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Small Molecule Somatostatin Receptor Subtype-2 Antagonists

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Abstract—The first potent small molecule sst2 antagonists are reported. Altering known sst2 agonist molecules yielded compounds with high sst2 binding affinity and full antagonist activity. Compound **7a**, for example, displaced somatostatin binding to the sst2 receptor with an $IC_{50} = 2.9$ nM and antagonized somatostatin action with an $IC_{50} = 29$ nM.

The somatostatin peptide is known to have multiple functions in the endocrine system. Some of the many physiological functions of somatostatin include the inhibition of growth hormone, glucagon, and insulin secretion, and the regulation of gastrin secretion from the gastrointestinal tract.[1] There are five main receptor subtypes that have been cloned from human tissue.[2] Of these, the sst2 receptor subtype has attracted some of the most interest because of the role it plays in growth hormone regulation.

We were interested in developing a small molecule sst2 antagonist in the belief that this would upregulate growth hormone levels in livestock. Starting from our initial high-throughput screening lead **1**, we were able to develop compounds such as **2** with good receptor binding affinities ($IC_{50} = 85$ nM). It was intriguing to find that we could make both functional agonists and antagonists in this series, and that function would often flip between agonist and antagonist with small changes in the molecule (Fig. 1).

A series of small molecule sst2 agonists has recently been disclosed by Merck scientists.[3] These molecules, exemplified by L-054,522 (**3**), are very potent and selective agonists that inhibit growth hormone release in vitro. However, no antagonists were reported in this series. Indeed, small molecule sst2 antagonists have been elusive in the literature.[4] The only examples of sst2 antagonists are peptide derivatives of somatostatin,[5] exemplified by **4** and **5**.

Based on experience with our series, we introduced small changes in the scaffolding of the L-054,522 series of sst2 agonists in an attempt to discover structurally related antagonists.

Compounds were made by standard amide and urea coupling methods as shown in Scheme 1. D-Trp-Lys(-BOC)-*O*-*t*-Bu was synthesized by an EDC mediated coupling of Z-D-Trp and HLys(BOC)-*O*-*t*-Bu followed by hydrogenolysis of the Z group. The BOC protected derivatives of ureas **7a–d** were formed by reaction of the D-Trp-Lys(BOC)-*O*-*t*-Bu substrate with *N,N′*-disuccimidyl carbonate followed by the requisite monosubstituted piperazine. The BOC protected derivative of amide **7e** was formed by an EDC mediated coupling of D-Trp-Lys(BOC)-*O*-*t*-Bu with the *N*-substituted isonipecotic acid. The BOC groups were removed by reaction with 5% TFA in DCM at room temperature for 10 min to yield the finished products **7a–e**.

The sst2 receptor binding assay was conducted as previously described[6] with minor modification. The assay utilized Neuro2A cells transiently expressing the full length porcine sst2 amino acid sequence.[7] Membranes were incubated with [^{125}I]-somatostatin 14 (15 nCi) and test compounds for 1 h at 37 °C, before vacuum filtration through glass fiber filters and quantitation of bound radioactivity by liquid scintillation counting. Cyclic AMP (cAMP) content of pituitary cells was used to differentiate somatostatin agonists from antagonists as previously described,[8] with minor modifications. GH_4C_1 cells at $1–2 \times 10^6$/mL were incubated for 20 min at 37 °C with test compound dose-titrations in the presence of 100 nM vasoactive intestinal peptide (VIP) and

