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SSTR1- and SSTR3-Selective Somatostatin Analogues

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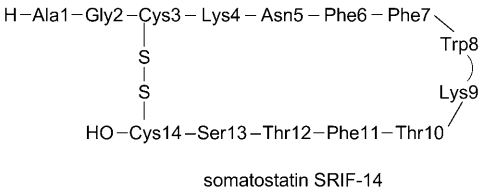
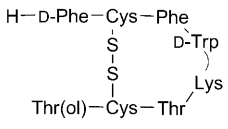
We prepared the two enantiomers of 3-(3′-quinolyl)-alanine (Qla, **1**) in multigram scale by asymmetric hydrogenation. These amino acids, protected as Fmoc derivatives, were then used in the solid-phase synthesis of two new somatostatin 14 (SRIF-14) analogues **8a** and **8b**, tetradecapeptides in which the tryptophan residue (Trp8) is replaced by one of the two enantiomers of 3-(3′-quinolyl)-alanine (Qla8) and therefore lack the N–H bond in residue 8. The selectivity of these new analogues for the somatostatin receptors, SSTR1–5, was measured. Substitution with L-Qla8 yielded peptide **8a**, which was highly selective for SSTR1 and SSTR3, with an affinity similar to that of SRIF-14. Substitution by D-Qla gave the relatively selective analogue **8b**, which showed high affinity for SSTR3 and significant affinity for SSTR1, SSTR2 and SSTR5. The biological results demonstrate that bulky and electronically poor aromatic amino acids at position 8 are compatible with strong activity with SSTR1 and SSTR3. Remarkably, these high affinity levels were achieved with peptides in which the conformational mobility was increased with respect to that of SRIF-14. This observation suggests that conformational rigidity is not required, and might be detrimental to the interaction with receptors SSTR1 and SSTR3. The absence of an indole N proton in Qla8 might also contribute to the increased flexibility observed in these analogues.

Introduction

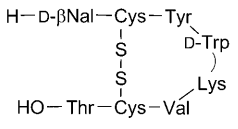
Somatostatin, a 14-residue peptidic hormone produced in the hypothalamus, was discovered in 1973 in an active form.[1] Since then other forms of various lengths have been identified,[2] thus the name "somatostatin" refers to a family of heterogeneous peptide hormones that differ in both length (from 14 to 37 residues) and amino acid composition, depending on the species. In mammals, the different hormone forms are N-terminal variations that result from differential processing of the same precursor, preprosomatostatin I.[2] Somatostatin is also known as somatotropin release-inhibiting factor (SRIF) and growth-hormone-inhibiting hormone (GHIH).



somatostatin SRIF-14



octreotide



lanreotide

SRIF-14 is involved in multiple biological functions that are mediated by direct interactions between the hormone and at least five characterized G protein-coupled receptors, SSTR1 to SSTR5.[3] These receptors differ in their tissue distribution and physiological functions. For instance, receptors SSTR2 and SSTR5 participate in the inhibition of secretion of growth hormone,[4] glucagon, and insulin.[5] SSTR3 and, to a lesser extent, SSTR2 are involved in cellular apoptosis, while SSTR1 arrests the cell cycle and regulates angiogenesis.[6]

SRIF-14 has been thoroughly studied and successfully synthesized by the pharmaceutical industry as a therapeutic drug.

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*Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbic.201000597: ^{1}H and ^{13}C NMR spectra of compounds **4a–b, 5a–b**, **6** and **7**. ^{1}H NMR TOCSY/NOESY spectra of **SOM-14, 8a** and **8b**.*

It has three main applications in clinical practice: 1) as a gastric anti-secretory drug in the treatment of esophageal varices and in acute variceal bleeding in cirrhotic patients; 2) in the treatment of growth hormone secretion disorders; and 3) in the treatment of primary thyroid-stimulating hormone (THS)-secreting pituitary tumors and neuroendocrine tumors of the gastrointestinal tract.[7] As it is a natural hormone, SRIF-14 has very low toxicity compared to synthetic drugs. However, because of its low serum stability (1–3 min half-life in human plasma)[8] it has found limited clinical use since continuous infusion is needed. This fact, coupled with its broad spectrum of biological activity, has fostered research into SRIF-14 analogues with distinct selectivity profiles and/or improved stability; but only octreotide (trade name: Sandostatin) and lanreotide (trade name: Somatuline) have reached the market.

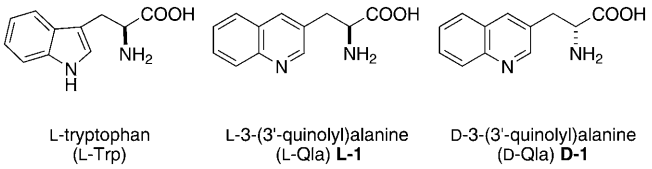
Several structural studies[9] have provided information about the conformational flexibility of SRIF-14 in solution. Although SRIF-14 shows some intrinsic flexibility, which probably accounts for its functional versatility,[10] residues Phe7-Trp8-Lys9-Thr10 constitute a conserved β-turn that is stabilized by a network of interactions between the Trp8 and Lys9 side chains and with neighboring residues.[11] The pioneering studies of Vale et al. demonstrated that a region including the turn is the pharmacophore of the hormone.[12] Therefore, attempts to develop molecules that retain these residues and the conformational structure of the hormone but of reduced size have yielded a myriad of shorter SRIF-14 analogues, including octreotide and lanreotide.[13]

Recent advances in solid-phase peptide synthesis (SPPS) have prompted us to revisit the full-length SRIF-14 structure in order to design new analogues with the aim of overcoming some of the limitations of the short-ring molecules. For instance, octreotide and its derivatives do not retain SRIF-14's recognition for all receptors. Thus a gain of stability and rigidity has come at a cost, namely a loss of function with certain receptors. A second focus in the design of full length SRIF-14 analogues concerns the use of these derivatives as tumor markers: around 80% of gastroenteropancreatic neuroendocrine tumors (GEP-NET) express SSTR receptors. SRIF-14 is known to interact with all five receptors while octreotride derivatives recognize just SSTR2 with high binding affinity, and to a lesser extent SSTR5 and SSTR3.[7d] Furthermore, tumors that express SSTRs often lose particular receptor subtypes, thus making full length SRIF-14 analogues an attractive choice as potential markers for tumor identification and monitoring their progression.[14]

We therefore centered our attention on the production of full-length SRIF-14 analogues with specificity towards some of the receptors and improved stability. Since the presence of the turn and the contacts between residues Trp8 and Lys9 are critical for this function, we focused on modifying these residues. However, the critical role of Trp8 in the formation and stabilization of the β-turn is still not fully understood, since a change in the configuration of this amino acid does not have a significant effect on the activity. Indeed, the substitution with D-Trp8 in SRIF-14 and octreotride appears simply to increase the stability of these molecules, either by favoring the proximity of the indole and aliphatic Lys9 side chain,[15] or possibly by inhibiting or delaying the recognition of these molecules by the cellular degradation machinery.

