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Optimization of a Somatostatin Mimetic via Constrained Amino Acid and Backbone Incorporation

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Abstract—Constrained analogues **5**–**7** of the potent and subtype selective somatostatin mimetic **1** were prepared by incorporating conformational constraints into the nine-membered heterocyclic scaffold. Each constrained peptidomimetic showed an altered activity profile relative to lead compound **1**, with compound **7** exhibiting a 25-fold and 2-fold binding enhancement against somatostatin receptor subtypes sst4 and sst5, respectively.

Although peptides and proteins mediate numerous biological processes, their use as therapeutic agents and biological probes is often accompanied by several drawbacks, including poor biostability, poor receptor subtype selectivity, and unfavorable absorption properties.[1] Consequently, much effort has been dedicated to the design and synthesis of selective, high-affinity ligands by the display of appropriate amino acid side chains upon peptidomimetic scaffolds.[2,3] Previously we reported the first successful design and implementation of a β-turn mimetic scaffold that displays side chains corresponding to the $i+1$, $i+2$, and $i+3$ positions of the β-turn structure, and that is amenable to the rapid and parallel solid-phase synthesis of multiple derivatives in high yield.[4] We have since demonstrated the efficacy of this scaffold for producing small molecule ligands to several distinct receptor targets.[4b,5]

In one effort we targeted the human somatostatin receptors 1–5.[4b] The endogenous peptide somatostatin (SRIF) is a cyclic tetradecapeptide that has attracted considerable attention as a potential therapeutic agent. SRIF modulates the secretion of several hormones, including growth hormone, insulin, and glucagon.[6] However, the very short biological half-life and lack of subtype specificity of SRIF necessitates that longer acting and more selective small molecule mimetics be developed, and the identification of potent and selective non-peptidic sst4[7a] and sst5[7b] ligands has only recently been achieved. To address this problem, we synthesized and evaluated a collection of somatostatin mimetics based on our β-turn scaffold resulting in the identification of the selective and potent sst5 ligand **1** ($IC_{50} = 87$ nM) as shown in Figure 1.[4b] Herein we report a study to further enhance potency and selectivity of **1** by introducing conformational constraints into the flexible nine-membered heterocyclic scaffold.

The lysine side chain in mimetic **1** (Fig. 1) presented an opportunistic position for the incorporation of constrained amino acids. Since both proline and pipecolic acid could be used effectively in the solid-phase synthesis sequence, constrained lysine analogues of these amino acids (Fig. 2) were selected. Ligand **1** possesses the unnatural D stereochemistry at the $i+2$ position, therefore the five-membered lysine analogues **2** and **3** retained this stereochemistry at the α-carbon, but differed in the stereochemistry at the 4-position. It was hoped that this variable would probe the conformational preference of the side chain while locking the heterocyclic backbone. The six-membered lysine derivative **4** was also designed to retain the unnatural D stereochemistry at the α-carbon. In addition, the side chain would be oriented in a manner *cis* to the carboxylate in order to 'tie back' the primary amine. The three constrained lysine surrogates were synthesized according to literature methods with only minor modifications,[8] and were then incorporated into the protected turn mimetics according to our previously reported solid-phase synthesis sequence.[4] Standard functional group

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manipulation/global deprotection afforded the desired mimetics **5**, **6**, and **7** (Fig. 3), which were then purified by preparative HPLC and characterized prior to biological evaluation.[9]

The flexible alkanethiol backbone provided an additional site for introducing conformational constraints. To assess the efficacy of backbone constraints, we designed a solution-phase synthesis that employed the aromatic disulfide **8**. This backbone should constrain the heterocyclic mimetic while simultaneously introducing an additional hydrophobic contact. Notably, SRIF and potent cyclic peptide analogues of SRIF require hydrophobic residues at the entering i residue of their corresponding β-turn regions.[10] Constrained mimetic **10** was synthesized according to Scheme 1 and purified by preparative HPLC prior to biological evaluation.[9]

The four individual mimetics were then tested at a concentration of 1 μM in a competitive radioligand binding assay ({^{125}I}-{Tyr11}-SRIF) in membranes from CHO-K1 cells expressing human sst1–sst5. As displayed in Table 1, control compound **1** showed complete inhibition of SRIF binding to sst5 at 1 μM, while compounds **5** and **6** inhibited binding to sst5 at 50 and 56%, respectively. Although both second-generation mimetics exhibited considerably reduced levels of inhibition, it is interesting to note that the highest inhibitory value for sst5 was conserved. Bicyclic compound **7** exhibited near-complete and complete inhibition of sst4 and sst5, respectively, while showing moderate to low inhibition for the remaining subtypes. Finally, the backbone-constrained heterocycle **10** possessed modest activity for the various subtypes.

