Identification of Potent Non-Peptide Somatostatin Antagonists with sst_3 Selectivity

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Using a solution-phase parallel synthesis strategy, a series of non-peptide somatostatin analogues were prepared, and their binding affinities to the five human somatostatin receptor subtypes (sst_{1-5}) were determined. Imidazolyl derivatives **2** were found to bind with moderate affinity but with high selectivity to the sst_3 receptor subtype. Further modifications of these structures led to a more potent class of ligands, the tetrahydro-β-carboline derivatives **4**. Among these, compounds **4k** (BN81644) and **4n** (BN81674) bind selectively and with high affinity to the sst_3 receptor subtype (K_i = 0.64 and 0.92 nM, respectively). Furthermore, **4k** and **4n** reverse the inhibition of cyclic AMP accumulation induced by 1 nM somatostatin via sst_3 receptors, with IC_{50} = 2.7 and 0.84 nM, respectively. The most potent compound **4n** was shown to be a competitive antagonist of human sst_3 receptors by increasing the EC_{50} of SRIF-14-mediated inhibition of cAMP accumulation with a K_B of 2.8 nM (where K_B is the concentration of antagonist that shifts the agonist dose−response 2-fold). These new derivatives are, to our knowledge, the first potent and highly selective non-peptide human sst_3 antagonists known and, as such, are useful tools for investigating the physiological role of sst_3 receptors.

Introduction

Somatostatin (somatotropin-release-inhibiting factor, SRIF) is a peptide hormone originally isolated from the hypothalamus as an inhibitor of growth hormone release from the pituitary.[1] Widely distributed in the central nervous system, endocrine, and peripheral tissues, SRIF comprises two physiological forms, a tetradecapeptide (SRIF-14; Figure 1) and an N-terminal extended 28 amino acid peptide (SRIF-28). They exhibit multiple biological effects[2−4] which include the inhibition of the pancreatic secretion of insulin and glucagon,[5] and the release of gastrin by the gut.[6] In addition, SRIF possesses inhibitory effects on cell proliferation[7] and is also a neurotransmitter or neuromodulator, acting on cognitive function,[8] locomotor activity,[9] and the release of other neurotransmitters.[10,11] The biological functions of SRIF are mediated by a number of specific receptors. To date, five SRIF receptor subtypes have been cloned and characterized (sst_{1-5}) with distinct distributions in various tissues.[12] They all belong to the G-protein-coupled superfamily of receptors with seven hydrophobic transmembrane domains. So far, only sst_2 and sst_5 have been associated with specific physiological functions. sst_2 receptors have a predominant role in mediating the release of growth hormone (GH),[13] while the inhibition of insulin secretion from rat pancreatic islets is thought to be mediated through sst_5 receptors.[14,15]

The limitations of therapeutic applications of SRIF-14 due to its poor bioavailability and rapid proteolytic degradation have led to the search for peptide analogues with higher metabolic stability and improved selectivity with respect to the different receptor subtypes. Long-acting preparations of octreotide[16] and lanreotide[17]

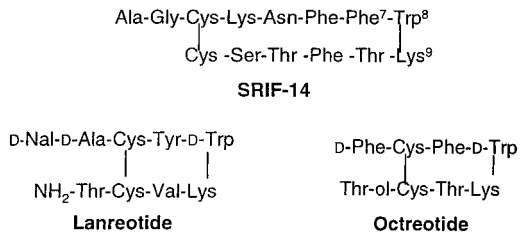

Figure 1. Chemical structures of SRIF-14, lanreotide, and octreotide.

(Figure 1) are now available for use in the treatment of acromegaly, neuroendocrine tumors, and gastrointestinal disorders. However, these analogues require parenteral administration. Recent efforts have therefore been focused on the search for potent, selective, and orally active non-peptide derivatives. On the basis of the knowledge of the crucial role of the tripeptide sequence[18] Phe^7-Trp^8-Lys^9 in the binding at ssts, non-peptide peptidomimetics, using a sugar or a benzodiazepinone core to mimic the β-turn of SRIF and exhibiting micromolar affinities for SRIF receptors, have been reported.[19−22] More recently, a thiourea derivative, NNC 26-9100 (**I**; Figure 2) was described as a potent and selective sst_4 agonist,[23] and a series of non-peptide agonists, with moderate to high selectivity for each of the five receptor subtypes, were also developed.[24,25] Among them, the derivative L-054,522 (**II**; Figure 2) was reported to bind selectively and with high affinity to the sst_2 receptor subtype.[26,27]

We initiated a synthetic program aimed at the identification of novel non-peptide SRIF agonists or antagonists for each subtype, since such compounds could be useful not only as potential therapeutic agents but also as tools for studying the physiological roles of individual SRIF receptor subtypes.

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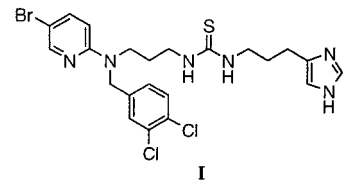
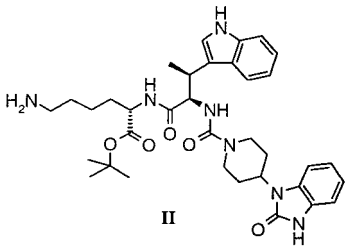


I

K_i sst$_4$ = 6 nM



II

K_i sst$_2$ = 0.01 nM

Figure 2. Chemical structures of **I** (NNC 26-9100) and **II** (L-054,522) and their inhibition constants (K_i) on somatostatin receptor subtypes.

From our compound collection we chose different templates suitable for parallel synthesis and displaying various possibilities of attachments. Among them imidazolyl derivatives **2** (Scheme 1) were selected. Using solution-phase parallel synthesis, a library of more than 1500 members was synthesized, and some of these imidazolyl derivatives were found to bind selectively to the sst$_3$ receptor subtype with moderate affinity.[28] These encouraging results validated the choice of imidazolyl derivatives **2** as potential peptidomimetics as well as the use of an indole moiety as a key side chain and prompted us to develop chemistry around this structural class to identify more potent compounds.

To introduce conformational rigidity into derivatives **2** and to provide a different spatial orientation to the side chains, we submitted imidazolyl compounds **3** to Pictet−Spengler cyclization[29] with various aldehydes to afford the corresponding tetrahydro-β-carbolines **4**. This reaction was successfully carried out in parallel in solution phase using resin scavengers to trap the excess of the reagents.[30] More than 400 compounds of structure **4** (as mixtures of C_1-diastereomers) were readily synthesized. Biological screening was carried out on the five SRIF receptor subtypes and led to the discovery of a set of compounds with high affinity and selectivity for sst$_3$ receptors.

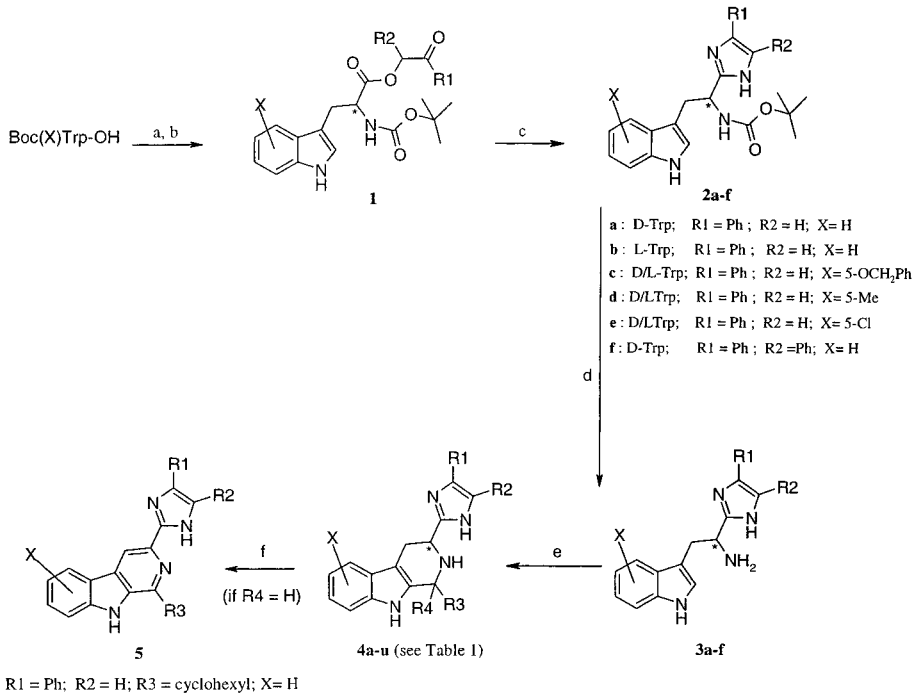
To further enhance the potency and selectivity of derivatives **4** for sst$_3$ receptors, several structural modifications were studied including the use of symmetric ketones instead of aldehydes in the Pictet−Spengler reaction, to avoid formation of diastereomeric mixtures. Replacement of the imidazole and indole rings by thiazole and benzothiophene, respectively, was investigated as well as substitution on the indole moiety and oxidation of the tetrahydro-β-carboline to its fully aromatized counterpart.

We will describe herein the synthesis of a novel series of tetrahydro-β-carbolines, their structure−activity relationships (SARs), and their affinities for sst$_3$ receptors. The most potent compounds were then evaluated for their agonist/antagonist properties.

Chemistry

Imidazole derivatives **3a**−**f** were prepared according to Scheme 1.[31,32] *N*-Boc-protected amino acids were treated with cesium carbonate followed by condensation with the appropriate α-bromoketone. Cyclization of the resulting ketoesters of structure **1** using ammonium acetate in refluxing xylene yielded the desired Boc-protected scaffolds **2a**−**f**, which upon acidic deprotection afforded amines **3a**−**f**. Pictet−Spengler cyclization with various aldehydes was carried out in solution-phase

Scheme 1[a]



[a] Reagents: (a) Cs_2CO_3, EtOH/H_2O; (b) $R_1COCH(Br)R_2$, DMF; (c) NH_4OAc, xylene, reflux; (d) 1 N HCl/EtOAc; (e) R_3CHO, TFA, $CHCl_3$, rt and then aminomethylpolystyrene resin; or R_3COR_4, acidic medium, reflux; (f) MnO_2, $CHCl_3$, reflux.