Attempts to clarify the relevance of the Trp residue to the biological activity of SRIF-14 and its analogues have been described.[16] For instance, by measuring gastric acid and pepsin inhibitory activities of several analogues of SRIF-14 that incorporated Trp8 modifications, Hirst et al. concluded that the N–H bond in the indole ring plays a crucial role.[17] However, questions remain concerning the effect of an electron-deficient heterocycle,[18] and whether the N–H bond is strictly necessary for activity with all receptors.

To further characterize these components, we set out to change the electronic properties of the heterocycle by replacing the native SRIF-14 Trp8 with 3-(3′-quinolyl)-alanine (Qla, **1**). Although Trp and Qla have similar shapes, the electronic properties of the two rings are reversed. Furthermore, the quinolyl fragment lacks the N–H bond present in the indole ring, which seems to be essential for activity because of its involvement in either binding to its receptors, or in adopting the biologically active conformation of SRIF-14).[17] To elucidate the role of the amino acid at position 8 in receptor recognition and conformational stability, we replaced Trp8 with the two 3-(3′-quinolyl)alanine enantiomers (L-**1** and D-**1**). To this end, we developed a



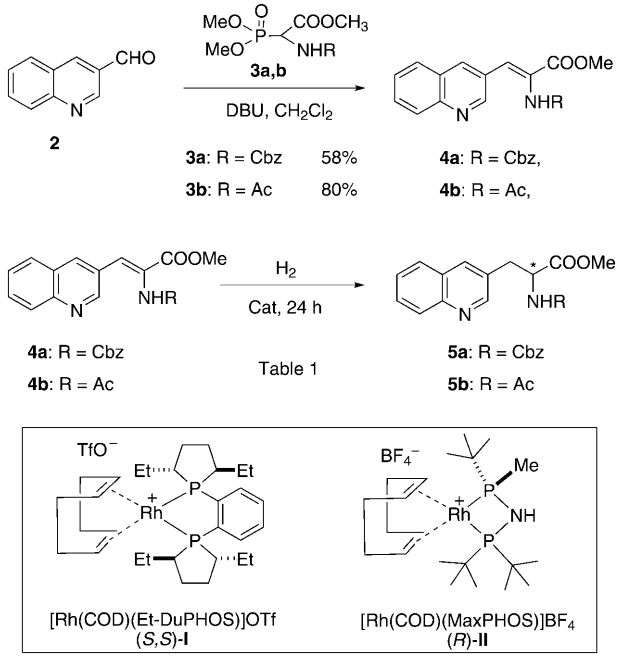
L-tryptophan (L-Trp) | L-3-(3'-quinolyl)alanine (L-Qla) **L-1** | D-3-(3'-quinolyl)alanine (D-Qla) **D-1**

new synthetic strategy to obtain these unnatural amino acids in multigram scale and high enantiomeric purity. Using NMR spectroscopy, we analyzed the conformational changes induced by the L-Qla and D-Qla substitution, and we performed receptor binding assays with membranes isolated from Chinese hamster ovary (CHO) cells that expressed each of the SSTR receptors (SSTR1–5).

Results and Discussion

Synthesis

The racemic synthesis of the unnatural amino acid 3-(3′-quinolyl)alanine[19] **1** and its enzymatic resolution[20,21] have been described previously. Nonetheless, we wished to develop an asymmetric multigram-scale synthesis method that would provide each of the two enantiomers separately. To this end, 3-quinoline carbaldehyde **2** was subjected to Horner–Emmons olefination by using **3a** and **3b,** phosphonates that differ only by the protecting groups at the nitrogen atom (Scheme 1). The resulting dehydroamino acids, **4a** and **4b,** were hydrogenated by using commercially available [Rh(COD)Et-DuPHOS]OTf (**cat-I**) as a pre-catalyst. The Cbz-protected dehydroamino acid **4a** gave low conversion both in methanol and in ethyl acetate

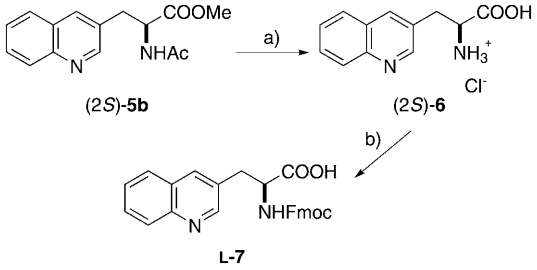


Scheme 1. Enantioselective synthesis of 3-(3′-quinolyl)alanine derivatives **5**. a) HCl, 110 °C, 100%; b) FmocOSu, Na_2CO_3, dioxane, 93%.

because of its low solubility (Table 1). Acetamido derivative **4b** also gave low conversions in ethyl acetate but its enantiomeric purity was high (96% *ee* at 25 °C). A lower enantiomeric excess was achieved when raising the temperature to increase the solubility (88% *ee* at 50 °C). However, working in methanol at room temperature the yield improved to 99%, and the enantiomeric excess was 95%. Lowering or increasing the temperature and/or pressure with **cat-I** as catalyst did not improve the enantiomeric excess. Finally, the hydrogenation was assayed with our recently developed rhodium complex (**cat-II**), which was derived from a chiral aminodiphosphine (MaxPHOS).[22] Gratifyingly, at 15 bar hydrogen with [Rh(COD)(MaxPHOS)]BF_4 (**cat-II**) as a precatalyst, the enantioselectivity improved to a noteworthy 99% *ee* (Table 1, entries 6 and 7).

