Discovery of a Novel Non-Peptide Somatostatin Agonist with SST_4 Selectivity

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Abstract: The discovery of novel non-peptide compounds with a high affinity for the peptide hormone somatostatin (SST) receptor is described. The compounds were tested for affinity at five human SST receptor subtypes individually expressed in mammalian cells. The compound NNC 26-9100 showed a K_i of 6 nM at SST_4 and more than 100 fold selectivity for SST_4 over SST_1, SST_2, SST_3, or SST_5. Competition binding studies and Scatchard analysis of the interaction by NNC 26-9100 with SST showed specificity at SST_4. Furthermore, NNC 26-9100 was highly selective for SST_4 over a variety of other G protein-coupled receptors, having affinities for M_1 muscarinic acetylcholin and D_3 dopamine receptors of around 500 and 1000 nM, respectively. Finally, NNC 26-9100 was found to fully inhibit forskolin-induced accumulation of adenosine 3′,5′-cyclic monophosphate in baby hamster kidney cells, expressing the human SST_4 receptor with an EC_{50} of 2 nM.

Somatostatin (somatotropin release inhibiting factor; SRIF), a tetradecapeptide originally isolated from ovine hypothalamus on the basis of its ability to inhibit growth hormone (GH) release from anterior pituitary cells,[1] has been shown to be present in several other tissues.[2] Somatostatin appears to have widespread functions as a modulator of neuronal activity as well as of endocrine and exocrine secretion. Inhibitory effects of this peptide on the release of a variety of hormones such as GH, prolactin, glucagon, insulin, gastrin, and thyroid-stimulating hormone have been described.[3] SRIF is best regarded as belonging to a phylogenetically ancient, multigene family of peptides with two important bioactive products, namely, SRIF-14 and SRIF-28, a congener of SRIF extended at the N terminus.

The regulatory functions of SRIF are mediated by specific membrane receptors. Currently, only agonists are available to study the pharmacology of SRIF receptors. High-affinity saturable binding sites have been demonstrated in a number of tissues, e.g., pituitary gland,[4] brain,[5] and pancreas,[6] Within the last few years, the cloning and isolation of five SST receptor genes have been reported for various species (human, rat, and mouse).[7] Structural considerations of the encoded proteins revealed that the SST receptor proteins (SST_1−SST_5) represent a distinct receptor subfamily belonging to the superfamily of G protein-coupled receptors with seven putative membrane-spanning regions.

Since the discovery of SRIF, the biological effects have gained a lot of pharmacological attention in the development of bioactive compounds with selective modes of action. Many cyclic peptides have been synthesized (Figure 1) which bind with high affinity to the SST receptors, but so far, no compound has been reported to bind selectively to one of the five cloned SST receptors.

The smallest SST analogue which retains biological activity is the hexapeptide MK 678.[8] The octapeptide analogue SMS 201-995 (Octreotide) is used clinically in long-acting preparations for the diagnosis and treatment of a variety of neuroendocrine tumors and gastrointestinal disorders.[9]

Recent works on the development of non-peptide structures substituting the peptide backbone of small cyclic peptides with β-D-glucose,[10] xylofuranose,[11] or mannitol scaffolds[12] have demonstrated low SST receptor affinity. However, these

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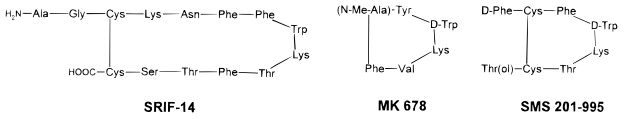

Figure 1.

structures are nonselective, displaying higher affinities for both β_2-adrenergic receptors and tachykinin receptors. Thus, there have been no reports in the literature on the successful development of a selective, competitive SST receptor ligand of non-peptide origin.

The structure−activity relationships of SRIF have suggested that the amino acid residues Phe7, Trp8, Lys9, and Thr10, which comprise a β turn, are necessary for biological activity, with residues Trp8 and Lys9 being essential, whereas Phe7 and Thr10 can undergo minor substitutions.[13]

We initiated a screening program to identify new non-peptide chemical entities with SST_1-SST_5 affinities, based on a scaffold containing two aromatic groups and a basic group. We now report our findings and the subsequent optimization which led to a small non-peptide SST receptor agonist with high affinity and selectivity for SST_4.

Results and Discussion

Our broad screening was based on a query consisting of two aromatic groups, one which was heteroaromatic, postulated to mimic the Trp8, and one nonheteroaromatic, mimicing the Phe7 of SRIF. On the basis of previous observations,[10] we wanted not only to mimic the Lys9 by primary amino groups but extend the query to contain all sorts of nitrogen containing functional groups. The initial screening identified the thiourea **1**, which showed moderate affinity at SST_4 and a 20-fold selectivity for SST_4 over SST_2 (Figure 2).