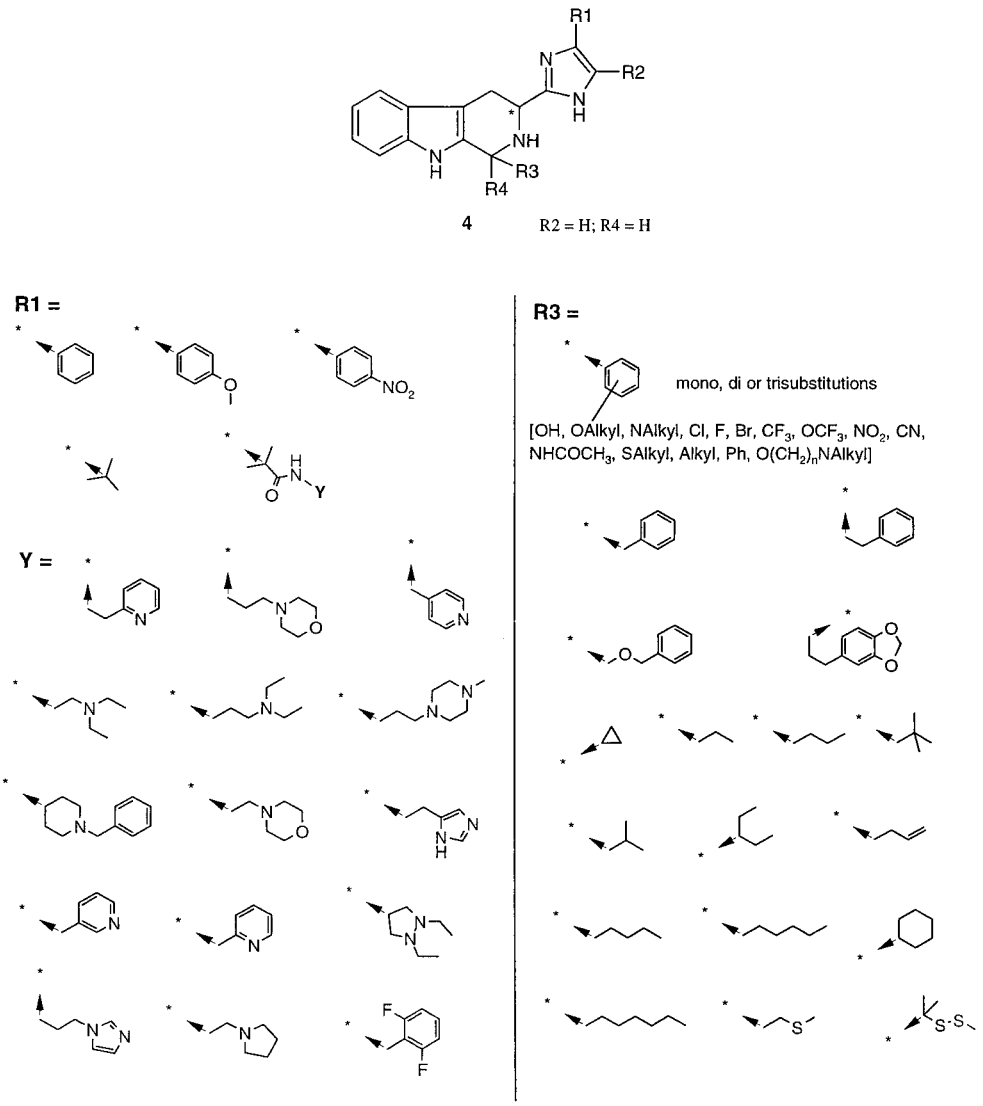
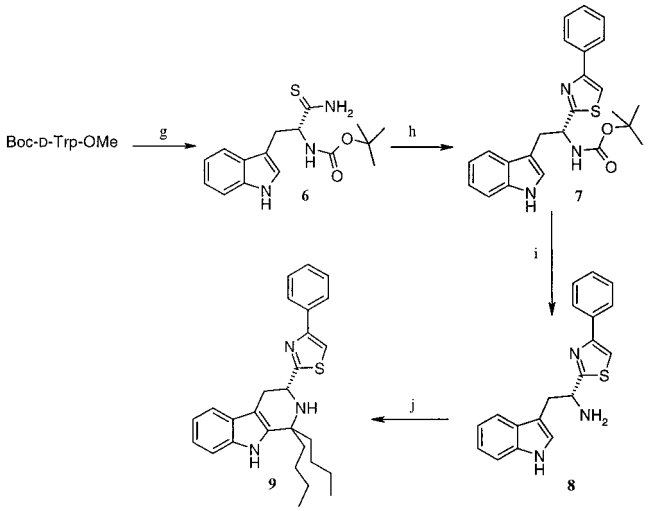


Figure 3. Set of compounds of structure **4** synthesized by solution-phase parallel synthesis.

parallel synthesis. Condensation of amines **3a**−**f** with an excess of aldehyde in dichloromethane, in the presence of trifluoroacetic acid, followed by evaporation of the mixture and subsequent trapping of the remaining aldehyde by aminomethylpolystyrene resin, yielded the desired tetrahydro-β-carbolines of structure **4** (R4 = H). The purity of the crude mixtures of diastereomers, as determined by LC/MS, was in the range 70−97%. Following this procedure, more than 400 compounds were prepared (Figure 3) and tested as mixtures of diastereomers (ratios varied from 60:40 to 90:10 as determined by LC/MS and/or ^{1}H NMR). Compound **4f** was isolated, after recrystallization of the fumarate salt of the mixture from methyl ethyl ketone, as the major and pure diastereomer, which we assumed to be the *trans*-1,3-tetrahydro-β-carboline.[29] Pictet−Spengler cyclization of amines **3a**−**f** with ketones required more vigorous conditions and afforded the corresponding tetrahydro-β-carbolines **4g**−**u**. Oxidation of compounds of general structure **4** (when R4 = H) to **5** was achieved using manganese dioxide in refluxing chloroform[33] followed by filtration of the mixture on a Celite pad. This convenient procedure allowed us to perform the reaction in parallel and to prepare a set of 50 fully aromatized carbolines of general structure **5**.

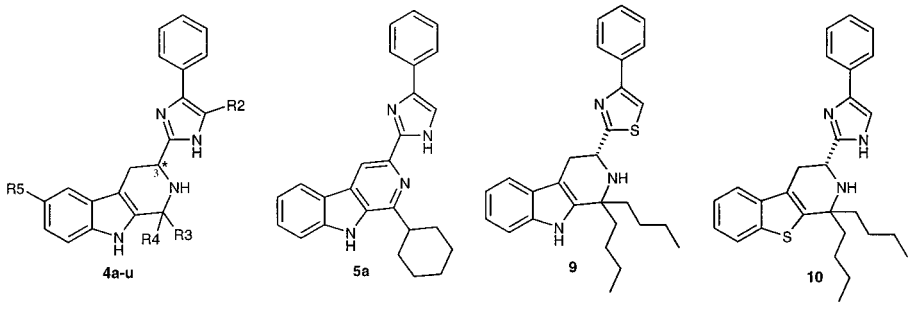
Scheme 2[a]



[a] Reagents: (g) (i) NH_3/MeOH, (ii) $NaHCO_3$, P_2S_5, $(CH_3OCH_2)_2$; (h) 2-bromoacetophenone, 90 °C; (j) 1 N HCl/EtOAc; (k) 5-nonanone, 1-butanol, reflux.

The thiazole derivative **9** (Scheme 2) was prepared by conversion of Boc-D-Trp-OMe to the corresponding

Table 1. Binding of Compounds **4a**−**u**, **5a**, **9**, and **10** to Human sst$_3$ Receptors



compd	C_3 config	R2	R3	R4	R5	salt	K_i(sst$_3$) (nM)[a]
4a	R	H	2-NO_2Ph	H	H		200 ± 4.0
4b	R	H	4-MeOPh	H	H		60 ± 8.1
4c	R	H	4-NMe_2Ph	H	H		33 ± 7.0
4d	R	H	4-pyridinyl	H	H		320 ± 76
4e	R	H	*n*-pentyl	H	H		11 ± 4.2
4f	R	H	cyclohexyl	H	H	$C_4H_4O_4$	1.7 ± 0.11
4g	R	H	2-adamantyl		H	2HCl	84 ± 0.5
4h	R	H	2-indanyl		H		13 ± 2.6
4i	R	H	1-acetyl-4-piperidine		H		71 ± 31
4j	R	H	ethyl	ethyl	H		11 ± 1.4
4k	R	H	*n*-butyl	*n*-butyl	H		0.64 ± 0.12
4l	R	H	*n*-butyl	*n*-butyl	H	$C_4H_4O_4$	0.79 ± 0.38
4m	S	H	*n*-butyl	*n*-butyl	H		3.4 ± 1.0
4n	R	H	*n*-pentyl	*n*-pentyl	H		0.92 ± 0.45
4p	R	H	*n*-hexyl	*n*-hexyl	H		28 ± 6.0
4q	R	H	methyl	cyclohexyl	H		0.90 ± 0.41
4r	*R*/*S*	H	*n*-butyl	*n*-butyl	$PhCH_2O$		17 ± 5.4
4s	*R*/*S*	H	*n*-butyl	*n*-butyl	Cl		3.4 ± 0.39
4t	*R*/*S*	H	*n*-pentyl	*n*-pentyl	CH_3		6.9 ± 1.2
4u	R	Ph	*n*-butyl	*n*-butyl	H		5500 ± 3300
5a							750 ± 83
9						HCl	3800 ± 2400
10							430 ± 190

[a] Results are expressed as the mean ± SEM of 2−4 experiments performed in duplicate (compound concentrations ranged from 0.01 nM to 10 μM).

thioamide **6** using a saturated methanolic solution of ammonia under pressure followed by treatment with P_2S_5. Condensation of **6** with 2-bromoacetophenone and subsequent cyclization to thiazole **7** afforded amine **8** after acidic deprotection. Pictet−Spengler cyclization with 5-nonanone gave rise to the desired thiazolyl tetrahydro-β-carboline **9**. Compound **10** was prepared analogously to derivative **4k**, starting from Boc-D-benzothienylalanine.