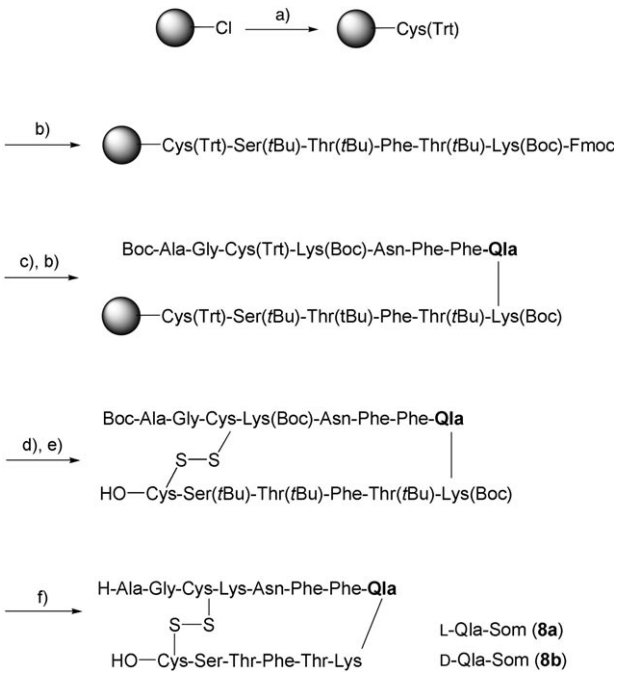
Enantiomerically enriched acetamido esters **5b** were hydrolyzed with hydrochloric acid, and the amino group was conveniently protected for use in solid-phase synthesis (Scheme 2). The Fmoc derivatives, L-**7** and D-**7**, were obtained in excellent yield under standard conditions.

The syntheses of [L-Qla8]-SRIF **8a** and [D-Qla8]-SRIF **8b** were performed by SPPS on a 2-chlorotrityl chloride resin by using



Scheme 2. Protection of (3-3′quinolyl)-alanines **5** as Fmoc derivatives **7**. a) HCl, 110 °C (100%); b) FmocOsu, Na_2CO_3, dioxane (93%).

the Fmoc/tBu, strategy (Scheme 3). The coupling of the first amino acid, Fmoc-Cys(Trt)-OH, was performed in CH_2Cl_2 in the presence of *N*-ethyldiisopropylamine (DIPEA). After coupling, the remaining free chlorides were capped with methanol. The next five Fmoc-protected amino acids (those present in the natural hormone, up to Lys9) were added by using *N*,*N*′-diisopropylcarbodiimide (DIPCDI) and 1-hydroxybenzotriazole (HOBt) in DMF, with 20% piperidine in DMF used for Fmoc removal. The resin was split in two, and L-**7** or D-**7** amino acids were incorporated into each half. The remaining protected amino acids were coupled following the natural sequence. The



Scheme 3. SPPS of somatostatin analogues. a) i: Fmoc-L-Cys(Trt)-OH (3 equiv), DIPEA (3 equiv); ii: MeOH; b) i: piperidine 20% DMF; ii: Fmoc-AA-OH (3 equiv), DIPCDI (3 equiv), HOBt (3 equiv), DMF; c) i: piperidine 20% DMF; ii: Fmoc-Qla-OH (3 equiv), DIPCDI (3 equiv), HOBt (3 equiv), DMF; d) CH_2Cl_2/TFE/AcOH; e) I_2; f) TFA/CH_2Cl_2/anisole/H_2O.

Table 1. Asymmetric hydrogenation of dehydroquinolylalanine derivatives (**4**). Reaction conditions, yields and enantiomeric excesses of compound **5**.

	SM	Catalyst[a]	Solvent	*T* [°C]	*P* [bar]	Yield [%]	*ee* [%] (conf)[b]
1	**4a**	(*S*,*S*)-**I**	MeOH	25	10	60	n.d. [c]
2	**4a**	(*S*,*S*)-**I**	AcOEt	50	5	60	n.d. [c]
3	**4b**	(*R*,*R*)-**I**	AcOEt	25	40	n.d.[c]	96.0 (*R*)
4	**4b**	(*R*,*R*)-**I**	AcOEt	50	40	n.d.[c]	88.0 (*R*)
5	**4a**	(*S*,*S*)-**I**	MeOH	25	5	99	95.0 (*S*)
6	**4b**	(*R*)-**II**	MeOH	25	25	99	98.6 (*S*)
7	**4b**	(*R*)-**II**	MeOH	25	15	99	99.0 (*S*)

n.d.: not determined. [a] 3 mol% catalyst. [b] Measured by HPLC (CHIRALPAK-IA) [c] Incomplete reaction.

disulfide bridge in the two analogues was formed in solution at room temperature after cleavage (CH_2Cl_2/TFE/AcOH) of the fully protected linear peptide from the resin with iodine. Finally, side-chain deprotection by using TFA/CH_2Cl_2/anisole/H_2O for 4 h afforded SRIF-14 analogues **8a** and **8b** respectively, in good yield (74 and 67%) and purity (95 and 84%), as assessed by RP-HPLC.

Structure

In order to examine the functional effects of the Trp8 mutations at the structural level, we analyzed the NOE pattern of SRIF-14 (trifluoroacetate counterion) and **8a** and **b** (also as trifluoroacetates) in aqueous solution (buffered at pH 6.5, 12 °C). Spin systems and sequential assignments for the two molecules were obtained from 2D TOCSY and NOESY homonuclear experiments.

Under our experimental conditions, the NMR data for SRIF-14 showed a pattern of NOEs consistent with the presence of a highly populated beta hairpin for residues 6 to 11 (Figure 1; a larger image is available in the Supporting Information). Along the backbone we observed HN–HN and HA–HN contacts across the turn. In addition, the turn was stabilized by a network of contacts involving several side chains, for example NOEs between Trp8 and Lys9 were abundant, and further stabilized by interactions between the Phe6 and Phe11 aromatic side chains. These phenylalanines also showed contacts with the tryptophan residue. Some of the contacts observed between side chains are compatible with the presence of several rotamers, as previously described for SRIF-14 under other experimental conditions.[9a]

The substitution of Trp8 with L-Qla8 (**8a**) dramatically reduced the rigidity of this molecule compared to that of SRIF-14, as reflected in differences in the intensities of several medium- and long-range contacts. Although both Phe6 and Phe11 still displayed weak NOEs with the Lys9 side chain, those corresponding to Phe11 were weaker than their Phe6–Lys9 counterparts. No NOEs were detected from L-Qla8 to the