As seen in Table 1, our initial lead compound **1**, containing one heteroaromatic, one aromatic, and one imidazole group connected through a thiourea scaffold, had a K_i of 118 nM at SST_4 and showed a 20 fold selectivity for SST_4 over the other subtypes. This finding prompted us to screen our in-house databases for all similar compounds, and the most selective compound turned out to be a simple *S*-methyl derivative of **1**. The *S*-methylated thiourea **2** was slightly more potent than **1** at SST_4, but more interestingly, it showed about a 100-fold selectivity for SST_4 over SST_2, yet only a 3-fold selectivity for SST_4 over SST_5. This encouraged us to derivatize **2**, and a series of thiourea analogues[14] led us to our most potent compound, NNC 26-9100 (**3**), in which we have replaced the pyridinyl moiety with 5-bromopyridinyl and the 4-bromobenzyl moiety with 3,4-dichlorobenzyl, respectively. NNC 26-9100 showed a 20 fold increase in affinity at SST_4 (K_i = 6 nM) and was also the most potent compound at SST_2, although NNC 26-9100 showed more than 100 fold selectivity for SST_4 over SST_2. We compared the binding profile of NNC 26-9100 to that of SRIF at SST_4 as depicted in Figure 3. The shape of the displacement curve with a Hill coefficient close to unity

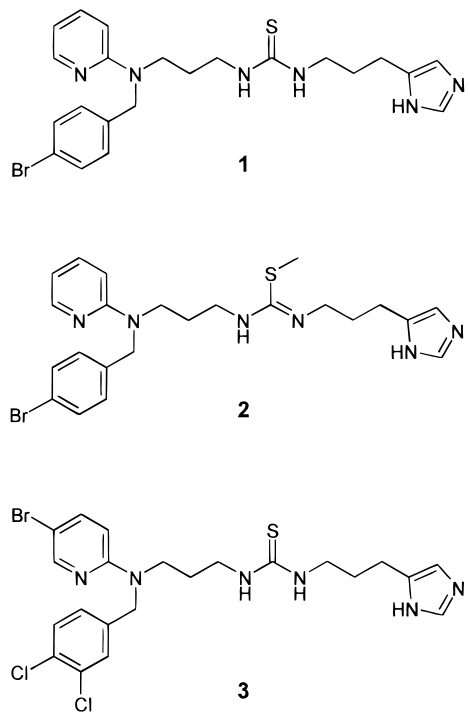

Figure 2.

Table 1. Dissociation Constants for a Number of SST Analogues Assayed by ^{125}I-Tyr11-SRIF Radioligand Binding to Membranes from Transfected BHK and HEK 293 Cells

compd	$K_i(SST_1)^a$	$K_i(SST_2)^b$	$K_i(SST_3)^a$	$K_i(SST_4)^a$	$K_i(SST_5)^a$
SRIF-14	0.8	0.2	0.6	1.4	0.3
SMS 201-995	1 200	0.3	108	1904	1.2
MK 678	>10 000	0.3	20	3200	3.4
1		2500	7 000	118	
2		4600	12 000	50	145
NNC 26-9100 (**3**)	1 800	621	1 400	6	1900

[a] *Ki* (nM) in transfected BKH cells. [b] *Ki* (nM) in transfected HEK 293 cells.

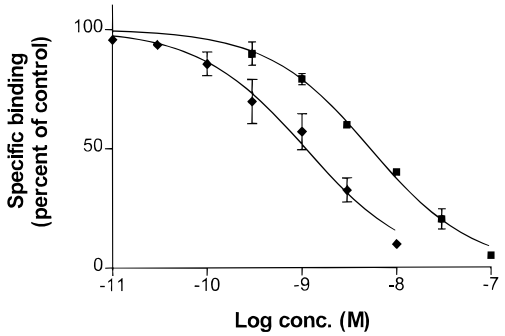

Figure 3. Displacement by NNC 26-9100 (■) and SRIF (◆) of ^{125}I-Tyr11-SRIF binding to membranes from BHK cells expressing human SST_4 receptors.

suggested competitive inhibition by NNC 26-9100 of ^{125}I-Tyr11-SRIF. This was further substantiated by Scatchard analysis of ^{125}I-Tyr11-SRIF binding in the absence or presence of 5 nM NNC 26-9100. The pK_d of ^{125}I-Tyr11-SRIF for SST_4 was unaffected ($p > 0.10$, students t test) by NNC 26-9100 (8.86 ± 0.12 vs 8.55 ± 0.10, $n = 3$), whereas B_{max} was significantly lower (data not shown).

NNC 26-9100 was further tested in a functional assay based on inhibition of forskolin-induced accumulation of adenosine cyclic 3′,5′-monophosphate (c-AMP) in BHK cells expressing the human SST_4 receptor. Like SRIF, NNC 26-9100 was able

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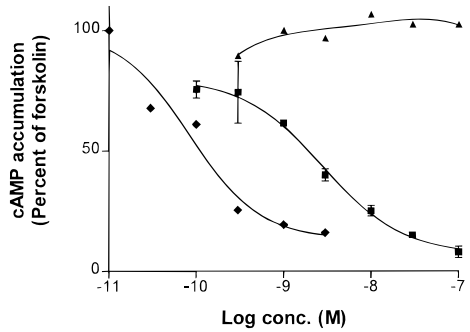

Figure 4. Inhibition by NNC 26-9100 (■) and SRIF (◆) of forskolin induced accumulation of c-AMP in BHK cells expressing SST_4. The effects by NNC 26-9100 of c-AMP accumulation in wild-type BHK cells are indicated by ▲.