Pharmacology

The affinity of the compounds for human SRIF receptors was determined by measuring the inhibition of [^{125}I-Tyr^{11}]SRIF-14 binding to membranes isolated from CHO-K1 cells expressing each of the cloned human SRIF receptor subtypes. The library screening was performed in 96-well plate format. Percentage inhibitions were first determined at 10 μM, and compounds with greater than 70% inhibition at this concentration were selected for inhibition constant (K_i) determination. The biological activity of the derivatives **4** (Figure 3) was confirmed by resynthesizing and retesting a selected set of molecules with moderate to high affinity for sst$_3$ receptors (see Table 1, **4a**−**f**).

Among this first set of compounds, **4e** (K_i = 11 nM) and **4f** (K_i = 1.7 nM) were the most potent leads (Table 1). Removal of the C_1 chiral center by using symmetrical ketones (**4k** and **4n**) increased the activity (K_i = 0.64 and 0.92 nM, respectively). Shortening the alkyl chain of **4k** (compound **4j**) or extending it by two carbons (**4p**) caused a 17−40-fold drop in affinity. There was a preference for the *R* stereochemistry at the C_3 center since **4k** was 6 times more potent than the *S*-isomer **4m**. Substitution on the indole moiety did not increase potency (**4r**, **4s**, **4t**), while replacing indole by benzothiophene caused an important decrease in affinity (**10**, K_i = 430 nM), the latter result confirming the importance of the indole ring as a mimic of Trp^8 in SRIF. Replacement of the basic imidazole moiety by a thiazole (compound **9**) resulted in a loss of activity (K_i = 3800 nM), suggesting its key role in sst$_3$ binding affinity. Imidazole substitution seemed to be critical: biological screening of libraries of tetrahydro-β-carbolines **4** with various R1 groups (Figure 3) showed that compounds with a phenyl substituent exhibited the highest affinity whereas substitution on the phenyl ring resulted in a dramatic drop in activity (percentage inhibition at 10 μM, <30%; data not shown). When R2 is a phenyl instead of hydrogen, the compound **4u** is about 8000 times less active than **4k**.

Attempts to further increase the rigidity of the tetrahydro-β-carboline template by synthesizing the corresponding fully aromatized planar β-carbolines **5** resulted in a decrease in affinity, suggesting that compounds **4** display an optimal binding conformation and/or that the basic nitrogen is necessary for sst$_3$ binding (compare **4f** and **5a**).

Binding affinities of the most potent compounds **4f**, **4k**, **4m**, and **4n** toward sst$_{1-5}$ receptors (Table 2) revealed a 1000−10000-fold selectivity for sst$_3$. The

Table 2. Binding Affinities (K_i, nM) of Selected Tetrahydro-β-carbolines to Human sst_{1-5} Receptors

	K_i (nM)				
compd	sst_1	sst_2	sst_3	sst_4	sst_5
4f	1800	>10000	1.7	>10000	>10000
4k	3700	>10000	0.64	7100	2200
4m	>10000	>10000	3.4	9800	3700
4n	>10000	>10000	0.92	>10000	>10000
4q	4400	>10000	0.90	>10000	>10000
SRIF-14	0.29	0.035	0.10	0.63	0.12

Table 3. Inhibition of SRIF-14 (1 nM) Induced Reduction of cAMP Accumulation Due to Forskolin (1 μM) in CHO-K1 Cells Expressing the Human sst_3 Receptor

compd	IC_{50} (nM)[a]	compd	IC_{50} (nM)[a]
4f	19 ± 9.7	**4s**	43 ± 25
4k	2.7 ± 0.7	**4q**	5.4 ± 4.2
4m	6.8 ± 5.1	**4t**	450 ± 150
4n	0.84 ± 0.27		

[a] Results are expressed as the mean ± SEM of three experiments performed in triplicate (compound concentrations ranged from 0.1 nM to 10 μM).

functional activity of tetrahydro-β-carboline derivatives **4** with the highest binding activity was assessed by measuring the modulation of intracellular adenosine cyclic 3′−5′-monophosphate (cAMP) production stimulated by forskolin on CHO-K1 cells expressing the human sst_3 receptor subtype.

While derivatives **4** were unable to reduce the cAMP production elicited by forskolin as expected for agonists, they blocked the inhibitory effect of 1 nM SRIF-14. Compound **4n** was the most potent antagonist with an IC_{50} value of 0.84 nM (Table 3). In another set of experiments, the dose−response decrease of cAMP levels induced by SRIF-14 was measured in the presence of increasing concentrations of **4n**. Compound **4n** increased the EC_{50} of SRIF-14 in a dose-dependent manner without affecting the maximal inhibition observed (Figure 4). Schild regression analysis of the data led to a K_B value (concentration of compound **4n** that shifts SRIF-14 dose−response 2-fold) of 2.8 nM with a slope factor of 0.9. These results demonstrate that compound **4n** acts as a competitive antagonist at the human sst_3 receptors. So far, very few cyclic peptide SRIF analogues have been known to exhibit antagonist activity with partial subtype selectivity.[34−37] Meanwhile, during the revision of this paper, Reubi et al. reported the discovery of octapeptide derivatives as somatostatin sst_3 receptor antagonists.[38] But, the limited number of sst_3 selective or partially selective ligands and the lack of common structural features among them do not offer clear assumptions for the factors determining agonist/antagonist activity at sst_3 receptors.

Conclusion

Our approach consisted of the synthesis of an imidazolyl scaffold displaying various possibilities of attachment and which was amenable to parallel synthesis. The choice of these imidazolyl derivatives allowed us to introduce, via a Pictet−Spengler cyclization, a constrained indole moiety. Despite the knowledge of the crucial role of the indole group for somatostatin receptor binding, the high sst_3 selectivity of these new tetrahydro-β-carboline derivatives **4** remains puzzling. SAR analysis suggests a key role of the imidazole moiety

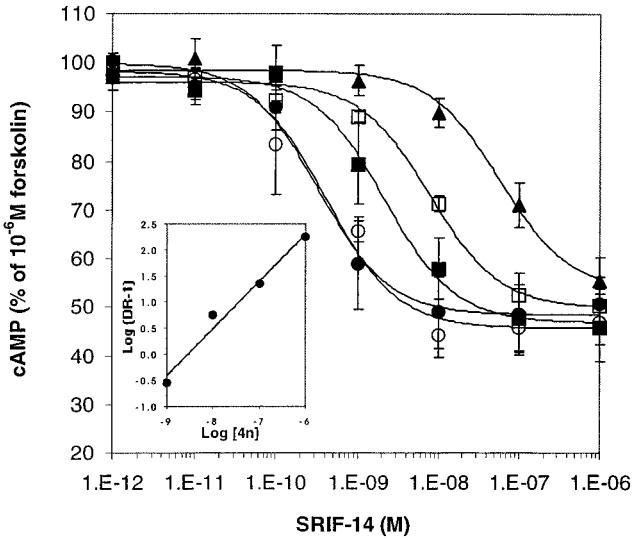


Figure 4. Inhibition by compound **4n** of SRIF-14 decreased cAMP accumulation induced by forskolin (1 μM) in CHO-K1 cells expressing the human sst_3 receptor. The dose−response reduction of cAMP levels induced by SRIF-14 was measured with 1 nM (O), 10 nM (■), 100 nM (□), and 1000 nM (▲) compound **4n** or without (●) compound **4n**. Data represent the mean ± standard error of three experiments performed in duplicate. The inset shows the Schild regression analysis of the data. DR is the ratio of the EC_{50} of SRIF-14 in the presence of compound **4n** over the EC_{50} of SRIF-14 alone. K_B is the concentration of compound **4n** when log(DR − 1) = 0.

probably due to its basicity compared to that of the thiazole. In addition, the β-carboline moiety is necessary for sst_3 binding; its replacement by a benzothienopyridine template proved to be detrimental to activity. On the basis of these observations and the knowledge of the crucial Phe^7-Trp^8-Lys^9-Thr^{10} segment which comprises a β-turn, essential for SRIF binding, two hypotheses may be proposed: (a) where the phenyl group and β-carboline of structures **4** mimic the Phe^7-Trp^8 moiety or (b) where the β-carboline and the imidazole, although less basic than the terminal amine of lysine, mimic the Trp^8-Lys^9 moiety of the tripeptide. However, as these assumptions do not provide a satisfactory explanation for their high selectivity and their antagonist behavior at sst_3 receptors, other hypotheses may be considered. Incorporation of constrained amino acids in a SRIF mimetic has been shown to provide more potent compounds with altered activity and selectivity profiles toward sst_{1-5}.[39] Thus, the conformational rigidity of the tetrahydro-β-carboline scaffold may play an important role in selective binding at sst_3. In addition, specific lipophilic and electrostatic interactions between the constrained derivatives **4** and crucial residues of the sst_3 receptor subtype, located in the extracellular and/or transmembrane domains, may be considered. A sequence alignment of the five human SRIF receptor subtypes revealed that several amino acids are conserved or conservatively exchanged in sst_1, sst_2, sst_4, and sst_5 but not in sst_3, particularly in the extracellular loop III and in transmembrane domain VII (e.g., Glu286−Glu287, Phe290−Phe291). Site-directed mutagenesis may be helpful in determining whether the latter residues are essential for tetrahydro-β-carboline **4** binding. This method was successfully used by Kaupmann et al.,[40] where advantage was taken between the binding

selectivity of octreotide (Figure 2) for sst$_2$ over sst$_1$ receptors, thus identifying two crucial residues located in the transmembrane domains VI and VII.