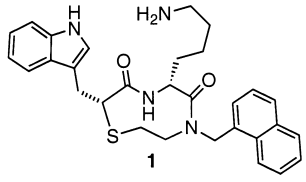


Figure 1. sst5 selective turn mimetic.

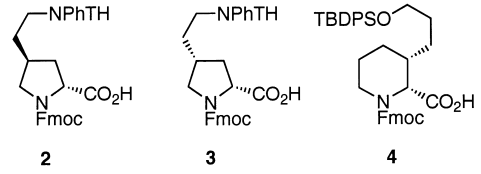


Figure 2. Constrained amino acids.

Since compound **7** showed very promising inhibition data, IC_{50} values of this compound were determined and compared to somatostatin for each receptor subtype (Table 2). The inhibitory constants of **7** for sst4 and sst5 are both 41 nM. The dual activity and selectivity for these subtypes over the other receptor subtypes is quite interesting given that these two receptors come from distinct groups.[6] Furthermore, the IC_{50} value of 41 nM is a 2-fold enhancement over compound **1** for sst5, but over 25-fold for sst4. Thus, while the constrained amino acid clearly imparts enhanced potency on mimetic **7** for the two subtypes, it has the effect of lowering the selectivity for sst5 over the non-homologous sst4 when compared to the parent compound **1**.

In summary, we have synthesized and evaluated a small collection of constrained second-generation mimetics based on lead SRIF mimetic **1**. Incorporation of the three constrained amino acids and aromatic disulfide backbone into the flexible synthetic sequence provided compounds with considerably altered biological profiles towards the somatostatin receptor subtypes 1–5. Furthermore, this effort resulted in the identification of the bicyclic mimetic **7** as a more potent sst5 ligand than parent compound **1**. Further optimization of the constrained SRIF mimetic will be reported in due course.

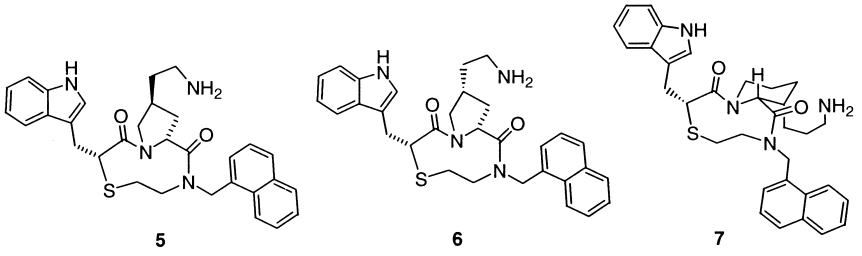
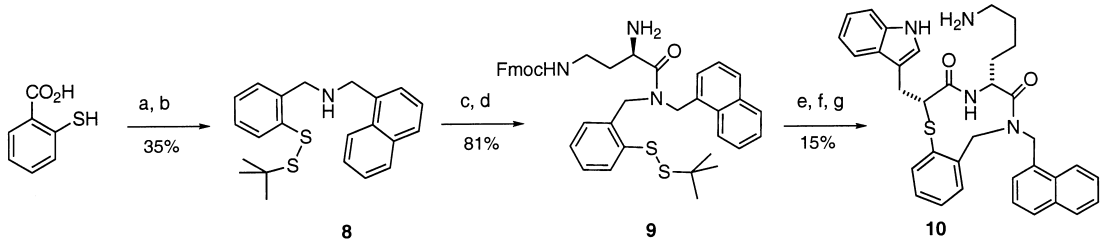


Figure 3. Second-generation constrained mimetics.



Scheme 1. Reagents. (a) BH_3, 0–25 °C; (b) (i) Dithiobisbenzothiazole, $CHCl_3$, then *t*-BuSH; (ii) Ms_2O, pyridine, CH_2Cl_2, then 1-napthylmethylamine; (c) Pybop, HOAt, Fmoc-D-Lys-(*N*-Phth)-OH, *i*-Pr_2EtN, DMF; (d) piperidine/CH_2Cl_2; (e) EDC, HOBt, α-Br-Trp-(*N*-Boc)-OH; (f) *i*-Bu_3P, 9:1 dioxane/H_2O, then TMG, 55 °C; (g) H_2NNH_2, EtOH, reflux.