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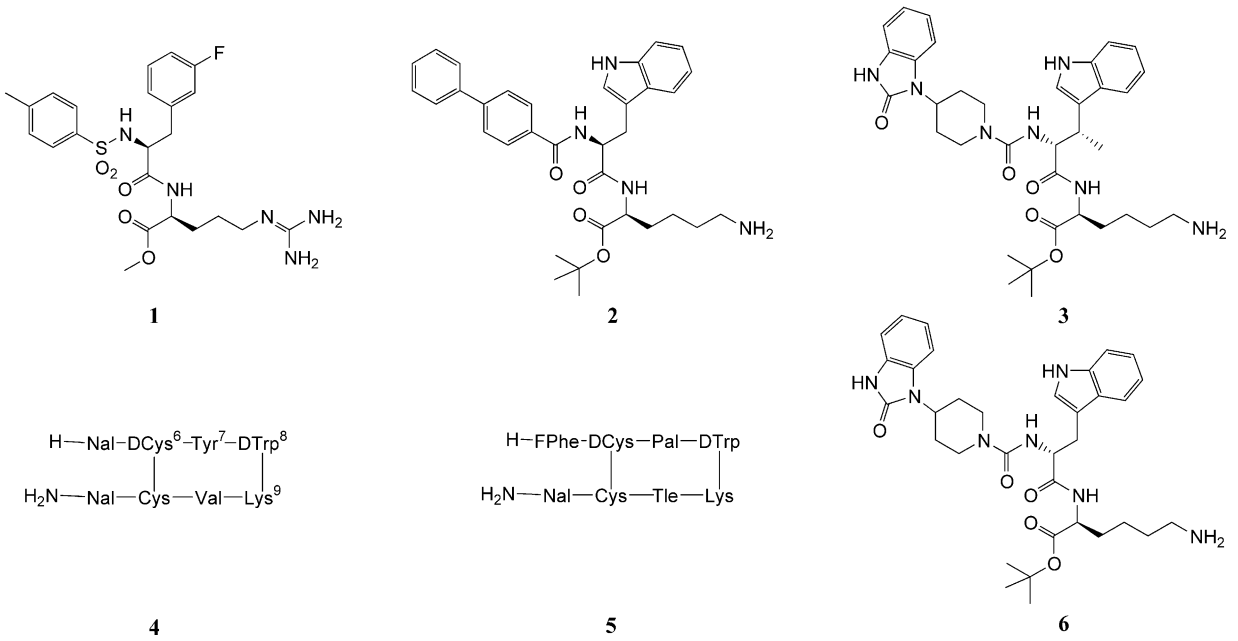
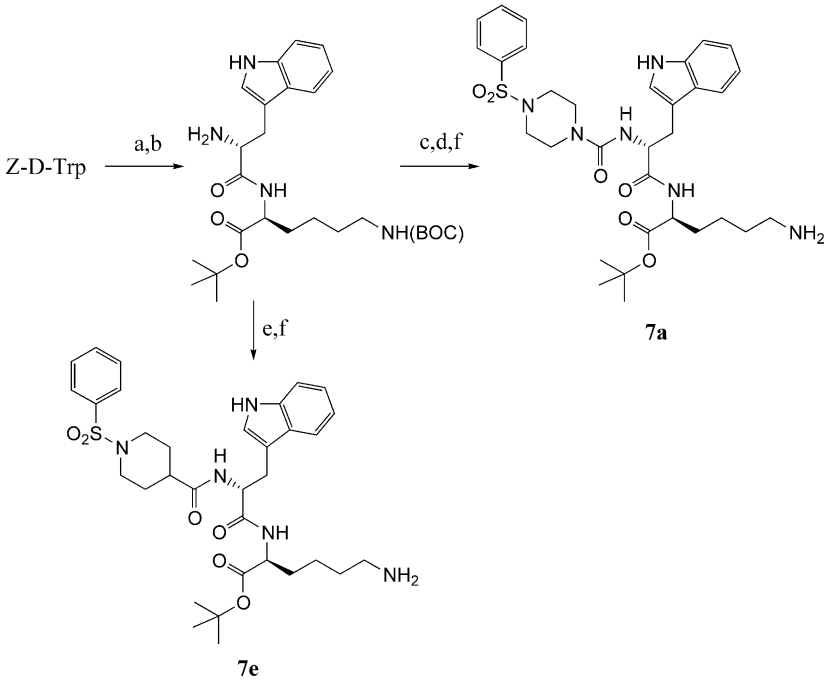


Figure 1. sst2 agonists and antagonists.