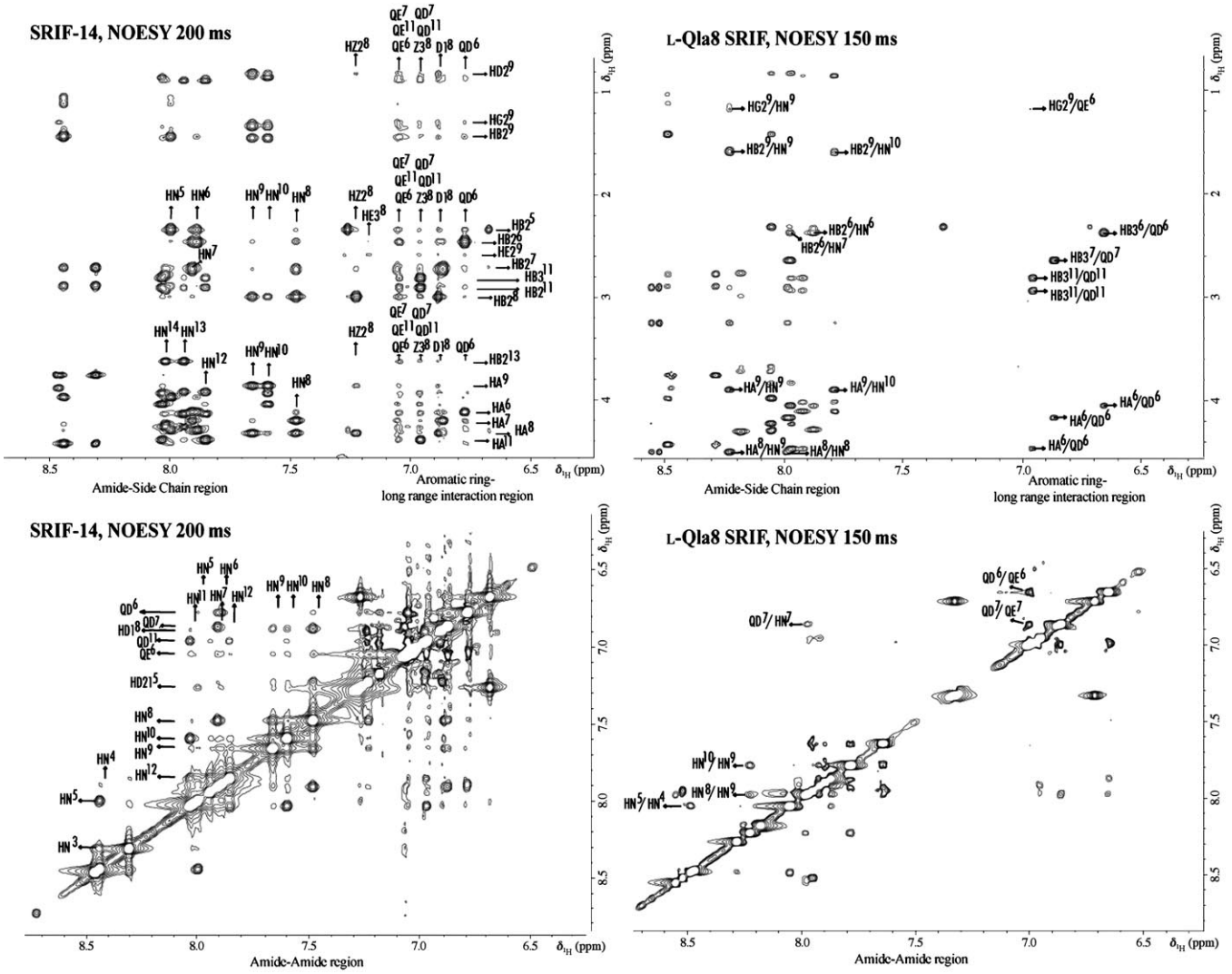


Figure 1. NOESY (600 MHz, D_2O) of SRIF-14 and [L-Qla8]-SRIF (**8a**). Characteristic NOEs in several regions of the spectrum of **8a** are assigned in order to compare them in number and intensity with those for SRIF-14. Upper left and lower left quadrants of the spectra are shown.

Lys9 side chain. The absence of this interaction was corroborated by the enhanced downfield shift of the Lys9 γ-methylene of the analogue (1.186 ppm) in comparison with SRIF-14 (0.887 ppm).[15] Also, the NOEs between Phe6 and Phe11 and from these to the L-Qla8 side chain were absent in **8a**. The lack of contacts from the L-Qla8 side chain to neighboring residues indicates that its bulky side chain is somehow forced to adopt a conformation with the side chain pointing away from the residues in the turn.

A similar situation was observed for **8b**, in which Trp8 was substituted by D-Qla8: no NOEs were detected for any of the quinoline-ring protons to those in the nearby side chains. The interactions with the Lys9 side chain, which are very strong in SRIF-14, were absent in the spectra of **8b.** This observation indicates that, independently of the configuration of Qla, the bulky and electron-poor side chain of **8a** cannot adopt a conformation that favors contacts with the neighboring residues. It is also noteworthy that interactions between the phenylalanine residues were minimal, as shown by the extremely weak NOEs between them. These two effects might contribute to the high flexibility of **8b**,[15] which is consistent with, for example, the absence of NOEs between facing residues and the disappearance of the NH–NH contacts.

Together, these features suggest that the flexibilities of **8a** and **8b** are much greater than that of SRIF-14, and that the Qla8 substitution introduces a rearrangement at the side-chain level that has a strong impact on the conformation variability of the whole molecule.

Biological activity

The receptor selectivities of **8a** and **8b** were assessed by binding assays. For comparative purposes, the same test was applied to SRIF-14, [D-Trp8]-SRIF and octreotide. Stable CHO cell lines, each of which expressed one of the five SSTR receptors, were cultured. Membrane preparations from these were used to evaluate the efficacy of the interaction with each receptor by competitive binding assays with ^{125}I-labeled and unlabeled SRIF-14 (Table 2).

The two analogues showed negligible affinity for SSTR4. Previously, Hirschmann and co-workers reported a correlation between the electrostatic potentials of various simulated substitutions at position 8 of SRIF-14, and the resulting binding to the SSTR pocket.[18] This suggested that electron-rich π systems are required for efficient interactions between residue 8 and the binding pocket of SSTR4, by means of a predicted aromatic-aromatic interaction to a residue within the receptor. A lack of affinity for SSTR4 would thus be expected for **8a** and **8b**, which contain electron-poor quinoline heterocycles at position 8, and this was indeed fully confirmed here. Analogue **8a** also had no affinity for SSTR2 and SSTR5 although, interestingly, it retained the SRIF-14 binding affinity for SSTR1 and SSTR3, with K_i values in the nanomolar range. Thus, **8a** presents a binding profile that is complementary to that of octreotide. (Octreotide has high affinity for SSTR2 and is moderately active with SSTR3 and 5, while peptide **8a** was highly active with SSTR1 and 3 and showed no affinity for SSTR2, 4 or 5.) In contrast, **8b** showed a broader binding profile. It retained the binding affinity of SRIF-14 for SSTR3, and showed a remarkable activity with SSTR1. Moreover, in contrast to **8a**, **8b** also had binding affinity for SSTR2 and 5 with K_i values in the nanomolar range (although much lower than SRIF-14). The binding profile of **8b** is quite similar to Pasireotide (SOM-230), a cyclohexapeptide developed by Novartis that has completed phase II trials.[23]

Finally, the stability in serum was measured. Half-lives in human serum of 3.4 h and 10.0 h were found for peptides **8a** and **8b**, respectively. Somewhat surprisingly, although the stabilities of these new peptides were superior to that of native SRIF-14 (2.7 h under the same conditions), they were lower than that of D-[Trp8]-SRIF-14 (19.7 h).

