL of DMSO (total binding), or 50 μL of the compound to be investigated in DMSO and 100 μL of binding buffer. The assay was started by the addition of 750 μL of membrane suspension.

Cos-7 cell membranes were thawed, homogenized, and diluted with binding buffer with composition 10 mM *N*-tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), pH 7.4; 1 mM EGTA; 0.14 g/L bacitracin; 54 μg/mL chymostatin; and 0.2% BSA. The assay mixture comprised 100 μL of [^{3}H]-BK in binding buffer, 50 μL of DMSO (total binding), or 50 μL of the test compound in DMSO. The assay was started by addition of 750 μL of membrane suspension to give a membrane protein concentration of 5 μg/mL.

For all binding assays, nonspecific binding was determined in the presence of 10 μM BK and the concentration of [^{3}H]BK in displacement assays was 1 nM. Samples were incubated for 60 min at 4 °C and then filtered through GF/B filters and presoaked in polyethylenimine (6 g/L), using a Brandel Harvester. The filters were washed with ice cold wash buffer (25 mM potassium phosphate, pH 6.5, for the NG108-15 cells and 50 mM tris(hydroxymethyl)aminomethane (TRIS), pH 7.4, for the Cos-7 cells), placed in scintillation vials, and counted in "Ready-Micro" liquid scintillation fluid. Binding parameters were calculated using LIGAND.[46] K_i values of compounds were calculated from the equation $K_i = (IC_{50})/1 + S$ where S = (concentration of radioligand)/(K_d of radioligand).[47] Data are the means ± SEM from at least three experiments performed in triplicate.

FCA-Induced Mechanical Hyperalgesia in the Rat Knee Joint.[32] Female Sprague−Dawley rats (100−120 g) were lightly anaesthetized with Enflurane, and 100 μL of FCA was injected into one knee. Three to six days later, the animal was placed with each hind paw on a pressure transducer, and a downward force was exerted until the uninjected leg was bearing 100 g. The force the animal would bear on the injected leg was determined, and the reduction in the load tolerated indicated the degree of hyperalgesia.[48] Half-hourly measurements were taken, and test compounds were administered intravenously, subcutaneously, or orally after three control readings. The increase in load tolerated by the injected leg resulting from a test compound was expressed as a percentage maximal reversal of the hyperalgesia (with 100% reversal representing an equal load tolerated by the control and injected leg). The ED_{50} value (defined as the dose that reversed the hyperalgesia by 50%) for groups of 8−10 animals was calculated from the peak response. For determination of maximum efficacy, the maximum reversal produced by the test compound was expressed as a percentage of the maximal possible reversal of hyperalgesia in this model. Statistical analysis was performed by two way analysis of variance (ANOVA) to compare pre- and posttreatment values.

Turpentine-Induced Mechanical Hyperalgesia in the Rat Paw. The nociceptive pressure threshold was determined on the left hind paw (Analgesymeter, Ugo Basile, Milan) of male Sprague−Dawley rats (180−220 g), and the end point was taken as paw withdrawal. The mean of two readings was taken as the withdrawal threshold. Immediately after the control reading, the rats received an intraplantar injection in the left hind paw of 50 μL of a turpentine/paraffin mixture (1:1). Three days later, the test compounds or vehicle were administered orally or intravenously. A further threshold reading was then taken 1 h later. For potency estimation, an ED_{50} value was calculated as the dose producing 50% of the maximum reversal of hyperalgesia for each test compound. The efficacy of each test compound was the maximum reversal of hyperalgesia obtained, expressed as a percentage of the withdrawal threshold of the animals before injection with turpentine; 100% represented complete reversal of hyperalgesia. The duration of action of a test compound was assessed following a dose of 1 μmol/kg po of the compound or of vehicle. The withdrawal threshold of both test compound- and vehicle-treated animals (n = 10) were then measured every hour for 4 h after administration of test compound.

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(44) Compound **19c** was screened against a number of receptors, including human B_1 receptors. It showed very weak activity at the rat M_1 muscarinic receptor (IC_{50} 3.2 ± 0.5 μM, $n = 3$) and the human NK_1 receptor (K_i 2.19 ± 0.3 μM, $n = 3$) but no detectable activity at the other receptors tested, up to a concentration of 10 μM.

(45) Compound **19c** was submitted for genotoxicity screening in V79 Chinese hamster cells and was positive in one of the test systems. This is in contrast to the result obtained with (*S*)-**4**,[31] which did not exhibit mutagenic potential despite incorporating a nitro group in its structure.

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