In conclusion, the tetrahydro-β-carboline derivatives BN 81644 (**4k**; K_i = 0.64 nM, IC_{50} = 2.7 nM) and BN 81674 (**4n**; K_i = 0.92 nM, IC_{50} = 0.84 nM, K_B = 2.8 nM) are believed to be the first non-peptide sst$_3$ antagonists known so far. These new highly potent and selective sst$_3$ antagonists are useful tools for investigating the physiological role of the sst$_3$ receptors. Few biological data are so far available: expression studies have revealed a high level of sst$_3$ receptor mRNA in the cerebellum,[41,42] gastrointestinal tract,[43] rat adrenal PC12 cells,[44] mouse insulinoma MIN6 cells,[45] and human Jurkat T cells.[46] Moreover, a subtype-selective induction of apoptosis by sst$_3$ receptors has been reported,[47] suggesting a possible effect of sst$_3$ antagonists as anti-apoptotic agents.

Studies are in progress to evaluate the therapeutic potential of this new class of non-peptidic somatostatin receptor ligands.

Experimental Section

Chemistry. Anhydrous dichloromethane and *N*,*N*-dimethylformamide (DMF) were purchased in septum-sealed bottles, and all other solvents were of reagent grade and used as received from various commercial sources. Aminomethylpolystyrene resin was purchased from Novabiochem. Analytical thin-layer chromatography was performed on silica gel 60 F254 coated glass plates, visualized under UV light. Melting points were determined using a Büchi 535 apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Brücker ARX 400 spectrometer. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane, and spin multiplicities are given as s (singlet), d (doublet), dd (double of doublets), t (triplet), or m (multiplet). Optical rotation was measured at 20 °C with a Jasco DIP-370 polarimeter. LC/MS was recorded on a Micromass Plateform II. Elemental analyses were performed on a Fison EA 1108 apparatus. The percentage of water included in the elemental analyses was determined by a coulometric Karl Fisher titration using a Metrohm 737 KF coulometer.

Representative Procedure for the Synthesis of Imidazole Derivatives of Structure 2: Preparation of *tert*-Butyl (1*R*)-2-(1*H*-Indol-3-yl)-1-(4-phenyl-1*H*-imidazol-2-yl)ethylcarbamate (*2*a). A solution of Boc-D-Trp-OH (50 g, 164 mmol) and cesium carbonate (27 g, 83 mmol) in EtOH (350 mL) was shaken for about 30 min at room temperature, and then evaporated under reduced pressure. To the resulting salt in DMF (600 mL) was added 2-bromoacetophenone (32.6 g, 164 mmol). The mixture was stirred for about 1 h at room temperature under argon and then concentrated under reduced pressure. Ethyl acetate (400 mL) was added, the mixture filtered, and the CsBr washed with ethyl acetate. The filtrate was then concentrated under reduced pressure. A solution of the resulting oil and ammonium acetate (246 g, 320 mmol) in xylene (2 L) was refluxed for 45 min. Excess NH_4OAc and H_2O were removed using a Dean−Stark trap. The mixture was then cooled to room temperature, diluted with ethyl acetate (1 L), and washed twice with water, 10% $NaHCO_3$ aqueous solution until basic pH, and brine. The organic layer was then dried (Na_2SO_4), and concentrated under reduced pressure until ca. 400 mL of solvent remained. The organic layer was allowed to stand at room temperature, upon which crystals appeared that were collected by filtration and washed with diethyl ether to afford 57.5 g (89%) of **2a** as a white powder: mp 208−210 °C; ^{1}H NMR (DMSO-d_6) δ 1.32 (s, 9H), 3.15 (dd, 1H, ^3J = 7 Hz, ^2J = 14 Hz), 3.35 (m, 1H), 4.88 (m, 1H), 6.94−7.77 (m, 12H), 10.77 (s, 1H), 11.87 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 28.34, 30.22, 49.99, 78.04, 110.55, 111.37, 112.47, 118.30, 118.49, 120.90, 123.54, 124.01, 124.36, 125.93, 127.64, 128.48, 128.93, 135.21, 136.13, 139.53, 149.21, 155.19; $[\alpha]_D$ = +6.0° (*c* 1.3, DMSO). Anal. ($C_{24}H_{26}N_4O_2 \cdot 0.75H_2O$) C, H, N.

***tert*-Butyl (1*S*)-2-(1*H*-Indol-3-yl)-1-(4-phenyl-1*H*-imidazol-2-yl)ethylcarbamate (2b).** Using the same procedure as described for **2a**, Boc-L-Trp-OH (50 g, 164 mmol) gave an oil. Crystallization in dichloromethane afforded 53 g (80%) of **2b** as a white solid: mp 208−210 °C; ^{1}H NMR (DMSO-d_6) δ 1.32 (s, 9H), 3.15 (dd, 1H, ^3J = 7 Hz, ^2J = 14 Hz), 3.35 (m, 1H), 4.88 (m, 1H), 6.94−7.77 (m, 12H), 10.77 (s, 1H), 11.87 (s, 1H).

***tert*-Butyl 2-[5-(Benzyloxy)-1*H*-indol-3-yl]-1-(4-phenyl-1*H*-imidazol-2-yl)ethylcarbamate (2c).** Using the same procedure as described for **2a**, Boc-5-BzO-Trp-OH (7 g, 17 mmol) gave an oil. Purification by flash chromatography using AcOEt/heptane (1:1) as the eluent afforded 3.8 g (44%) of **2c** as an off-white foam: ^{1}H NMR (DMSO-d_6) δ 1.32 (s, 9H), 3.11 (dd, 1H, ^3J = 7.5 Hz, ^2J = 14 Hz), 3.30 (m, 1H), 4.85 (m, 1H), 4.99 (m, 2H), 6.75−7.78 (m, 16H), 10.61 (s, 1H), 11.80 (s, 1H).

***tert*-Butyl 2-(5-Methyl-1*H*-indol-3-yl)-1-(4-phenyl-1*H*-imidazol-2-yl)ethylcarbamate (2d).** Using the same procedure as described for **2a**, Boc-5-Me-Trp-OH (6.6 g, 20 mmol) gave 2.2 g (27%) of **2d** as an off-white foam: ^{1}H NMR (DMSO-d_6) δ 1.39 (s, 9H), 2.39 (s, 3H), 3.19 (dd, 1H, ^3J = 6.5 Hz, ^2J = 14 Hz), 3.30 (m, 1H), 4.92 (m, 1H), 6.90−7.83 (m, 11H), 10.64 (s, 1H), 11.84 (s, 1H).

***tert*-Butyl 2-(5-Chloro-1*H*-indol-3-yl)-1-(4-phenyl-1*H*-imidazol-2-yl)ethylcarbamate (2e).** A mixture of 5-Cl-Trp-OH (10 g, 42 mmol) and di-*tert*-butyl dicarbonate (10.05 g, 46 mmol) in dioxane (120 mL) was refluxed for 2 h and then cooled to room temperature. The solvent was evaporated and the resulting oil crystallized in diisopropyl ether to afford 12 g (84%) of Boc-5-Cl-Trp-OH.

Using the same procedure as described for **2a**, Boc-5-Cl-Trp-OH (12 g, 35 mmol) gave an oil which was crystallized in diisopropyl ether/heptane to afford 10 g (65%) of **2e** as an off-white foam: ^{1}H NMR (DMSO-d_6) δ 1.36 (s, 9H), 3.17 (dd, 1H, ^3J = 6.5 Hz, ^2J = 14 Hz), 3.30 (m, 1H), 4.90 (m, 1H), 7.05−7.83 (m, 11H), 11.01 (s, 1H), 11.85 (s, 1H).

***tert*-Butyl (1*R*)-1-(4,5-Diphenyl-1*H*-imidazol-2-yl)-2-(1*H*-indol-3-yl)ethylcarbamate (2f).** Using the same procedure as described for **2a**, Boc-D-Trp-OH (9.1 g, 30 mmol) and 2-bromo-2-phenylacetophenone (8.26 g, 30 mmol) gave an oil. Purification by flash chromatography using dichloromethane/methanol (97:3) as the eluent followed by crystallization of the resulting oil in diisopropyl ether afforded 7.2 g (50%) of **2f** as an off-white powder: mp 143−145 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 3.45 (dd, 1H, ^3J = 6 Hz, ^2J = 14 Hz), 3.65 (m, 1H), 5.07 (m, 1H), 5.47 (s, 1H), 7.01−7.62 (m, 15H), 8.09 (s, 1H), 9.48 (s, 1H).

***tert*-Butyl (1*R*)-2-(1-Benzothiophen-3-yl)-1-(4-phenyl-1*H*-imidazol-2-yl)ethylcarbamate (2g).** Using the same procedure as described for **2a**, Boc-D-3-benzothienylalanine (5 g, 15 mmol) gave an oil. Crystallization in isopentane afforded 4 g (64%) of **2g** as a white powder: mp 116−120 °C; ^{1}H NMR (DMSO-d_6) δ 1.30 (s, 9H), 3.30 (m, 1H), 3.59 (dd, 1H, ^3J = 6 Hz, ^2J = 12 Hz), 5.03 (m, 1H), 7.19−7.96 (m, 12H), 11.93 (s, 1H).

Representative Procedure for the Synthesis of Amino Imidazole Derivatives 3. Preparation of (1*R*)-2-(1*H*-Indol-3-yl)-1-(4-phenyl-1*H*-imidazol-2-yl)-1-ethanamine (3a). Into a suspension of **2a** (57 g, 142 mmol) in ethyl acetate (500 mL) at 0 °C was bubbled dry HCl until TLC (eluent ethyl acetate, 100%) showed complete disappearance of starting material. The resulting mixture was then evaporated under reduced pressure. Diethyl ether was added to the resulting solid, and the mixture was filtered. The dihydrochloride was washed with dichloromethane followed by diethyl ether to afford 57 g (99%) of a white foam.

3a was used in the next step either as its dihydrochloride salt or as its free base.