Conclusions

In summary, we prepared two new tetradecapeptide SRIF-14 analogues, **8a** and **8b,** in which the tryptophan residue (Trp8) was replaced with each of the two enantiomers of 3-(3′-quinolyl)alanine (Qla8) and which therefore lack the N–H bond in residue 8. The selectivity for the receptors SSTR1–5 of these new somatostatin analogues was measured. Substitution of Trp8 by L-Qla8 in SRIF-14 provided peptide **8a**, which was highly selective for receptors SSTR1 and SSTR3, with an affinity similar to that of SRIF-14. Substitution by D-Qla gave a relatively selective analogue **8b**, which showed high affinity for SSTR3 and considerable affinity for receptors SSTR1, SSTR2 and SSTR5. The biological results demonstrated that bulky and electronically poor aromatic amino acids at position 8 do not compromise strong activity with receptors SSTR1 and SSTR3. Remarkably, this was achieved in spite of the increased conformational mobility compared to that of SRIF-14. This observation suggests that conformational rigidity is not required and might even be detrimental to interaction with SSTR1 and SSTR3. The absence of the indole NH proton of Qla8 may also contribute to the flexibility increase observed in these analogues.[18]

Table 2. Affinity of **8a**, **8b**, somatostatin, [D-Trp8]-SRIF and octreotide to receptors SSTR1–5. Values are mean ± S.E.M.

	K_i [nM]				
	SSTR1	SSTR2	SSTR3	SSTR4	SSTR5
SRIF-14	0.43 ± 0.08	0.0016 ± 0.0005	0.53 ± 0.21	0.74 ± 0.07	0.23 ± 0.04
[D-Trp8]-SRIF	0.32 ± 0.11	0.001 ± 0.0007	0.61 ± 0.02	5.83 ± 0.44	0.46 ± 0.24
octreotide	300 ± 85	0.053 ± 0.011	15.2 ± 5.9	> 1000	11.53 ± 1.91
[L-Qla8]-SRIF **8a**	1.33 ± 0.60	> 1000	1.95 ± 0.58	> 1000	> 1000
[D-Qla8]-SRIF **8b**	13.66 ± 4.32	1.16 ± 0.39	0.65 ± 0.19	> 1000	14.52 ± 2.97
K_d [nM]	0.68 ± 0.10	0.09 ± 0.01	0.41 ± 0.21	0.96 ± 0.15	0.27 ± 0.005

Experimental Section

General procedures: Dry solvents were distilled before use. All other reagents were used as received. All reactions were performed with flame-dried glassware under argon. Optical rotations were measured at room temperature (23 °C) in a Perkin–Elmer 241 MC polarimeter. Infrared spectra were recorded by using an NaCl film in a Nicolet Nexus FTIR spectrometer. ^{1}H and ^{13}C NMR were recorded on a Varian Mercury 400 spectrometer. ^{1}H NMR spectra were obtained at 400 MHz with tetramethylsilane as internal standard. ^{13}C NMR spectra were obtained at 100.6 MHz in $CDCl_3$, and referenced to the solvent signal. 2D TOCSY and NOESY homonuclear experiments were performed in a Bruker Avance III spectrometer. Chemical shifts were recorded in ppm.

Methyl (*Z*)-2-benzyloxycarbonylamino-3-(3-quinolyl)-2-propenoate, 4a: 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU; 0.57 mL, 3.82 mmol) was added dropwise to a solution of methyl (±)-*Z*-α-phosphonoglycine trimethyl (1.27 g, 3.82 mmol) in CH_2Cl_2 (10 mL). After 10 min of stirring at room temperature, a solution of quinolyl-3-carboxaldehyde (500.0 mg, 3.18 mmol) in CH_2Cl_2 (8 mL) was added. After 2 h of stirring at room temperature, the reaction was complete. The solvent was removed at reduced pressure, and the residue was diluted with EtOAc (23 mL). The organic layer was washed with 1 M HCl and brine, and then dried and evaporated. The crude product was purified by chromatography (SiO_2/NEt_3 2.5% *v/v*) with hexanes/AcOEt mixtures to afford 580.0 mg of **4a** (50% yield) as a white solid. M.p. 118–120 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.02 (s, 1H), 8.20 (s, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.65–7.76 (m, 2H), 7.53 (t, J = 7.2, 7.4 Hz, 2H), 7.30 (s, 5H), 6.81 (s, 1H), 5.09 (s, 2H), 3.87 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 165.6, 164.3, 153.6, 151.2, 141.2, 136.8, 135.9, 135.8, 135.3, 130.7, 129.4, 128.8, 128.7, 128.6, 128.5, 127.7, 127.3, 125.7, 120.2, 68.0, 53.2 ppm; IR (film): ν_{max} = 3295(b), 2904, 1721, 1260 cm^{-1}; MS (ESI) *m/z* (%): 363.0 (100) $[M+H]^+$; elemental analysis calcd (%) for $C_{21}H_{18}N_2O_4$: C 69.60, H 5.01, N 7.73; found: C 69.30, H 5.09, N 7.77.

Methyl (*Z*)-2-acetamide-3-(3-quinolyl)-2-propenoate, 4b: DBU (3.20 mL, 21.00 mmol) was addded drop-wise to a solution of methyl-2-*N*-(acetylamino)dimethylphosphonoacetate (4.35 g, 18.21 mmol) in CH_2Cl_2 (10 mL). After 30 min of stirring at room temperature, a solution of quinolyl-3-carboxaldehyde (2.20 g, 14.00 mmol) in CH_2Cl_2 (8 mL) was added. After 3 h of stirring at room temperature, the reaction was complete. The solvent was removed at reduced pressure, and the residue was diluted with EtOAc (23 mL). The organic layer was washed with 1 M HCl and brine, then dried and evaporated. The crude product was purified by chromatography (SiO_2/NEt_3 2.5% *v/v*) with hexanes/AcOEt to afford 2.90 g of **4b** (80% yield) as a white solid. M.p. 165–168 °C. ^{1}H NMR (400 MHz, CD_3OD): δ = 8.99 (s, 1H; CH Ar), 8.49 (s, 1H; CH Ar), 7.96–8.02 (m, 2H; CH Ar), 7.80–7.81 (m, 1H; CH Ar), 7.61–7.67 (m, 1H; CH Ar), 7.57 (s, 1H; CHβ), 3.86 (s, 3H; CH_3), 2.14 ppm (s, 3H; CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ = 171.8 (CO), 165.5 (CO), 150.4 (CH), 147.0 (C), 137.6 (CH), 131.0 (CH), 129.3 (CH), 128.7 (CH), 128.0 (C), 127.8 (CH), 127.7 (C), 127.6 (CH), 127.6 (C), 48.5 (CH_3), 21.4 ppm (CH_3); IR (film): ν_{max} = 3244(b), 1718, 1652, 1283 cm^{-1}; MS (ESI) *m/z* (%): 271.1 (100) $[M+H]^+$; HRMS: calcd for $C_{15}H_{15}N_2O_3$: 271.1077 $[M+H]^+$, found: 271.1082; elemental analysis calcd (%) for $C_{15}H_{14}N_2O_3$: C 66.66, H 5.22, N 10.36; found: C 66.14, H 5.08, N 10.15.