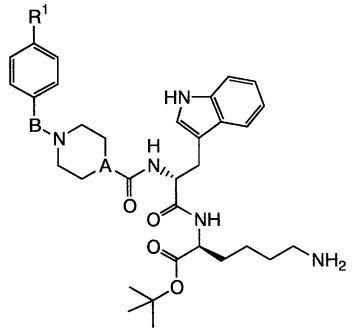
Scheme 1. (a) HLys(BOC)-*O*-*t*-Bu·HCl, DMAP, EDC, HOBt, DCM; (b) H_2, Pd/C, MeOH; (c) *N*,*N*′-disuccimidyl carbonate, DIEA, THF; (d) benzenesulfonylpiperazine, DIEA, THF; (e) 4-benzenesulfonylisonipecotic acid, DMAP, EDC, HOBt, DCM; (f) 5% TFA, DCM, 10 min.

10 nM somatostatin 14. Following incubation, cAMP content was determined using Adenylyl Cyclase Activation FlashPlate® plates (NEN) in comparison with cAMP standards. VIP increased cAMP content of the GH_4C_1 cells, and somatostatin caused a partial inhibition. Somatostatin antagonists increased cAMP content in comparison to control wells containing VIP and somatostatin alone. Somatostatin agonists decreased cAMP content.

All calculations were performed using standard protocols available through the SYBYL forcefield from Tripos Associates. A three-dimensional structure was determined for each of the compounds, and conformers were generated by systematically rotating every rotatable bond in 5° increments. The conformers were then clustered into families, and the lowest energy member of each family was taken to be representative of that cluster. Conformations were not determined for the long alkylamine chains corresponding to the lysine residue; the flexible nature of this chain allows it to be placed in an equivalent orientation in each of the compounds. Moreover, we chose to model it in its extended form

because of the known activity of a more rigid form[3] of **3**. Each of the representative conformers was minimized and placed into an ensemble. This ensemble of conformations was then compared to the NMR structure of Sandostatin® (octreotide acetate),[9] and a pharmacophore was determined by finding common conformations that were consistent with the solution structure.

Table 1. Structural data for compound **7a–e**



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Entry	A	B	R^1
7a	N	SO_2	H
7b	N	SO_2	CH_3
7c	N	CO	H
7d	N	CO	CH_3
7e	CH	SO_2	H

Table 2. Sst2 receptor binding and functional data for compounds **1–2**, **5–6** and **7a–e**. Binding data are from duplicate 12-point titrations, each with triplicate wells per concentration. Functional data are from individual experiments, or are means from 2–4 replicate experiments

Entry	Sst2 Binding IC_{50} (nM)	Functional activity	Functional IC_{50} (nM)
1	> 2000	Antagonist	6500
2	85	Antagonist	1200
5	4.1	Antagonist	270
6	0.26	Agonist	6.5
7a	2.9	Antagonist	29
7b	9.2	Antagonist	7700
7c	6.9	Antagonist	1600
7d	4.4	Antagonist	67
7e	3.2	Antagonist	120

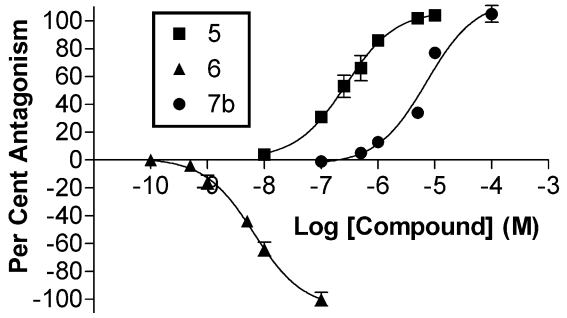

Figure 2. Agonism (**6**) and antagonism (**5** and **7b**) of somatostatin action in GH_4C_1 cells. Data are mean ± standard error from three to four experiments.