The free base **3a** was obtained after treatment of the salt with 10% $NaHCO_3$ aqueous solution and the usual workup: ^{1}H NMR (CD_3OD) δ 3.36 (dd, 1H, ^3J = 7 Hz, ^2J = 14.3 Hz), 3.45 (dd, 1H, ^3J = 7 Hz, ^2J = 14.3 Hz), 4.51 (t, 1H, ^3J = 7 Hz), 6.95−7.68 (m, 11H); $[\alpha]_D$ = −114° (*c* 1.2, DMSO).

(1*S*)-2-(1*H*-Indol-3-yl)-1-(4-phenyl-1*H*-imidazol-2-yl)-1-ethanamine Dihydrochloride (3b). Using the same proce-

dure as described for **3a**, **2b** (53 g, 132 mmol) gave 49 g (98%) of dihydrochloride **3b** as a hygroscopic foam: ^{1}H NMR (DMSO-d_6) δ 3.65 (dd, 1H, ^3J = 6 Hz, ^2J = 14 Hz), 3.80 (dd, 1H, ^3J = 10 Hz, ^2J = 14. Hz), 4.98 (m, 1H), 6.95−8.03 (m, 11H), 9.31 (s, 3H), 11.02 (s, 1H).

2-[5-(Benzyloxy)-1*H*-indol-3-yl]-1-(4-phenyl-1*H*-imidazol-2-yl)ethylamine Dihydrochloride (3c). Using the same procedure as described for **3a**, **2c** (3.8 g, 7.5 mmol) gave 2.8 g (78%) of dihydrochloride **3c** as an off-white powder: mp 180−182 °C; ^{1}H NMR (DMSO-d_6) δ 3.62 (dd, 1H, ^2J = 14 Hz, ^3J = 5.5 Hz), 3.74 (dd, 1H, ^2J = 14 Hz, ^3J = 10 Hz), 4.99 (m, 3H), 6.74−8.05 (m, 15H), 9.31 (m, 3H), 10.89 (s, 1H).

2-(5-Methyl-1*H*-indol-3-yl)-1-(4-phenyl-1*H*-imidazol-2-yl)ethylamine Dihydrochloride (3d). Using the same procedure as described for **3a**, **2d** (1.6 g, 3.8 mmol) gave 1.12 g (75%) of dihydrochloride **3d** as a hygroscopic foam: ^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3H), 3.62 (dd, 1H, ^2J = 14 Hz, ^3J = 5.5 Hz), 3.74 (dd, 1H, ^2J = 14 Hz, ^3J = 10 Hz), 4.94 (m, 1H), 6.84−8.01 (m, 10H), 9.31 (m, 3H), 10.88 (s, 1H).

2-(5-Chloro-1*H*-indol-3-yl)-1-(4-phenyl-1*H*-imidazol-2-yl)ethylamine Dihydrochloride (3e). Using the same procedure as described for **2a**, **2e** (10 g, 23 mmol) gave 6.5 g (69%) of dihydrochloride **3e** as a white solid: mp 198−200 °C; ^{1}H NMR (DMSO-d_6) δ 3.59 (dd, 1H, ^2J = 14 Hz, ^3J = 5.5 Hz), 3.74 (dd, 1H, ^2J = 14 Hz, ^3J = 10 Hz), 4.94 (m, 1H), 6.84−8.01 (m, 10H), 9.20 (m, 3H), 11.21 (s, 1H).

(1*R*)-1-(4,5-Diphenyl-1*H*-imidazol-2-yl)-2-(1*H*-indol-3-yl)-1-ethanamine Dihydrochloride (3f). Using the same procedure as described for **3a**, **2f** (3.6 g, 7.5 mmol) gave 2.4 g (71%) of dihydrochloride **3f** as an off-white solid: mp 260−262 °C; ^{1}H NMR (DMSO-d_6) δ 3.64 (dd, 1H, ^2J = 14 Hz, ^3J = 6 Hz), 3.74 (dd, 1H, ^2J = 14 Hz, ^3J = 9 Hz), 4.88 (m, 1H), 6.93−7.47 (m, 15H), 9.25 (s, 3H), 11.07 (s, 1H).

(1*R*)-2-(1-Benzothiophen-3-yl)-1-(4-phenyl-1*H*-imidazol-2-yl)-1-ethanamine Dihydrochloride (3g). Using the same procedure as described for **3a**, **2g** (4,0 g, 9.5 mmol) gave 3.0 g (80%) of dihydrochloride **3g** as an off-white powder: mp 190−192 °C; ^{1}H NMR (DMSO-d_6) δ 3.80 (dd, 1H, ^2J = 14 Hz, ^3J = 5.5 Hz), 3.93 (dd, 1H, ^2J = 14 Hz, ^3J = 10 Hz), 5.05 (m, 1H), 7.35−8.05 (m, 11H), 9.40 (m, 3H).

General Procedure for Tetrahydro-β-carboline 4 Library Synthesis. In a vial, to a solution of an amine of structure **3** (0.05 mmol) in chloroform (0.8 mL) were successively added an aldehyde (0.10 mmol) and TFA (0.5 mmol). The mixture was stirred for 5 h at room temperature on an orbital shaker and then concentrated under reduced pressure. The resulting residue was diluted in THF (3 mL), and aminomethylpolystyrene resin (loading 2.75 mmol/g, 0.15 mmol) was added. The resulting suspension was shaken overnight on an orbital shaker at room temperature and then filtered on cartridges with frits. The filtrate was concentrated under reduced pressure, and the resulting residue was filtered on a silica gel pad (with ethyl acetate as the eluent) to afford the tetrahydro-β-carboline of structure **4** as a mixture of diastereomers. An aliquot was analyzed by LC/MS.

The following compounds were prepared using this procedure. However, for confirmation of chemical structure and biological activity, they were resynthesized on a larger scale and purified by flash chromatography on silica gel.

(3*R*)-1-(2-Nitrophenyl)-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline (4a). Free base **3a** (900 mg, 2.97 mmol), 2-nitrobenzaldehyde (896 mg, 5.95 mmol), and TFA (2.25 mL, 29.7 mmol) gave an oil. Basic workup followed by flash chromatography on silica gel using AcOEt/heptane (60:40) as the eluent afforded 1.0 g (78%) of the title product as a yellow foam: diastereomeric ratio 57:43; ^{1}H NMR (DMSO-d_6) δ 3.10 (m, 2H, CH_2), 4.09, 4.34 (2m, 1H, H_3), 5.85, 5.91 (2m, 1H, H_1), 6.98−8.03 (m, 14H), 10.41; 10.76 (2s, 1H), 11.82; 12.05 (2s, 1H). Anal. ($C_{26}H_{21}N_5O_2\cdot 1H_2O$) C, H, N.

(3*R*)-1-(4-Methoxyphenyl)-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline (4b). Free base **3a** (700 mg, 2.3 mmol), 4-anisaldehyde (562 μL, 4.62 mmol), and TFA (1.7 mL, 23 mmol) gave an oil. Basic workup followed by flash chromatography on silica gel using AcOEt as the eluent afforded 805 mg (83%) of the title product as a foam: diastereomeric ratio 88:12; ^{1}H NMR (DMSO-d_6) δ 3.10 (m, 2H, CH_2), 3.73 (s, 3H, OCH_3), 4.16 (m, 1H, H_3), 5.24; 5.23 (2s, 1H, H_1), 6.89−7.74 (m, 14H), 10.64; 10.69 (2s, 1H), 11.91; 12.13 (2s, 1H). Anal. ($C_{27}H_{24}N_4O\cdot 0.5H_2O$) C, H, N.

(3*R*)-1-(4-Dimethylaminophenyl)-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline (4c). Free base **3a** (800 mg, 2.64 mmol), 4-(dimethylamino)benzaldehyde (788 mg, 5.28 mmol), and TFA (2.0 mL, 26.4 mmol) gave an oil. Basic workup followed by flash chromatography on silica gel using AcOEt as the eluent afforded 998 mg (87%) of the title product as a foam: diastereomeric ratio 58:42; ^{1}H NMR (DMSO-d_6) δ 2.85, 2.88 (2s, 6H, N(CH_3)$_2$), 3.06 (m, 2H, CH_2), 4.25 (m, 1H, H_3), 5.23 (s, 1H, H_1), 6.72−7.78 (m, 14H), 10.25 (s, 1H), 12.01 (2s, 1H). Anal. ($C_{28}H_{27}N_5\cdot 0.5H_2O$) C, H, N.

(3*R*)-3-(4-Phenyl-1*H*-imidazol-2-yl)-1-(4-pyridinyl)-2,3,4,9-tetrahydro-1*H*-β-carboline (4d). A solution of free amine **3a** (1,5 g, 5 mmol), pyridine-4-carbaldehyde (954 μL, 10 mmol), and TFA (3.85 mL, 50 mmol) in dichloromethane (25 mL) was stirred for 2 h at room temperature and evaporated under reduced pressure. The resulting residue was diluted in EtOAc (80 mL), washed successively with 10% $NaHCO_3$ aqueous solution and brine, dried (Na_2SO_4), and evaporated to yield an oil. Flash chromatography on silica gel using dichloromethane/ethanol (90:10) as the eluent afforded 250 mg (13%) of **4d** as an off-white powder: mp 204−206 °C; diastereomeric ratio 85:15; ^{1}H NMR (CD_3OD) δ 3.20 (m, 2H, CH_2), 4.25, 4.48 (2 dd, 1H, ^3J = 4.9 Hz, ^2J = 10 Hz, H_3), 5.38, 5.43 (2s, 1H, H_1), 7.01−7.71 (m, 12H), 8.49−8.55 (m, 2H). Anal. ($C_{25}H_{21}N_5\cdot 0.5H_2O$) C, H, N.

(3*R*)-1-(*n*-Pentyl)-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline (4e). Free base **3a** (500 mg, 1.65 mmol), hexanal (395 μL, 3.30 mmol), and TFA (1.27 mL, 33.2 mmol) gave an oil. Basic workup followed by flash chromatography on silica gel using AcOEt/heptane (90:10) as the eluent afforded 320 mg (50%) of the title product as an off-white foam: diastereomeric ratio 65:35; ^{1}H NMR (DMSO-d_6) δ 0.85 (s, 3H), 1.33−1.46 (m, 6H), 1.63 (m, 1H), 2.04 (m, 1H), 2.95 (m, 2H, CH_2), 4.10 (m, 1H, H_3), 4.19 (m, 1H, H_1), 6.93−7.80 (m, 10H), 10.73 (s, 1H), 11.97 (s, 1H). Anal. ($C_{25}H_{28}N_4\cdot 0.25H_2O$) C, H, N.