Methyl (2*S*)-2-benzyloxycarbonylamino-3-(3-quinolyl)-propanoate, (2*S*)-5a: (*S,S*)-[[(COD)Et-DuPHOS]Rh^I]OTf (9.7 mg, 0.01 mmol) was added to a solution of dehydro amino acid **4a** (500.0 mg,1.37 mmol) in MeOH (200 mL). The reaction mixture was purged and pressurized to 7 bar with hydrogen. After four days at room temperature the reaction mixture was purged with nitrogen, the solvent was then evaporated, and the resulting crude product was purified by chromatography (SiO_2/NEt_3 2.5% *v/v*) with hexanes/AcOEt to afford 0.20 g of (2*S*)-**5a** as a white solid and 0.17 g of starting material **4a** (34% conversion, 40% yield). $[\alpha]_D$ = +10.1 (c = 0.50, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.68 (s, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.89 (s, 1H), 7.64–7.77 (m, 2H), 7.53 (t, J = 7.2, 7.4 Hz, 1H), 7.32 (s, 5H), 5.47 (d, J = 6.8 Hz, 1H), 5.10 (s, 2H), 4.76 (q, J = 7.2, 6.6 Hz, 1H), 3.73 (s, 3H), 3.21–3.41 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 171.7, 155.9, 151.8, 147.4, 136.3, 129.6, 129.4, 129.1, 128.8, 128.5, 128.3, 128.1, 127.8, 127.1, 67.3, 54.9, 52.8, 35.9 ppm; IR (film): ν_{max} = 3328(b), 2925, 1717, 1215, 1056 cm^{-1}; MS (CI-NH_3) *m/z* (%): 365.1 (100) $[M+H]^+$; HRMS: calcd for $C_{21}H_{21}N_2O_4$: 365.1496 $[M+H]^+$, found: 365.1489.

Methyl (2*S*)-2- acetamide-3-(3-quinolyl)-propanoate, (2*S*)-5b

Procedure A: (*S,S*)-[[(COD)Et-DuPHOS]Rh^I]OTf (7.33 mg, 0.01 mmol) was added to a solution of dehydro amino acid **4b** (100 mg, 0.37 mmol) in MeOH (6 mL). The reaction mixture was purged and pressurized with hydrogen to 5 bar. After 24 h of stirring at room temperature, the reaction mixture was purged with nitrogen, the solvent was evaporated, and the resulting crude product was purified by chromatography (SiO_2/NEt_3 2.5%, *v/v*) with hexanes/AcOEt to afford 99 mg of (2*S*)-**5b** as a white solid (99% conversion, 99% yield, *ee* > 95%).

Procedure B: (*R*)-[Rh(COD)(MaxPHOS)]BF_4 (6.25 mg, 0.011 mmol) was added to a solution of the dehydro amino acid **4b** (100 mg, 0.37 mmol) in MeOH (12 mL). The reaction was carried out as described above but pressurized to 25 or 15 bar. (2*S*)-**5b** was obtained in 99% yield with an enantiomeric purity of 98.6%.

$[\alpha]_D$ = +104.5 (c = 1.00, $CHCl_3$); m.p. 120 °C; ^{1}H NMR (400 MHz, CD_3OD): δ = 8.72 (s, 1H; CH Ar), 8.19 (s, 1H; CH Ar), 7.99 (d, J = 8.4 Hz, 1H; CH Ar), 7.90 (d, J = 8.0 Hz, 1H; CH Ar), 7.74 (t, J = 7.2, 8.4 Hz, 1H; CH Ar), 7.60 (t, J = 7.2, 8.4 Hz, 1H; CH Ar), 5.48 (s, 1H; NH), 4.80–4.82 (m, 1H; CHα), 3.71 (s, 3H; CH_3), 3.37–3.44 (m, 1H; CH_2 (CHβ)), 3.12–3.23 (m, 1H; CH_2 (CH_2β)), 1.89 ppm (s, 3H; CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ = 171.9 (CO), 171.8 (CO), 151.4 (CH), 146.5 (C), 136.8 (CH), 130.6 (C), 129.7 (CH), 128.4 (C), 127.8 (CH), 127.7 (CH), 127.1 (CH), 53.5 (CH_3) , 51.7 (CH), 34.5 (CH_2), 21.0 ppm (CH_3); IR (film):ν_{max} = 3274(b), 3033, 2948, 1744, 1658, 1541 cm^{-1}; MS (CI-NH_3) *m/z* (%): 273.1 (100) $[M+H]^+$; HRMS: calcd for $C_{15}H_{17}N_2O_3$: 273.1233 $[M+H]^+$, found: 273.1239; elemental analysis calcd (%) for $C_{15}H_{16}N_2O_3$: C 66.16, H 5.92, N 10.29; found: C 65.92, H 5.94, N 9.91; HPLC (CHIRALPAK-IA, heptane/EtOH 70:30, 0.5 mL min^{-1}, λ = 254 nm, t_R(*S*) = 24 min, t_R(*R*) = 29 min).