Table 2. Inhibition by NNC 26-9100 of Radioligand Binding on a Variety of G Protein-Coupled Receptors

receptor	ligand	IC_{50}, nM
angiotensin II	[^{3}H]angiotensin II	>10 000
bradykinin B_2	[^{3}H]bradykinin	>10 000
dopamine D_3	[^{3}H]spiperone	1 000
CCK_A	[^{3}H]L-364718	>10 000
CCK_B	[^{3}H]CCK-8	>10 000
endothelin ET_A	[^{125}I]endothelin	>10 000
endothelin ET_B	[^{125}I]endothelin	>10 000
galanin	[^{125}I]galanin	>10 000
muscarinic M_1	[^{3}H]pirenzepine	540
muscarinic M_2	[^{3}H]NMS	>10 000
neurokinin NK_1	[^{3}H]substance P	>10 000
neurokinin NK_1	[^{3}H]substance P	>10 000
neuropeptide Y_2	[^{125}I]NPY	>10 000
serotonin 5-HT_{1A}	[^{3}H]8-OH-DPAT	>10 000
serotonin 5-HT_3	[^{3}H]GR-65630	>10 000
VIP	[^{125}I]VIP	>10 000

to almost completely inhibit (efficacy 95%) the accumulation of c-AMP with an EC_{50} of 2 nM (Figure 4). Mock-transfected BHK cells, on the other hand, did not respond to NNC 26-9100 upon forskolin stimulation. These data demonstrate NNC 26-9100 to be a full agonist at the human SST_4 receptor.

NNC 26-9100 is also highly selective for SST_4 compared to other G protein-coupled receptors as outlined in Table 2. The initial binding affinity for the muscarinic M_1 receptor (IC_{50} = 540 nM) could not be confirmed using the rabbit vas deferens relaxation model with NNC 26-9100 in concentrations up to 100 μM (Panlabs Inc., data not shown).

Previously, non-peptides with SST receptor affinity have been reported.[15] These binding studies have been employed on pituitary and cerebral cortex membranes comprising mixtures of several subtypes of SST receptors. To our knowledge, no reports of non-peptides evaluated at the individual cloned SST receptor subtypes have ever been reported.

Hirschmann et al.[10] have previously postulated that the His7 analogue of c-(Phe-D-Trp-Lys-Thr-Phe-Pro) displayed a 1.6-fold increase in potency when assayed using homogenates from AtT-20 cells. This gives rise to speculation to whether the imidazole in NNC 26-9100 mimics the Phe7 (His7) of SRIF or the basic amine of Lys9.

The identification of SST_4's biological and pharmacological properties has lagged behind due to the lack of selective SST_4 ligands. Recently, in situ hybridization showed predominant SST_4 expression in the posterior iris epithelium and ciliary body, suggesting functional roles of somatostatin in the autonomic nervous system in the anterior segments of the eye.[16] Since NNC 26-9100 shows such a superior selectivity to SST_4, it now should open up the possibility to further evaluate the physiological role of this receptor.

The low molecular weight and non-peptide nature of NNC 26-9100 seem promising for the development of a drug candidate for treatment of various diseases (e.g., glaucoma) related to the malfunction of SST receptors.

Conclusion

We have described the synthesis and biological properties of compounds **1, 2**, and NNC 26-9100. These compounds bind to five individual SST receptor subtypes with various affinities. The most potent compound, NNC 26-9100, binds to SST_4 with an affinity of 6 nM and at least a 100 fold selectivity for SST_4 over the other four SST receptor subtypes. In a functional assay, NNC 26-9100 showed full SST receptor agonism (95% of SRIF) with an EC_{50} of 2 nM.

Chemistry

A convergent synthesis−strategy was chosen for the preparation of compounds **1** and **3,** using the amine **8** and the isothiocyanates **17** or **18,** respectively, to form the thiourea in the last step. Compound **2** was prepared from **1** by simple *S*-methylation. The intermediate 3-[1-(triphenylmethyl)imidazol-4-yl]propylamine (**8**; Scheme 1) was prepared from the ester **4**, which has previously been described by Sellier et al.[17] The imidazole of **4** was protected with trityl chloride and the ester was reduced to the alcohol **6.** The alcohol **6** was converted *via* a Mitsunobu reaction with DEAD, phthtalimide, and triphenylphosphine to the phthalimide **7**. By refluxing **7** in hydrazine hydrate, the propylamine **8** was obtained in 85% yield.

The amines **15** and **16** were prepared by alkylation of 1,3-diaminopropane with 2-bromopyridine (**9**) and 2,5-dibromopyridine (**10**), respectively, followed by alkylation of the obtained secondary amines **11** and **12** with 4-bromobenzyl bromide (**13**) and 3,4-dichlorobenzyl bromide (**14**), respectively. The isothiocyanates **17** and **18** were obtained by a DCC coupling in carbon disulfide of **15** and **16**, respectively, similar to a procedure described by Jochims and Seeliger[18] (Scheme 2).