(3*R*)-1-Cyclohexyl-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline Fumarate (4f). A solution of free amine **3a** (3.6 g, 12 mmol), cyclohexanecarboxaldehyde (2.68 g, 24 mmol), and TFA (9.2 mL, 120 mmol) in dichloromethane (90 mL) was stirred for 2 h at room temperature and evaporated under reduced pressure. The resulting residue was diluted in dichloromethane (100 mL), washed successively with 10% $NaHCO_3$ aqueous solution and brine, dried (Na_2SO_4), and evaporated under reduced pressure to give an oil. Flash chromatography on silica gel using AcOEt/heptane (70:30) as the eluent afforded 2.15 g (45%) of the title product as its free base (diastereomeric ratio 85:15). To the free base (1.18 g, 3 mmol) in methyl ethyl ketone (15 mL) was added fumaric acid (345 mg, 3 mmol). The mixture was warmed to 50 °C to obtain a solution. The white crystals obtained when the solution was allowed to stand overnight at room temperature were collected by filtration, washed with methyl ethyl ketone and diethyl ether, and dried to afford 1.2 g (80%) of white crystals of **4f** as its fumarate salt: mp 200−202 °C; one single diastereomer; ^{1}H NMR (DMSO-d_6) δ 1.18−1.79 (m, 10H), 2.10 (m, 1H), 2.92 (t, 1H, ^2J = ^3J = 11 Hz, H_4), 3.01 (dd, 1H, ^2J = 4 Hz, H_4), 4.12 (dd, 1H, ^3J = 11 Hz, ^3J = 4 Hz, H_3), 4.22 (s, 1H, H_1), 6.62 (s, 2H), 6.94−7.78 (m, 10H), 10.71 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 26.36, 26.83, 27.50, 29.68, 40.94, 52.96, 58.52, 108.09, 111.32, 115.17, 117.62, 118.63, 120.78, 124.55, 126.34, 127.11, 128.76, 134.21, 134.47, 135.28, 136.50, 137.85, 149.92, 166.63. Anal. ($C_{26}H_{28}N_4$, $C_4H_4O_4\cdot 0.25H_2O$) C, H, N.

Representative Procedure for Pictet−Spengler Cyclization with Ketones. Method A: Preparation of (3*R*)-1,1-Dibutyl-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-β-carboline as Its Free Base (4k) and Its Fumarate Salt (4l). A mixture of dihydrochloride **3a** (10 g, 33 mmol), 1-butanol (150 mL), and 5-nonanone (28.4 g, 165 mmol) was

refluxed for 4 h, and 10 mL of 1-butanol was then removed using a Dean−Stark apparatus. After being refluxed for a further 2 h, the mixture was heated at 100 °C overnight and then cooled to room temperature. The solvent was evaporated and the resulting residue partitioned between ethyl acetate (100 mL) and 10% $NaHCO_3$ solution (50 mL). After decantation, the organic layer was washed successively with 10% $NaHCO_3$ aqueous solution (50 mL) and water and then dried ($MgSO_4$). Evaporation of the solvent afforded a brown residue which was purified by flash chromatography on silica gel using dichloromethane/AcOEt (90:10) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after being washed with diisopropyl ether, 3.6 g (26%) of a white powder as the free base **4k** (26%): mp 160−162 °C. Anal. ($C_{28}H_{34}N_4 \cdot 1.75H_2O$) C, H, N.

The free base **4k** (1.3 g, 3 mmol) was dissolved in acetone (5 mL). Fumaric acid (448 mg, 3 mmol) was added, and the mixture was warmed to 50 °C to obtain a solution. The solution was allowed to stand overnight, upon which white crystals appeared. Diethyl ether (20 mL) was added, and the suspension was filtered to afford 1.05 g of the fumarate salt **4l** as a white solid: mp 168−170 °C; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, 3H), 0.86 (t, 3H), 1.22−1.44 (m, 8H), 1.70−1.90 (m, 4H), 2.88 (dd, 1H, ^2J = 14 Hz, ^3J = 11 Hz, H$_4$), 3.04 (dd, 1H, ^2J = 14 Hz, ^3J = 3.3 Hz, H$_{4'}$), 4.37 (m, 1H, H$_3$), 6.61 (s, 2H), 6.93−7.78 (m, 10H), 10.74 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 14.28, 22.95, 23.19, 25.52, 26.32, 27.81, 38.49, 48.90, 58.01, 106.94, 111.24, 117.87, 118.54, 120.84, 124.59, 126.38, 126.70, 128.76, 134.11, 134.50, 136.27, 138.70, 149.71, 166.69; $[\alpha]_D$ = +6.8° (*c* 1.3, DMSO). Anal. ($C_{28}H_{34}N_4$, $C_4H_4O_4 \cdot 0.75H_2O$) C, H, N.

Method B: Preparation of (3*S*)-1,1-Dibutyl-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline (4m). A solution of free base **3b** (3 g, 10 mmol), 5-nonanone (3.5 mL, 20 mmol), and TFA (7.7 mL, 100 mmol) in dichloroethane (90 mL) was refluxed for 8 h. The mixture was then cooled to room temperature and evaporated under reduced pressure. The resulting residue was partitioned between dichloromethane (100 mL) and 10% $NaHCO_3$ aqueous solution (50 mL). After decantation, the organic layer was washed successively with 10% $NaHCO_3$ aqueous solution (50 mL) and water and dried ($MgSO_4$). Evaporation of the solvent afforded a brown residue which was purified by flash chromatography on silica gel using AcOEt/heptane (70:30) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after being washed with isopentane, 1.0 g (23%) of a white powder as the free base **4m**: mp 136−138 °C; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, 3H), 0.86 (t, 3H), 1.22−1.42 (m, 8H), 1.62−1.90 (m, 4H), 2.08 (s, 1H), 2.78 (dd, 1H, ^2J = 14 Hz, ^3J = 11 Hz, H$_4$), 3.04 (dd, 1H, ^2J = 14 Hz, ^3J = 6 Hz, H$_{4'}$), 4.37 (m, 1H, H$_3$), 6.93−7.78 (m, 10H), 10.67 (s, 1H), 11.95 (s, 1H); $[\alpha]_D$ = −20.8° (*c* 1.9, MeOH). Anal. ($C_{28}H_{34}N_4 \cdot 0.25H_2O$) C, H, N.

(3*R*)-3-(4-Phenyl-1*H*-imidazol-2-yl)spiro[*β*-carboline-1,2′-adamantyl] Dihydrochloride (4g). Using method A, dihydrochloride **3a** (1.12 g, 3 mmol) and 2-adamantanone (2.25 g, 15 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using AcOEt as the eluent. The pure fractions were collected and concentrated under reduced pressure. The resulting oil was dissolved in diethyl ether, and 1 N HCl in ethyl acetate was added. The dihydrochloride was collected by filtration and washed with diethyl ether to give 0.2 g (15%) of **4g** as a white solid: mp 158−160 °C; ^{1}H NMR (DMSO-d_6) δ 1.61−2.56 (m, 13H), 2.80 (m, 1H), 3.37−3.63 (m, 2H), 3.63 (m, 1H), 7.03−8.18 (m, 10H), 10.79 (s, 1H); $[\alpha]_D$ = +19.5° (*c* 1.4, DMSO). Anal. ($C_{29}H_{30}N_4 \cdot 2HCl \cdot 0.75H_2O$) C, H, N.

2′,3′-Dihydro-(3*R*)-(4-phenyl-1*H*-imidazol-2-yl)spiro[*β*-carboline-1,2′-1*H*-indene] (4h). Using method A, dihydrochloride **3a** (1.12 g, 3 mmol) and 2-indanone (1.98 g, 15 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using AcOEt/heptane (80:20) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after crystallization in diisopropyl ether, 0.3 g (24%) of **4h** as a white solid: mp 198−200 °C; ^{1}H NMR (DMSO-d_6) δ 2.93−3.07 (m, 3H), 3.24, 3.36 (AB, 2H, J = 16 Hz), 3.73 (d, 1H, J = 16 Hz), 4.27 (m, 1H, H$_3$), 6.95−7.73 (m, 14H), 10.91 (s, 1H), 11.83 (s, 1H); $[\alpha]_D$ = +43.0° (*c* 1.4, DMSO). Anal. ($C_{28}H_{24}N_4 \cdot 0.25H_2O$) C, H, N: calcd 13.31, found 12.50.

1′-Acetyle-(3*R*)-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydrospiro[*β*-carboline-1,4′-piperidine] (4i). Using method B, free base **3a** (1.5 g, 5 mmol), *N*-acetylpiperidone (1.23 mL, 10 mmol), and TFA (3.9 mL, 50 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using dichloromethane/methanol (95:5) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after crystallization in dichloromethane/diethyl ether, 0.3 g (14%) of the title compound as a gray powder: mp 248−250 °C; ^{1}H NMR (DMSO-d_6) δ 1.63−1.93 (m, 3H), 2.06 (s, 3H, CH_3), 2.20−2.40 (m, 2H), 2.88−3.25 (m, 3H), 3.62−3.69 (m, 2H), 4.19 (m, 1H), 4.29 (m, 1H), 6.95−7.82 (m, 10H), 10.82 (s, 1H), 11.89 (s, 1H); $[\alpha]_D$ = +44.4° (*c* 1.2, DMSO). Anal. ($C_{26}H_{27}N_5O \cdot 0.5\ H_2O$) C, H, N.