(2*S*)-2-amino-3-(3-quinolyl)-propanoic acid, (2*S*)-6: A solution of (2*S*)-**5b** (1.30 g, 4.78 mmol) in concentrated HCl (32 mL) was heated under reflux for 6 h. After cooling, the solution was concentrated under reduced pressure to furnish 1.20 g of (2*S*)-**6** (quantitative yield) as a white solid. $[\alpha]_D$ = +13.8 (c = 0.50, D_2O); m.p. 160 °C; ^{1}H NMR (400 MHz, D_2O): δ = 8.97 (s, 2H; CH Ar), 8.00–8.21 (m, 3H; CH Ar), 7.86 (t, J = 7.6, 8.0 Hz, 1H; CH Ar), 4.16–4.26 (m, 1H; CHα), 3.51 ppm (t, J = 4.4, 5.4 Hz, 2H; CH_2 (CH_2β)); ^{13}C NMR (100 MHz, D_2O): δ = 171.1 (CO), 148.1 (CH), 144.9 (C), 136.9 (C), 135.6 (CH), 130.7 (CH), 129.5 (CH), 129.2 (CH), 129.1 (CH), 129.0 (C), 120.2 (CH), 53.7(CH_3), 32.9 ppm (CH_2); IR (film): ν_{max} = 3264(b), 1720, 1530 cm^{-1}; MS (CI-NH_3) *m/z* (%): 217.1 (100) $[M+H]^+$; HRMS: calcd for $C_{12}H_{13}N_2O_2$: 217.0899 $[M+H]^+$, found: 217.0971; elemental analysis calcd (%) for $C_{12}H_{21}ClN_2O_6$: C 44.38, H 6.02, N 8.63; found: C 43.95, H 5.54, N 8.28.

***N*-Fmoc-L-3-(3-quinolyl)-alanine, L-7:** A solution of FmocOsu (2.72 g 8.07 mmol) in dioxane (25 mL) was added to a suspension of (2*S*)-**6** (1.36 g, 5.38 mmol) in 10% Na_2CO_3 (25 mL) at 0 °C. The mixture was stirred for 48 h at room temperature. Water (40 mL) was then added and the mixture was extracted with hexanes (3× 20 mL). The remaining aqueous phase was cooled and adjusted to pH 2 with 1 M hydrochloric acid and then extracted with ethyl acetate (3×20 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to yield 2.20 g of L-**7** (93% yield) as a white solid. $[\alpha]_D = +56.0$ ($c = 1.00$, $CHCl_3$); m.p. 146–148 °C. ^{1}H NMR (400 MHz, DMSO): $\delta = 8.96$ (s, 1H; CH), 8.34 (s, 1H; CH), 8.08 (d, $J = 8.3$ Hz, 1H; CH), 7.94 (d, $J = 8.6$ Hz, 1H; CH), 7.87 (d, $J = 7.5$ Hz, 2H; CH), 7.78 (t, $J = 7.4$ Hz, 1H; CH), 7.62 (m, 3H; CH), 7.39 (dd, $J =$ 13.6, 6.9 Hz, 2H), 7.28 (t, $J = 7.3$ Hz, 1H), 7.20 (t, $J = 7.4$ Hz, 1H), 4.40 (m, 1H; CHα), 4.19 (m, 3H; CH, CH_2), 3.41 (dd, $J = 4.2$, 13.9 Hz, 1H; $CH_2\beta$), 3.15 ppm (m, 1H; $CH_2\beta$); ^{13}C NMR (100 MHz, DMSO): $\delta =$ 173.6 (CO), 173.4 (CO),156.7 (C), 152.2 (CH), 146.1 (C), 144.3 (C), 144.2 (C), 141.3(2C), 137.1 (CH), 131.9 (C), 130.1 (CH), 128.5 (2CH), 128.3 (2CH), 127.7 (2CH), 127.5 (CH), 125.9 (CH), 125.8 (CH), 120.7 (2CH), 66.6 (CH_2), 55.8 (CHα), 47.2 ppm (CH), 34.5($CH_2\beta$); IR (film): ν_{max} 3319 (b), 3010, 1715, 1441 cm^{-1}; MS (CI-NH_3) *m/z* (%): 439.2 (100) $[M+H]^+$; HRMS: calcd for $C_{27}H_{23}N_2O_4$: 439.1658 $[M+H]^+$, found: 439.1647.

General procedure for the synthesis of peptides L-8 and D-8: The syntheses were performed by SPPS on a 2-Cl-Trt resin (1.60 mmol g^{-1}) by using the Fmoc/*t*Bu, strategy. The first amino acid, Fmoc-L-Cys-OH, (3 equiv) was coupled for 2 h in the presence of DIPEA (6 equiv) in CH_2Cl_2 as solvent, then end-capped with methanol (0.8 mL g^{-1}). Fmoc removal was then performed by treating the peptidyl resin with 20% piperidine in DMF (2×15 min). The second amino acid Fmoc-Ser-OH (3 equiv) was coupled using DIPCDI (3 equiv) and HOBt (3 equiv), as activating reagents, in DMF for 1–2 h. The Kaiser test was used to check for coupling completion.[24] This procedure was repeated for the following 11 Fmoc-protected amino acids and for the final Boc-Ala-OH. The fully protected linear peptide was then cleaved from the resin by a cleavage cocktail (CH_2Cl_2/TFE/AcOH, 16:5:2 (*v/v*)) for 2 h. The formation of the disulfide bridge in each of the two analogues was achieved by using iodine (4 equiv) in solution at room temperature for 30 min, then the reaction was quenched with an aqueous solution of sodium thiosulfate (1 N). The aqueous layer was extracted with CH_2Cl_2 (3×150 mL) and the combined organic layer was washed with a mixture of 5% aqueous citric acid and 5% sodium chloride (1:1) and evaporated under reduced pressure. Finally, total deprotection of the side chains was performed by using an acidic mixture (TFA/CH_2Cl_2/anisole/H_2O 12:6:2:1, *v/v*) for 4 h, and the remaining solution was then washed with heptane (5 mL) and the aqueous layer was precipitated in Et_2O (−10 °C) to afford SRIF-14 analogues **8a** and **8b**.

[L-Qla8]-Somatostatin, 8a: SRIF-14 analogue **[L-Qla8]-SRIF** was synthesized from 0.33 g of 2-Cl-Trt resin (1.60 mmol g^{-1}) following the general procedure and with Fmoc-L-Qla-OH, L-**7**, affording 0.43 g of **8a** in 74% yield and 95% purity. NMR: see Table 3; HPLC: $t_R = 8.32$ min [gradient from 25% to 60% of B in 20 min (A: H_2O-HCOOH (99.9: 0.1), B: ACN-HCOOH (99.3:0.07)), flow: 2 mL min^{-1}, $\lambda = 220$ nm]; HRMS: calcd for $C_{77}H_{106}N_{18}O_{19}S_2$: 825.3583; found: 825.6195.