The desired compounds **1** and **3** were prepared by reacting **8** with **17** and **18**, respectively, followed by deprotection in hydrochloric acid (Scheme 3).

When compound **1** was treated with sodium hydride for 2 days followed by methyl iodide, the *S*-methylated derivative **2** was obtained (Scheme 4). This compound exhibited a singlet in ^{1}H NMR at d 2.71, which is characteristic for *S*-methylated thioureas.[19]

Experimental Section

All reactions were carried out under a nitrogen atmosphere. Melting points were determined on a Thomas Hoover melting point apparatus and were not corrected. The NMR spectra were recorded on a JEOL FX 90Q spectrometer or on a Bruker DPX200, and the chemical shifts were recorded in parts per million (d) relative to tetramethylsilane. Analytical data were obtained from Oneida Research Services, Inc. Whitesboro, NY. All reagents and chemicals were used without further purification except where indicated. Flash chromatography[20] was performed on Ace glass columns (i.d. = 5 cm, length = 45 cm) with 150-200 g of silica gel (40 mm). All solvents which were used for recrystallizations were of analytical grade.

***N*-(Pyrid-2-yl)propane-1,3-diamine (11).** To a solution of propane-1,3-diamine (310 mL, 3.6 mol) in dry pyridine (75 mL) was added

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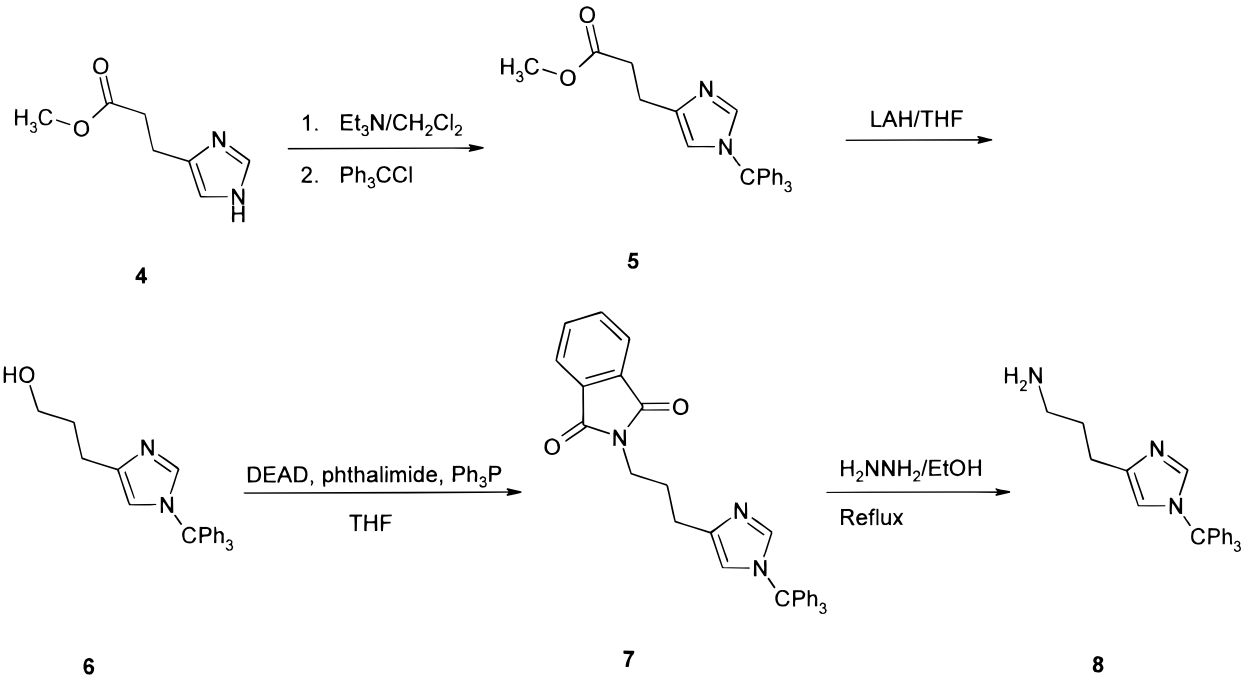
Scheme 1