(3*R*)-1,1-Diethyl-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline (4j). Using method B, free base **3a** (1.5 g, 5 mmol), 3-pentanone (1 mL, 10 mmol), and TFA (3.9 mL, 50 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using AcOEt/heptane (70:30) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after crystallization in dichloromethane and being washed with isopentane, 0.5 g (27%) of the title compound as a white powder: mp 218−220 °C; ^{1}H NMR (CD_3OD) δ 0.79 (t, 3H), 1.02 (t, 3H), 1.76−2.05 (m, 4H), 2.94 (dd, 1H, ^2J = 14 Hz, ^3J = 11 Hz, H$_4$), 3.10 (dd, 1H, ^2J = 14 Hz, ^3J = 4 Hz, H$_{4'}$), 4.47 (dd, 1H, ^3J = 11 Hz, ^3J = 4 Hz, H$_3$), 6.92−7.71 (m, 10H). Anal. ($C_{24}H_{26}N_4$) C, H, N.

(3*R*)-1,1-Dipentyl-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline (4n). Using method B, free base **3a** (1.5 g, 5 mmol), 6-undecanone (2 mL, 10 mmol), and TFA (3.9 mL, 50 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using AcOEt/heptane (70:30) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after crystallization in isopentane, 0.4 g (17%) of the title compound as a white powder: mp 170−172 °C; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, 3H), 0.86 (t, 3H), 1.12−1.51 (m, 12H), 1.62−1.88 (m, 4H), 2.09 (s, 1H, NH), 2.78 (dd, 1H, ^2J = 14 Hz, ^3J = 11 Hz, H$_4$), 3.00 (dd, 1H, ^3J = 3 Hz, ^2J = 14 Hz, H$_{4'}$), 4.26 (m, 1H, H$_3$), 6.92−7.79 (m, 10H), 10.67 (s, 1H), 11.95 (s, 1H); $[\alpha]_D$ = +37.1° (*c* 1.1, DMSO). Anal. ($C_{30}H_{38}N_4 \cdot 0.25H_2O$) C, H, N.

(3*R*)-1,1-Dihexyl-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline (4p). Using method A, dihydrochloride **3a** (1.12 g, 3 mmol) and dihexyl ketone (2.69 g, 15 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using dichloromethane/AcOEt (90:10) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after crystallization in diethyl ether, 0.2 g (14%) of **4p** as a white powder: mp 166−167 °C; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, 3H), 0.83 (t, 3H), 1.10−1.49 (m, 16H), 1.61−1.84 (m, 4H), 2.05 (s, 1H, NH), 2.77 (dd, 1H, ^2J = 14 Hz, ^3J = 11 Hz, H$_4$), 2.99 (dd, 1H, ^2J = 14 Hz, ^3J = 4 Hz, H$_{4'}$), 4.23 (m, 1H, H$_3$), 6.93−7.78 (m, 10H), 10.65 (s, 1H), 11.93 (s, 1H); $[\alpha]_D$ = +26.8° (*c* 1.6, DMSO). Anal. ($C_{32}H_{42}N_4$) C, H, N.

(3*R*)-1-Cyclohexyl-1-methyl-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline (4q). Using method B, free base **3a** (1.5 g, 5 mmol), cyclohexyl methyl ketone (1.4 mL, 10 mmol), and TFA (3.9 mL, 50 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using AcOEt/heptane (70:30) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after crystallization in diethyl ether, 0.2 g (10%) of the title compound as a white powder: mp 154−156 °C; ^{1}H NMR (DMSO-d_6) δ 0.92−1.32 (m, 6H), 1.48 (s, 3H, CH_3), 1.60 (m, 2H), 1.79 (m, 2H), 1.95 (m, 2H), 2.77

(dd, 1H, ^2J = 14 Hz, ^3J = 11 Hz, H_4), 2.97 (dd, 1H, ^2J = 14 Hz, ^3J = 4 Hz, $H_{4'}$), 4.19 (m, 1H, H_3), 6.88−7.79 (m, 10H), 10.65 (s, 1H). Anal. ($C_{27}H_{30}N_4$·0.25H_2O) C, H, N.

Benzyl 1,1-Dibutyl-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-*β*-carbolin-6-yl Ether (4r). Using method A, dihydrochloride **3c** (2.8 g, 5.8 mmol) and 5-nonanone (5 mL, 29 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using AcOEt/heptane (1:1) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after crystallization in diisopropyl ether, 230 mg (7%) of **4r** as a white powder: mp 138−140 °C; ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3H), 0.88 (t, 3H), 1.25−1.62 (m, 8H), 1.75−1.95 (m, 5H), 2.95 (dd, 1H, ^2J = 15 Hz, ^3J = 11 Hz, H_4), 3.45 (dd, 1H, ^2J = 15 Hz, ^3J = 4 Hz $H_{4'}$), 4.48 (dd, 1H, ^3J = 4 Hz, ^3J = 11 Hz, H_3), 5.12 (s, 2H), 6.91−7.73 (m, 16H). Anal. ($C_{35}H_{40}N_4O$) C, H, N.

1,1-Dibutyl-6-chloro-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline (4s). Using method A, dihydrochloride **3e** (1.2 g, 2.6 mmol) and 5-nonanone (2.23 mL, 13 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using dichloromethane/AcOEt (90:10) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after crystallization in diisopropyl ether, 500 mg (42%) of **4s** as a white powder: mp 152−154 °C; ^{1}H NMR (DMSO-d_6) δ 0.79 (t, 3H), 0.87 (t, 3H), 1.18−1.42 (m, 8H), 1.62−1.92 (m, 4H), 2.10 (s, 1H), 2.78 (dd, 1H, ^2J = 15 Hz, ^3J = 11 Hz, H_4), 2.99 (dd, 1H, ^2J = 15 Hz, ^3J = 4 Hz, $H_{4'}$), 4.25 (m, 1H, H_3), 7.0−7.79 (m, 9H), 10.91 (s, 1H), 11.95 (s, 1H). Anal. ($C_{28}H_{33}ClN_4$) C, H, N.

6-Methyl-1,1-dipentyl-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline (4t). Using method A, dihydrochloride **3d** (0.9 g, 2.3 mmol) and 6-undecanone (2.35 mL, 11.5 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using AcOEt/heptane (1:1) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after crystallization in diisopropyl ether, 0.35 g (35%) of **4t** as an off-white powder: mp 202−204 °C; ^{1}H NMR (DMSO-d_6) δ 0.79 (t, 3H), 0.84 (t, 3H), 1.16−1.48 (m, 12H), 1.63−1.85 (m, 4H), 2.35 (s, 3H), 2.79 (dd, 1H, ^2J = 15 Hz, ^3J = 11 Hz, H_4), 2.97 (dd, 1H, ^2J = 15 Hz, ^3J = 4 Hz $H_{4'}$), 3.31 (s, 1H), 4.27 (m, 1H, H_3), 6.82−7.77 (m, 9H), 10.54 (s, 1H). Anal. ($C_{31}H_{40}N_4$·0.25H_2O) C, H, N.

(3*R*)-1,1-Dibutyl-3-(4,5-diphenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline (4u). Using method A, dihydrochloride **3f** (1.30 g, 2.9 mmol) and 5-nonanone (2.5 mL, 14.5 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using dichloromethane/AcOEt (95:5) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after crystallization in diisopropyl ether, 0.6 g (38%) of **4u** as a white solid: mp 150−152 °C; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, 3H), 0.87 (t, 3H), 1.19−1.48 (m, 8H), 1.68−1.90 (m, 4H), 2.16 (s, 1H), 2.85 (dd, 1H, ^2J = 15 Hz, ^3J = 11 Hz, H_4), 3.01 (dd, 1H, ^2J = 15 Hz, ^3J = 4 Hz, $H_{4'}$), 4.25 (m, 1H, H_3), 6.94−7.51 (m, 14H), 10.70 (s, 1H), 12.20 (s, 1H); $[\alpha]_D$ = +3.4° (*c* 1.5, DMSO). Anal. ($C_{34}H_{38}N_4$·0.25H_2O) C, H, N.

(3*R*)-1,1-Dibutyl-3-(4-phenyl-1*H*-imidazol-2-yl)-1,2,3,4-tetrahydro[1]benzothieno[2,3-*c*]pyridine (10). Using method A, dihydrochloride **3g** (1 g, 2.5 mmol) and 5-nonanone (2.23 mL, 13 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using AcOEt/heptane (1:1) as the eluent. The pure fractions were collected and concentrated under reduced pressure to give, after crystallization in diisopropyl ether, 0.1 g (9%) of **10** as a white solid: mp 198−200 °C; ^{1}H NMR (DMSO-d_6) δ 0.80 (t, 3H), 0.89 (t, 3H), 1.19−1.48 (m, 8H), 1.62−1.90 (m, 4H), 2.88 (dd, 1H, ^2J = 15 Hz, ^3J = 11 Hz, H_4), 3.16 (dd, 1H, ^2J = 15 Hz, ^3J = 4 Hz, $H_{4'}$), 4.36 (m, 1H, H_3), 6.94−7.92 (m, 10H), 12.03 (s, 1H); $[\alpha]_D$ = +36.5° (*c* 1.5, DMSO). Anal. ($C_{28}H_{33}N_3S$·0.75H_2O) C, H, S, N: calcd 9.19, found 8.68.

General Procedure for *β*-Carboline Library Synthesis. A suspension of tetrahydro-*β*-carboline of structure **4** (R4 = H) (0.05 mmol) and MnO_2 (5 equiv, w/w) in chloroform was refluxed for 5 h. The mixture was cooled to room temperature and filtered on a Celite pad. The filtrate was evaporated under reduced pressure to afford the corresponding fully aromatized *β*-carboline of structure **5**.