It is widely accepted that the Phe^7-Trp^8-Lys^9 residues of somatostatin are critical for bioactivity. These same residues or suitable mimics are found in essentially all of the peptide analogues of somatostatin, including the commercial product Sandostatin® (as $Phe^{3'}$-$Trp^{4'}$-$DLys^{5'}$) and in all of the smaller sst2 peptidomimetic agonists reported to date. Compounds **2** and **3**, for example, each have a terminal aryl group, a Trp or modified Trp residue, and a Lys residue that correspond to the somatostatin Phe^7, Trp^8, and Lys^9 residues, respectively. Our strategy was to meld certain features of structures **2** and **3** in order to retain the potency of **3** and the antagonist activity of **2**. Starting with the agonist **6**, a simple des-methyl analogue of L-054,522 (**3**), we synthesized a series of derivatives where the benzimidazolonepiperidine group of **6** was replaced with an *N*-substituted piperazine or an *N*-substituted isonipecotic acid, yielding structures **7a–e**[10] (Table 1). As we hoped, structures **7a–e** retained most of the binding affinity of **6**, but were all full antagonists (Table 2, Fig. 2). All compounds had binding affinities in the low nanomolar range, with functional potency ranging from 12 to 7700 nM.[11]

Molecular modeling indicated that antagonists **7a–e** would have the terminal aryl group (Phe^7 equivalent) in a position closer to the antagonist **2** than the agonist **6**, as shown in Figure 3. The orientation of the terminal aryl group in antagonist **2** is expected to be different from agonist **6** because of the different stereochemistry at the Trp center. While antagonists **7a–e** have the same Trp stereochemistry as agonist **6**, it appears that the sulfonamide sp2 nitrogen in **7a**, **7b**, and **7e** (or amide sp2 nitrogen in **7c** and **7d**) can alter the geometry of **7a–e** enough to place the terminal aryl group in the hypothesized antagonist binding pocket.

Small structural changes have also been responsible for a functional change in a peptidic series of somatostatin analogues. Hocart et al.[5] found that changing the chirality of $DCys^6$ to L in cyclic peptide **4** switched function from antagonist to agonist. Their molecular modeling studies indicated that this would dramatically change the orientation of the exocyclic Nal residue with no apparent change in the relative positions of the Tyr^7, $DTrp^8$, and Lys^9 residues, contrary to what we observed

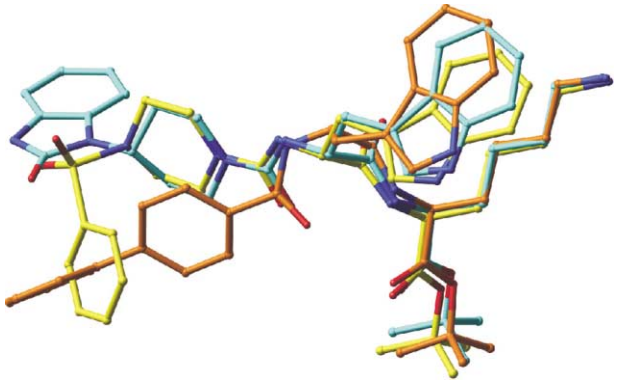

Figure 3. Overlap of three structures from the sst2 pharmacophore. Agonist **6** is shown in turquoise and antagonists **2** and **7a** are shown in orange and yellow, respectively.

with **6** versus **7a**–**e**. It may be that **7a**–**e** and **4** are hitting different antagonist binding pockets, or perhaps the inversion at Cys^6 induces subtle changes in the conformation of **4** in the Tyr^7, $DTrp^8$ and Lys^9 region.

In summary, we have discovered the first small molecule sst2 antagonists. An analysis of the in vivo effects of these molecules is the next step in defining their practical utility.

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10. After this work was completed, Merck scientists reported on potent sst2 binding compounds very similar to structure **6e**. Zhou, C.; Guo, L.; Morriello, G.; Pasternak, A.; Pan, Y.; Rohrer, S. P.; Birzin, E. T.; Huskey, S.-E. W.; Jacks, T.; Schleim, K. D.; Cheng, K.; Schaeffer, J. M.; Patchett, A.; Yang, L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 415
11. Functional potency was 1–3 orders of magnitude lower than receptor binding potency. The explanation could be that for certain compounds a particularly high degree of receptor occupancy is required for effective modulation of signal transduction.