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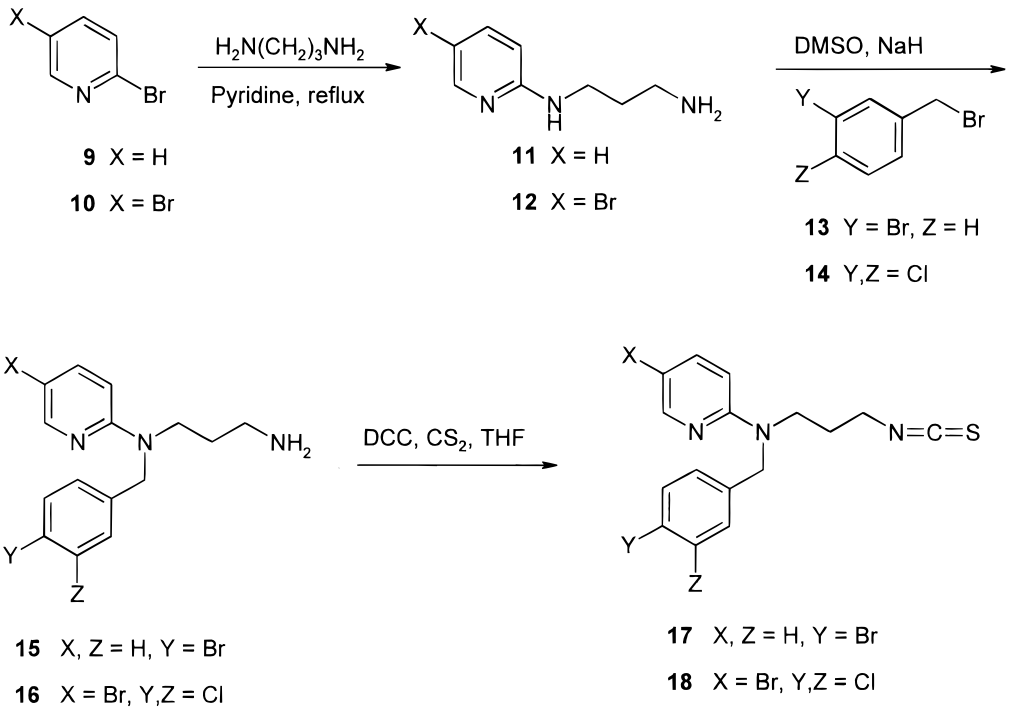

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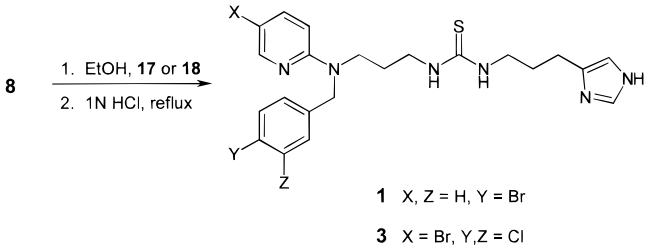
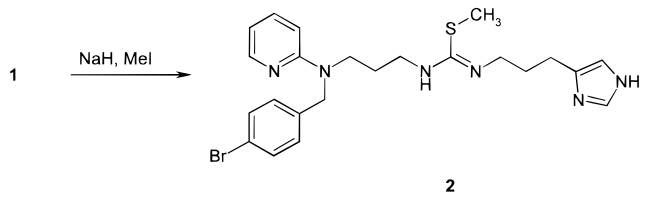


Scheme 4



2-bromopyridine (**9**, 70 mL, 0.73 mol). The reaction mixture was heated at reflux for 18 h and cooled, and the volatiles were evaporated *in vacuo*. To the residue was added tetrahydrofuran (1 L), and the precipitate was filtered off and washed with tetrahydrofuran (0.5 L). The solvent was evaporated *in vacuo* and the residue purified by distillation at 95-97 °C (0.02 mmHg) affording 83.4 g (76%) of **11**. ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.20 (br s, 2 H, NH_2), 1.74 (m, 2 H),

2.82 (t, 2 H), 3.34 (q, 2 H, CH_2NH), 4.86 (br s, 1 H, *NH*), 6.35 (dt, 1 H), 6.51 (ddd, 1 H), 7.37 (ddd, 1 H), 8.04 (ddd, 1 H).

***N*-(4-Bromobenzyl)-*N*-(pyrid-2-yl)propane-1,3-diamine (15).** To a mixture of sodium hydride (5.9 g, 60% dispersion in mineral oil, 0.14 mol) in dry dimethyl sulfoxide (250 mL) was slowly added a solution of **11** (20.0 g, 0.13 mol) in dry dimethyl sulfoxide (50 mL) at room temperature. The reaction mixture was stirred until gas evolution ceased. A solution of 4-bromobenzyl bromide (36.1 g, 0.14 mol) in dry dimethyl sulfoxide (100 mL) was slowly added at room temperature. The reaction mixture was stirred for 48 h at room temperature, and the mixture was poured onto ice water (500 mL) and extracted with ethyl acetate (3 × 250 mL). The combined organic extracts were washed with water (3 × 150 mL), dried ($MgSO_4$), filtered, and evaporated *in vacuo*. The residue (40.6 g) was washed with *n*-heptane (30 mL) which afforded 36.8 g of **15**. The crude product (20 g) was purified by column chromatography on silica gel (200 g) using a mixture of dichloromethane/methanol/triethylamine (90:5:5) as the eluent, affording 13.8 g (70%) of **15** as an oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.64 (s, 2 H, NH_2), 1.74 (t, 2 H), 2.72 (t, 2 H), 3.60 (t, 2 H, CH_2N), 4.67 (s, 2 H, CH_2Ph), 6.41 (d, 1 H), 6.53 (dd, 1 H), 7.07 (d, 2 H), 7.33−7.41 (m, 3 H), 8.13 (dt, 1 H).