1-Cyclohexyl-3-(4-phenyl-1*H*-imidazol-2-yl)-9*H*-*β*-carboline (5a). A suspension of free base 1-cyclohexyl-3-(4-phenyl-1*H*-imidazol-2-yl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline **4f** (300 mg, 0.75 mmol) and MnO_2 (1.8 g) in chloroform (15 mL) was refluxed for 5 h. The mixture was cooled to room temperature and filtered on a Celite pad. The filtrate was evaporated under reduced pressure to afford a solid. Flash chromatography on silica gel using AcOEt/heptane (1:1) gave, after crystallization in diisopropyl ether, 205 mg (69%) of the title compound as a white powder: mp 168−170 °C; ^{1}H NMR (DMSO-d_6) δ 1.32−1.60 (m, 3H), 1.81−2.04 (m, 7H), 3.32 (s, 1H), 7.19−8.83 (m, 11H), 11.61 (s, 1H), 12.21 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 25.90, 26.43, 31.20, 41.07, 108.50, 112.13, 114.08, 119.51, 121.60, 122.04, 124.54, 126.14, 128.17, 128.39, 133.07, 135.16, 138.19, 141.02, 147.61, 149.41. Anal. ($C_{26}H_{24}N_4$·0.25H_2O) C, H, N.

***tert*-Butyl (1*R*)-2-Amino-1-(1*H*-indol-3-ylmethyl)-2-thiooxoethylcarbamate (6).** To a reactor under 200 psi of pressure were added methyl (2*R*)-2-[(*tert*-butoxycarbonyl)amino]-3-(1*H*-indol-3-yl)propanoate (6.2 g, 22 mmol) and methanol (120 mL) saturated with NH_3. The solution was stirred at 85 °C for 24 h and then cooled to room temperature. The solvent was evaporated and the product precipitated by the addition of diisopropyl ether. Filtration gave 5.4 g (81%) of a white powder (mp 142−143 °C). To a solution of the amide (5 g, 16 mmol) in 85 mL of 1,2-dimethoxyethane were added $NaHCO_3$ (5.2 g, 62 mmol) and then P_2S_5 (7.3 g, 32 mmol) over a period of 45 min. The mixture was stirred overnight and the solvent evaporated. The resulting residue was suspended in ethyl acetate, washed successively with water and 10% $NaHCO_3$ aqueous solution, and dried ($MgSO_4$). The solvent was evaporated and the crude product precipitated by addition of isopentane/diisopropyl ether (1:1). Filtration gave 4.3 g (84%) of the title product as an off-white foam: ^{1}H NMR (DMSO-d_6) δ 1.29 (s, 9H), 2.93 (dd, 1H, ^2J = 14.5 Hz, ^3J = 11 Hz), 3.14 (dd, 1H, ^2J = 14.5 Hz, ^3J = 4 Hz), 4.47 (m, 1H), 6.72−7.64 (m, 6H), 9.17 (s, 1H), 9.62 (s, 1H), 10.81 (s, 1H).

***tert*-Butyl (1*R*)-2-(1*H*-Indol-3-yl)-1-(4-phenyl-1,3-thiazol-2-yl)ethylcarbamate (7).** A mixture of **6** (2.24 g, 7 mmol) and 2-bromoacetophenone (1.4 g, 7 mmol) was heated until complete melting (90 °C). The temperature was kept at 90 °C for 10 min and then the mixture allowed to cool to room temperature. Ethyl acetate (50 mL) and water (25 mL) were added. After decantation, the organic layer was washed with 10% $NaHCO_3$ aqueous solution and water and dried ($MgSO_4$). Evaporation of the solvent afforded a residue which was purified by flash chromatography on silica gel using dichloromethane/AcOEt (95:5) as the eluent. The pure fractions were collected and evaporated to give 1.1 g (38%) of the title compound as a foam: ^{1}H NMR (DMSO-d_6) δ 1.33 (s, 9H), 3.20 (dd, 1H, ^2J = 14.5 Hz, ^3J = 10 Hz), 3.48 (dd, 1H, ^2J = 14.5 Hz, ^3J = 4.6 Hz), 5.07 (m, 1H), 6.97−7.97 (m, 12H), 10.82 (s, 1H).

(1*R*)-2-(1*H*-Indol-3-yl)-1-(4-phenyl-1,3-thiazol-2-yl)-1-ethanamine Hydrochloride (8). To a solution of **7** (1.2 g, 2.85 mmol) in ethyl acetate (10 mL) was added 20 mL of a 1 N HCl solution in ethyl acetate. The mixture was stirred for 2 h at 20 °C and then heated to 50 °C for a further 2 h. The crystals which formed on cooling were collected by filtration and washed with diethyl ether to give an 89% yield of the title product as an orange powder: mp 170−172 °C; ^{1}H NMR (DMSO-d_6) δ 3.38 (dd, 1H, ^2J = 14.5 Hz, ^3J = 10 Hz), 3.56 (dd, 1H, ^2J = 14.5 Hz, ^3J = 5 Hz), 5.03 (dd, 1H, ^3J = 10 Hz, ^3J = 5 Hz), 6.95−8.07 (m, 11H), 8.95 (s, 3H), 11.04 (s, 1H). Anal. ($C_{19}H_{17}N_3S$·HCl·0.75H_2O) C, H, N, S: calcd 8.68, found 7.98.

(3*R*)-1,1-Dibutyl-3-(4-phenyl-1,3-thiazol-2-yl)-2,3,4,9-tetrahydro-1*H*-*β*-carboline Hydrochloride (9). Using method A, hydrochloride **8** (210 mg, 0.59 mmol) and 5-nonanone (0.52 mL, 3 mmol) gave a brown residue which was purified, after basic workup, by flash chromatography on silica gel using

dichloromethane/AcOEt (97:3) as the eluent. The pure fractions were collected and concentrated under reduced pressure. The resulting oil was dissolved in diethyl ether, and 1 N HCl in ethyl acetate was added. The hydrochloride was collected by filtration and washed with diethyl ether to give 85 mg (30%) of the title product as an orange powder: mp 134−136 °C; ^{1}H NMR (DMSO-d_6) δ 0.90 (m, 6H), 1.28−1.51 (m, 8H), 2.10−2.41 (m, 4H), 3.38 (m, 2H), 5.55 (m, 1H, H_3), 7.03−8.30 (m, 10H), 9.74 (s, 1H), 9.99 (s, 1H), 11.26 (s, 1H). Anal. ($C_{28}H_{33}N_3S$·HCl·0.25H_2O) C, H, N, S.

Human Somatostatin Receptor Expression. The genomic clones containing the human SRIF receptors (sst$_{1-5}$)[14,48−51] were kindly provided by Dr. Graeme Bell (University of Chicago). Human SRIF receptor cDNA fragments used were 1.5-kb *Pst*I−*Xmn*I, 1.7-kb *Bam*HI−*Hin*dIII, 2.0-kb *Nco*I−*Hin*dIII, 1.4-kb *Nhe*I−*Nde*I, and 1.2-kb *Hin*dIII−*Xba*I for sst$_1$, sst$_2$, sst$_3$, sst$_4$, and sst$_5$, respectively. These fragments were subcloned into the mammalian vector pCMV5 to produce expression plasmids, and clonal cell lines for each SRIF receptor subtype were obtained by transfection into CHO-K1 cells (American Type Culture Collection, ATCC, Rockville, MD) using calcium phosphate precipitation. The plasmid pRSV-neo (ATCC) was included as a cell-selectable marker. Clones that received an individual expression plasmid were selected in culture medium (RPMI 1640, Life Technologies) supplemented with 0.5 mg/mL Geneticin (G418, Life Technologies).

Human Somatostatin Receptor Binding Assay. CHO-K1 cells stably expressing each distinct SRIF receptor subtype were collected with 0.5 mM EDTA, and centrifuged at 500*g* for 5 min at 4 °C. The pellet was resuspended in 50 mM Tris, pH 7.4, and centrifuged twice at 500*g* for 5 min at 4 °C. The cells were lysed by sonication and centrifuged at 39000*g* for 10 min at 4 °C. The pellet was resuspended in the same buffer and centrifuged at 50000*g* for 10 min at 4 °C, and membranes in the resulting pellet were stored at −80 °C. An aliquot was taken for the assessment of protein content by Bradford's method.[52] Competitive inhibition experiments of [^{125}I-Tyr^{11}]-SRIF-14 (NEN Life Science Products) binding were run in polypropylene 96-well plates. Cell membranes (10−20 μg of protein/well) were incubated in 50 mM HEPES (pH 7.4), 0.2% bovine serum albumin (BSA), 5 mM $MgCl_2$, 200 KIU/mL Trasylol, 0.02 mg/mL bacitracin, and 0.02 mg/mL phenylmethanesulfonyl fluoride with 0.05−0.1 nM [^{125}I-Tyr^{11}]SRIF-14 for 50−90 min at 37 °C depending on the receptor subtype. Bound [^{125}I-Tyr^{11}]SRIF-14 was separated from free [^{125}I-Tyr^{11}]-SRIF-14 by filtration through 96-well GF/C glass fiber filter plates (Unifilter, Packard) presoaked with 0.1% (w/v) polyethylenimine, using a Packard Filtermate harvester. Filters were washed three times with 50 mM HEPES at 0−4 °C and assayed for radioactivity using a Packard Topcount scintillation counter. Specific binding was obtained by subtracting nonspecific binding (determined in the presence of 0.1 μM SRIF-14) from total binding. Binding data were analyzed by computer-assisted nonlinear regression analysis (data analysis toolbox, MDL Information Systems).

Functional Assay. CHO-K1 cells expressing the human sst$_3$ receptor were used at confluence in 96-well culture plates. The cells were washed twice with 100 μL/well of RPMI 1640 containing 0.2% BSA at 37 °C and incubated for 5 min with 1 μM isobutylmethylxanthine at 37 °C. Then, the plates were incubated for 20 min at 37 °C with 1 μM forskolin and increasing concentrations of the tested compound (measurement of agonist activity), or with 1 μM forskolin, 1 nM SRIF, and increasing concentrations of the tested compound (measurement of antagonist activity). Rapid aspiration and addition of 100 μL/well of 0.1 M HCl stopped the reaction.[53] The potency of the most potent antagonist was also assessed by performing dose−response measurements of SRIF-14 activity (concentration ranged from 1 pm to 1 μM) in the presence of fixed concentrations of the tested compound, leading to the determination of the K_B value (affinity of the antagonist expressed as the concentration that shifts the agonist dose−response 2-fold). Cyclic AMP levels were determined by radioimmunoassay using Flash Plates (NEN Life Science Products).

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