[D-Qla8]-Somatostatin, 8b: SRIF-14 analogue [D-Qla8]-SRIF was synthesized from 2-Cl-Trt resin (0.33 g, 1.60 mmol g^{-1}) following the general procedure and with Fmoc-D-Qla-OH, D-**7**, affording 0.42 g of **8a** in 67% yield and 84% purity. NMR: see Table 4; HPLC: $t_R = 8.94$ min [gradient from 25% to 60% of B in 20 min (A: H_2O-HCOOH (99.9: 0.1), B: ACN-HCOOH (99.3:0.07)), flow: 2 mL min^{-1}, $\lambda = 220$ nm]; HRMS: calcd for $C_{77}H_{106}N_{18}O_{19}S_2$: 825.3583; found: 825.6195.

Table 3. NMR data for **8a** from ^{1}H NMR, TOCSY, NOESY (D_2O, 600 MHz).

	Chemical shift (ppm)			
	H^N	H^α	H^β	other
1 Ala	7.894	3.898	1.291	
2 Gly	8.466	3.760		
3 Cys	8.284	4.430	2.841	
4 Lys	8.484	3.989	1.393	H^γ 1.087, H^δ 1.038
5 Asn	8.051	4.292	2.321	H^γ 7.021
6 Phe	7.871	4.060	2.385	H^γ 6.656, H^δ 6.995
7 Phe	7.973	4.184	2.634	H^D 6.861, H^E 6.995
8 Qla	7.988	4500	3.081	H^{D1} 8.560, H^{D2} 8.525, H^{H3} 6.972, H^{H2} 6.892, H^{E3} 7.895
9 Lys	8.226	3.903	1.591	H^γ 1.186, H^δ 1.387
10 Thr	7.785	4.107	4.013	$H^{\gamma1}$ 0.851, $H^{\gamma2}$ 0.870
11 Phe	7.919	4.473	2.809	H^D 6.952, H^E 7.000, H^Z 0838
12 Thr	7.966	3.989	3.836	
13 Ser	8.057	4.299	3.710	
14 Cys	8.1754	4.323	2.886	

Table 4. NMR data for **8b** from ^{1}H NMR, TOCSY, NOESY (D_2O, 600 MHz).

	Chemical shift (ppm)			
	H^N	H^α	H^β	other
1 Ala	7.906	3.898	1.305	
2 Gly	8.479	3.764		
3 Cys	8.295	4.433	2.849	
4 Lys	8.400	4.279	1.418	H^δ 1.274, H^E 2.596
5 Asn	8.287	4.528	2.450	H^γ 7.060
6 Phe	8.155	4.341	2.644	H^δ 6.722, H^E 6.998
7 Phe	8.050	4.191	2.648	H^D 6.798, H^E 6.610
8 Qla	8.448	4.433	3.022	H^{D1} 8.609, H^{D2} 8.505, H^{Z3} 7.714, H^{H2} 7.898, H^{E3} 7.961
9 Lys	8.344	4.100	1.213	H^γ 0.752, H^δ 1.485, H^E 2.462
10 Thr	7.815	4.161	3.911	$H^{\gamma1}$ 0.889
11 Phe	8.150	4.637	2.734	H^D 6.930, H^E 7.068
12 Thr	8.133	4.180	3.972	H^γ 0.928
13 Ser	8.044	4.301	3.669	
14 Cys	8.077	4.293	2.929	

Biological assays

Preparation of cells stably expressing each SRIF-14 receptor: CHO-K1 cells (American Type Culture Collection, Rockville, MD) were maintained in Kaighn's modification of Ham's F12 medium (F12K) supplemented with 10% fetal bovine serum. pcDNA3 vectors encoding each of the SSTR receptors were obtained from UMR cDNA Resource Center (University of Missouri–Rolla, MO, USA). CHO-K1 cells were stably transfected with these vectors by using Lipofectamine (Invitrogen). Colonies that were viable in F12K containing the antibiotic G418 (700 μg mL^{-1}) were screened for SRIF-14 receptor expression, and then maintained in a G418 (400 μg mL^{-1})-containing medium. Expression was detected by RT-PCR and Western blot, and confirmed by radio ligand binding assay.

Receptor ligand-binding assay: All receptor-binding assays were performed with membranes isolated from CHO-K1 cells expressing the cloned human SRIF-14 receptors, as previously described.[25] The assay buffer consisted of Tris (50 mM, pH 7.5) with EGTA (1 nM), $MgCl_2$ (5 mM), leupeptin (10 μg mL^{-1}), pepstatin (10 μg mL^{-1}), bacitracin (200 μg mL^{-1}), aprotinin (0.5 μg mL^{-1}), and BSA (0.2%). CHO-

K1 cell membranes, radiolabeled SRIF-14, and unlabeled test compounds were suspended/diluted in the buffer. Assays (200 μL) were performed in 96-well polypropylene plates. Ten micrograms of membrane protein were incubated (1 h, 30 °C) with ^{125}I-Tyr11-SRIF (0.1 nM, 2000 Ci mmol^{-1}) in the presence or absence of various concentrations of unlabeled peptides (1 pM–1000 nM). The binding reaction was terminated by vacuum filtration over Whatman GF/F glassfiber filters, pre-soaked in 0.5% (*w/v*) polyethyleneimine and 0.2% bovine serum albumin, by using a 98-well harvester (Inotech). The filters were washed with ice-cold Tris-HCl (50 mM, pH 7.5) and dried, after which scintillator sheets were adhered to the filter and the bound radioactivity was analyzed in a liquid scintillation counter (microβ plus, Wallac). Specific binding was defined as total bound ^{125}I-Tyr11-SRIF minus the amount bound in the presence of 1000 nM SRIF (nonspecific binding). Inhibition curves were analyzed, and IC_{50} values were calculated by using a curve-fitting program ("Prism", GraphPad, La Jolla, CA). K_i values were determined as described by Cheng and Prusoff.[26] Data are the mean ± S.E.M. of at least three separate experiments, each performed in triplicate.

Serum stability assay: A peptide solution of 6 mg mL^{-1} in water (1.8 mg in 300 μL) was sterilized by filtration (0.22 μm filter). 10 μL Aliquots from this solution were added to 90 μL serum (human male AB plasma, sterile filtered; SIGMA). These solutions were incubated at 35 °C and samples were taken at 0, 1, 7, 17, 24 and 48 h. Each sample was treated with acetonitrile (200 μL) and cooled to 0 °C for 30 min to precipitate the proteins. The suspensions were centrifuged (10000 rpm, 10 min, 4 °C). This procedure was repeated twice. Solutions were filtered (0.45 μm PVDF), and analyzed by RP-HPLC (eluent: 20–80% B (B = 0.07%TFA in acetonitrile); 20 min gradient; flow: 1 mL min^{-1}). For each peptide the experiment was repeated twice. The half-life of the peptide in serum was calculated from the analysis of these degradation data.

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