3-[*N*-(4-Bromobenzyl)-*N*-(pyrid-2-yl)amino]propyl Isothiocyanate (17). To a solution of *N*,*N*-dicyclohexylcarbodiimide (2.1 g, 10 mmol) in dry tetrahydrofuran (20 mL) was slowly added at -10 °C a solution of **15** (3.2 g, 10 mmol) and carbon disulfide (4.3 mL, 70 mmol) in dry tetrahydrofuran (20 mL). The mixture was stirred at -10 °C for 3 h and for 48 h at room temperature. The reaction mixture was filtered and evaporated *in vacuo*. The residue (5.3 g) was extracted with diethyl ether (3 × 20 mL), and the combined organic extracts were evaporated *in vacuo*, affording 3.2 g (88%) of **17** as an oil. TLC R_f = 0.72 [SiO_2; ethyl acetate/*n*-heptane (1:1)]. ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.00 (q, 2 H), 3.57 (t, 2 H), 3.68 (t, 2 H), 4.66 (s, 2 H), 6.41 (d, 1 H), 6.58 (dd, 1 H), 7.07 (d, 2 H), 7.35−7.43 (m, 3 H), 8.15 (dd, 1 H).

1-[3-[*N*-(4-Bromobenzyl)-*N*-(pyrid-2-yl)amino]propyl]-3-[3-(1*H*-imidazol-4-yl)propyl]thiourea Dihydrochloride (1). A mixture of **17** (1.0 g, 2.8 mmol) and 3-[(1-triphenylmethyl)imidazol-4-yl]propylamine (**8**, 1.0 g, 2.8 mmol) in chloroform (10 mL) was heated at reflux for 4 h. The solvent was removed by evaporation *in vacuo*, and the residue (3.1 g) was purified by column chromatography on silica gel (400 mL) using a mixture of ethyl acetate/methanol/triethylamine (90:5:5) as the eluent, affording 1.6 g (80%) of pure 3-[3-[(1-triphenylmethyl)imidazol-4-yl]propyl]-1-[3-[*N*-(4-bromobenzyl)-*N*-(pyridin-2-yl)-amino]propyl]thiourea. TLC R_f = 0.59 (SiO_2; ethyl acetate/methanol/triethylamine = 90:5:5). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.90 (m, 6 H), 2.62 (t, 2 H), 3.44−3.68 (m, 6 H), 4.55 (s, 2 H, CH_2Ph), 6.33 (d, 1 H), 6.49 (dd, 1 H), 6.56 (s, 1 H), 6.98−7.11 (m, 9H), 7.30−7.39 (m, 11 H), 8.06 (d, 1 H).

To a solution of 3-[3-[(1-(triphenylmethyl)imidazol-4-yl]propyl]-1-[3-[*N*-(4-bromobenzyl)-*N*-(pyridin-2-yl)amino]propyl]thiourea (1.6 g, 2.2 mmol) in ethanol (50 mL), 1 N hydrochloric acid (16 mL) was added, and the reaction mixture was heated to 50 °C for 10 h. The cooled reaction mixture was washed with diethyl ether (3 × 30 mL) and the aqueous phase evaporated *in vacuo*. The residue was extracted with absolute ethanol (3 × 20 mL) and evaporated *in vacuo* followed by drying *in vacuo* affording 1.2 g (99%) of **1** as an amorphous powder. ^{1}H NMR (200 MHz, MeOD-d_3) δ 2.0 (m, 4 H), 2.77 (t, 2 H), 3.57 (m, 4 H), 3.76 (t, 2 H), 4.90 (s, 2 H, CH_2Ph), 6.99 (t, 1 H), 7.19 (d, 2 H), 7.26 (d, 1 H), 7.36 (s, 1 H), 7.50 (d, 2 H), 7.94 (d, 1 H), 8.02 (dd, 1 H), 8.78 (d, 1 H).

3-[3-(Imidazol-4(5)-yl)propyl]-1-[3-[*N*-(4-bromobenzyl)-*N*-(pyrid-2-yl)amino]propyl]-*S*-methylisothiourea Hydroiodide Dihydrochloride (2). To a solution of **1** (0.58 g, 1.0 mmol) in absolute ethanol (50 mL) was added sodium hydride (0.10 mL, 1.28 mmol, 60% in mineral oil), and the reaction mixture was stirred for 60 h at room temperature. Iodomethane (0.025 mL, 0.32 mmol) was added, and the reaction mixture was stirred at room temperature for an additional 5 h. The volatiles were evaporated *in vacuo*, affording 0.7 g (97%) of **2** as an amorphous powder. TLC R_f = 0.40 (SiO_2; methanol/triethylamine = 75:25). HPLC retention time = 12.58 min (5 μM C18 4- × 250-mm column, eluting with a gradient of 15% acetonitrile/0.1 N aqueous ammonium sulfate to 25% acetonitrile/0.1 N aqueous ammonium sulfate, pH = 2.5, over 10 min at room temperature). ^{1}H NMR (90 MHz, MeOD-d_3) δ 2.12 (m, 4 H), 2.71 (s, 3 H, SCH_3), 2.85 (t, 2 H), 3.61 (m, 4 H), 3.84 (m, 2 H), 4.95 (s, 2 H, CH_2Ph), 7.02 (t, 1 H), 7.22 (d, 2 H), 7.30 (d, 1 H), 7.42 (br s, 1 H), 7.52 (d, 2 H), 7.98 (d, 1 H), 8.05 (br s, 1 H), 8.80 (d, 1 H).