Table 1. % Inhibition at 1 μM[a]

Compd	sst1	sst2	sst3	sst4	sst5
1	64	33	42	43	99
5	28	20	16	17	50
6	13	7	19	12	56
7	64	74	44	99	89
10	36	7	13	41	64

[a]Cell membranes (2–30 μg protein) were incubated with 0.03 nM [^{125}I]-[Tyr^{11}]-SRIF and increasing concentrations of SRIF or SRIF analogues for 90 min at rt. Nonspecific binding was defined with 1 μM SRIF. The assay was terminated by rapid filtration through Whatman GF/C glass fibre filters soaked in 0.5% polyethylenimine (PEI), followed by 3×3 mL washes of 50 mM Tris–HCl pH 7.4 containing 5 mg/mL bovine serum albumin (BSA). Radioactivity in the filters was determined using a Canberra Packard Cobra II Auto-© counter.

Table 2. IC_{50} values of compounds **1**, **7**, and **SRIF** (nM)[a]

Compd	sst1	sst2	sst3	sst4	sst5
SRIF	0.65	0.07	0.72	1.78	0.56
1	501	1585	3090	1047	87
7	407	275	1258	41	41

[a]See conditions for Table 1.

Acknowledgements

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9. (**5**) ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.83–7.77 (m, 3H), 7.59 (m, 1), 7.46 (m, 2H), 7.36 (d, J = 5.4 Hz, 2H), 7.28 (m, 1H), 7.12 (s, 1H), 7.07–6.92 (m, 2H), 5.78 (d, J = 13.7 Hz, 1H), 5.32 (m, 1H), 4.24 (m, 1H), 4.15–3.92 (m, 2H), 3.84 (m, 1H), 3.72–3.51 (m, 2H), 3.41 (m, 1H), 3.33–3.31 (m, 1H), 3.28–3.03 (m, 2H), 2.96–2.72 (m, 2H), 2.50 (m, 1H), 2.37 (m, 1H), 2.18 (m, 1H), 1.89 (m, 2H). HRMS (FAB) calcd for $C_{31}H_{34}N_4O_2S$ (M + 1): 527.2481. Found: 527.2480. (**6**) ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.92–7.74 (m, 3H), 7.62 (d, J = 7.8 Hz, 1H), 7.41–7.34 (m, 4H), 7.29 (d, J = 8.0 Hz, 1H), 7.00 (s, 1H), 7.08–6.92 (m, 2H), 5.73 (d, J = 14.8 Hz, 1H), 5.30 (d, J = 8.1 Hz, 1H), 4.19–3.38 (m, 4H), 3.43 (dd, J = 14.6, 6.2 Hz, 1H), 3.33–3.31 (m, 1H), 3.18–3.01 (m, 3H), 2.72–2.64 (m, 3H), 2.28 (dd, J = 11.6, 5.3 Hz, 1H), 2.17 (dd, J = 12.9, 7.3 Hz, 1H), 1.70 (m, 1H), 1.60 (m, 2H). HRMS (FAB) calcd for $C_{31}H_{34}N_4O_2S$ (M + 1): 527.2481. Found: 527.2475. (**7**) ^{1}H NMR (MeOH-d_4, 500 MHz) δ 8.02 (m, 1H), 7.96–7.80 (m, 2H), 7.60 (m, 1H), 7.55–7.40 (m, 4H), 7.25 (m, 1H), 7.11 (s, 1H), 7.03 (m, 1H), 6.96 (m, 1H), 5.73 (d, J = 14.7 Hz, 1H), 5.21 (d, J = 5.45 Hz, 1H), 4.41 (d, J = 10.7 Hz, 1H), 4.06 (m, 2H), 3.95 (m, 1H), 3.47 (m, 1H), 3.40 (dd, J = 7.02, 7.49 Hz, 1H), 3.11 (m, 2H), 2.67 (m, 1H), 2.59–2.41 (m, 3H), 1.98 (m, 1H), 1.82 (m, 1H), 1.73–1.58 (m, 2H), 1.48–1.07 (m, 5H). HRMS (FAB) calcd for $C_{33}H_{38}N_4O_2S$ (M + 1): 555.2794. Found: 555.2800. (**10**) ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.30 (br s, 1H), 7.87 (m, 1H), 7.78 (m, 1H), 7.77–7.63 (m, 2H), 7.59–7.30 (m, 5H), 7.27–7.16 (m, 5H), 7.12–6.89 (m, 2H), 6.00 (m, 1H), 5.18 (m, 1H), 4.87 (m, 1H), 4.66 (m, 1H), 4.06 (m, 1H), 3.97 (m, 2H), 3.70 (m, 1H), 3.63–3.37 (m, 3H), 3.34–3.12 (m, 2H), 1.88–1.65 (m, 3H), 1.65–1.50 (m, 3H). HRMS (FAB) calcd for $C_{35}H_{36}N_4O_2S$ (M + 1): 577.2637. Found: 577.2641.
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