***N*-(5-Bromopyrid-2-yl)propane-1,3-diamine (12).**[21] A mixture of 2,5-dibromopyridine (**12**, 4.4 g, 18.6 mmol), pyridine (1.9 g, 23.6 mmol), and 1,3-diaminopropane (25 mL) was refluxed for 18 h. The reaction mixture was evaporated *in vacuo* to yield an oil, which was vacuum distilled to give 2.9 g (63%) of **12** as an oil. Bp 135-139 °C (0.1 mmHg). ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.52 (br s, 2 H, NH_2), 1.72 (m, 2 H), 2.89 (t, 2 H), 3.36 (m 2 H), 5.30 (br s, 1 H, NH), 6.29 (d, J = 9 Hz, 1 H, pyridine H-3), 7.44 (dd, J = 2.4, 8.8 Hz, 1 H, pyridine H-4), 8.09 (d, J = 2.4 Hz, 1 H, pyridine H-6). ^{13}C NMR ($CDCl_3$) d 32.61, 39.98, 40.25, 106.45, 108.29, 139.50, 148.49, 157.48.

***N*-(5-Bromopyrid-2-yl)-1-(3,4-dichlorobenzyl)propane-1,3-diamine (16).** A mixture of sodium hydride (0.9 g, 22.9 mmol, 60% in mineral oil) and **12** (5.0 g, 21.7 mmol) in dimethyl sulfoxide (45 mL) was stirred for 2 h. The suspension was cooled to 0-5 °C and treated dropwise with a solution of 3,4-dichlorobenzyl chloride (**14**, 4.24 g, 21.7 mmol) in dimethyl sulfoxide (15 mL). The mixture was stirred overnight at room temperature and poured into ice−water (200 mL). The mixture was extracted with ethyl acetate (3 × 75 mL), and the combined ethyl acetate extracts were washed with water (2 × 50 mL), dried (Na_2SO_4), filtered, and evaporated to yield an oil. Flash chromatography using a mixture of dichloromethane/methanol/triethylamine (90:5:5) as the eluent afforded 4.9 g (58%) of **16** as an oil. ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.44 (s, 2 H, NH_2), 1.80 (m, 2 H), 2.73 (t, 2 H), 3.56 (t, 2 H), 4.66 (s, 2 H, $ArCH_2$), 6.37 (d, J = 9 Hz, 1 H, pyridine H-3), 7.31 (m, 4 H), 8.15 (d, J = 2.5 Hz, 1 H, pyridine H-6). ^{13}C NMR (90 MHz, $CDCl_3$) d 30.93, 39.44, 46.21, 50.71, 106.67, 107.16, 126.23, 128.72, 130.51, 130.89, 132.62, 138.90, 139.66, 148.55, 156.46.

3-[*N*-(5-Bromopyrid-2-yl)-*N*-(3,4-dichlorobenzyl)amino]propyl Isothiocyanate (18). To a solution of *N*,*N*-dicyclohexylcarbodiimide (1.9 g, 9.4 mmol) in dry tetrahydrofuran (20 mL) was slowly added at −10 °C a solution of **16** (3.6 g, 9.4 mmol) and carbon disulfide (7.6 mL, 100 mmol) in dry tetrahydrofuran (25 mL). The mixture was stirred at -10 °C for 3 h and for 48 h at room temperature. The reaction mixture was filtered and the solvent evaporated *in vacuo*. The residue was purified on silica gel using a mixture of hexane/ethyl acetate/triethylamine (70:30:1) as the solvent which afforded 3.2 g (79%) of **18** as an oil. ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.06 (m, 2 H), 3.62 (m, 4 H), 4.66 (s, 2 H), 6.40 (m, 1 H), 7.35 (m, 4 H), 8.22 (m, 1 H). ^{13}C NMR (90 MHz, $CDCl_3$) δ 28.06, 42.91, 46.10, 51.41, 107.21, 107.43, 126.17, 128.72, 130.72, 131.26, 138.31, 139.93, 148.76, 156.13. MS (CI, CH_4) 432 M^+. Anal. Calcd for $C_{16}H_{14}BrCl_2N_3S$: C, 44.56; H, 3.28; N, 9.75. Found: C, 44.39; H, 3.42; N, 9.79.

1-[3-[*N*-(5-Bromopyridin-2-yl)-*N*-(3,4-dichlorobenzyl)amino]propyl]-3-[3-(1*H*-imidazol-1-yl)propyl]thiourea (3). A suspension of 3-[1-(triphenylmethyl)imidazol-4-yl]propylamine (**8**, 1.3 g, 3.6 mmol) in tetrahydrofuran (50 mL) was treated dropwise with **18** (1.6 g, 3.6 mmol) in THF (25 mL) at 0−5 °C under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and was stirred overnight. Removal of the solvent under reduced pressure afforded an oil. Flash chromatography on silica gel using a mixture of ethyl acetate/methanol/triethylamine (92:4:4) afforded 2.1 g (73%) of the trityl-protected intermediate as an oil. The oil was suspended in 1 N hydrochloric acid (50 mL) and refluxed for 7 h. The precipitated triphenylmethanol was filtered off, and the filtrate was evaporated under reduced pressure to yield a foam. The hygroscopic hydrochloride was converted to the free base with 1 N sodium hydroxide, and the aqueous layer was extracted with ethyl acetate (3 × 75 mL). The combined extracts were washed with water (2 × 50 mL), dried (Na_2SO_4), filtered, and evaporated to afford 600 mg of a foam. Purification by flash chromatography on silica gel using a mixture of ethyl acetate/methanol/concentrated ammoniun hydroxide (85:15:1) as solvent afforded 0.5 g (27%) of **3** as a solid foam. ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.05 (m, 4

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H), 2.70 (m, 2 H), 3.59 (m, 6 H), 4.59 (s, 2 H, $ArCH_2$), 6.30 (d, J = 9.3 Hz, 1 H), 6.50-8.11 (m, 9 H). ^{13}C NMR (90 MHz, $CDCl_3$) δ 23.73, 27.03, 28.82, 41.88, 43.29, 46.10, 106.99, 107.70, 115.93, 126.01, 128.50, 130.72, 131.10, 132.78, 134.41, 137.39, 138.20, 140.04, 148.33, 156.56, 181.38. Anal. Calcd for $C_{22}H_{25}BrCl_2N_6S$: C, 47.49; H, 4.54; N, 15.11. Found: C, 47.48; H, 4.48; N, 14.96.

Biological Assay. Cell Lines Expressing SST Receptor Subtypes. BHK cells (tk-ts13, ATCC CRL No. 1632) and HEK 293 cells (ATCC CRL No. 1573) were grown to 20−40% confluency in a tissue culture dish in DMEM containing 1% penicillin/streptomycin, 10% fetal bovine serum, and 1% Glutamax. Prior to transfection, the cells were washed twice with calcium-free PBS, after which 20 mL of serum-free DMEM was added to the cells.

Transfection was carried out as described previously (product description: lipofectamin, Gibco BRL Catalog No. 18324-012). Briefly, 10 μg of c-DNA encoding a SST-receptor subtype inserted into the mammalian expression vector pcDNA3 (Invitrogen) was diluted in 300 μL of sterile water. Lipofectamin (30 μg) was diluted in 300 μL of sterile water. The c-DNA and lipofectamin solutions were mixed and left at room temperature for 15 min. The lipofectamin/c-DNA mixture was added dropwise to the cells (HEK 293 cells for SST_2, BHK for the other receptor subtypes) while the plates were gently swirled. The cells were then incubated for 16-24 h, after which the medium was replaced with standard medium containing 1 mg/mL Geneticin (G-418 sulfate). Resistant colonies appearing after 1-2 weeks were isolated and propagated for further characterization.

Binding Assay. Cells expressing individual SST receptor subtypes were resuspended in buffer (50 mM Tris−HCl (pH 7.4), 1 mM EGTA, 5 mM $MgCl_2$), and homogenized. Membranes were washed twice in buffer by homogenization and centrifugation. The final membrane pellets were resuspended at a protein concentration of 125 μg/mL in buffer. Binding assays using 75 pM ^{125}I-Tyr11-SRIF (Amersham, IM-161) were done in duplicates in minisorb polypropylene tubes in a volume of 250 μl. The assays were incubated at 30-37 °C for 30-90 min depending on the receptor subtype. Binding was terminated by filtration through Whatman GF/B glass fiber filters presoaked for 4 h in 0.5% poly(ethylenimine) and 0.1% BSA. The filters were washed three times with 5 mL of ice-cold 0.9% saline and counted in a Packard Cobra II gamma counter.

Assays for other G protein-coupled receptors were performed by Panlabs Inc., Bothell, WA (PT No. 118422) with the radioligands mentioned in Table 2 using plasma membrane preparations from rabbit adrenal gland (ATII), guinea pig ileum (B_2), rat pancreas (CCK_A), mouse brain (CCK_B), human recombinant (D_3), A10 cells (ET_A), rat cerebellum (ET_B), rat brain (galanin), rat brain cortex (M_1), rat heart (M_2), guinea pig submaxillary gland (NK_1), rabbit kidney medulla (Y_2), rat brain cortex (5-HT_{1A}), rabbit ileum (5-HT_3), and guinea pig lung (VIP).

Functional Assay. Cells expressing human SST receptors were seeded in 24-well tissue culture multidishes at 200 000 cells/well and grown for 16-20 h. The medium was removed, and fresh DMEM medium, supplemented with (1) 3-isobutyl-1-methylxanthine (IBMX), (2) forskolin or medium, and (3) medium, SRIF, SST analogue, or compound, was added. The plates were incubated for 15-30 min at 37 °C, the reaction medium was removed, and the cells were lysed with 0.1 M sodium hydroxide. Following neutralization with 0.1 M hydrochloric acid, an aliquot was removed for c-AMP determination using Amersham SPA RIA (RPA 538).

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