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Letters

Discovery of the First Nonpeptidic, Small-Molecule, Highly Selective Somatostatin Receptor Subtype 5 Antagonists: A Chemogenomics Approach

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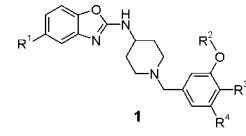
Abstract: We disclose the first selective, nonpeptidic, small-molecule somatostatin receptor subtype 5 (SST5R) antagonists that were identified by a chemogenomics approach based on the analysis of the homology of amino acids defining the putative consensus drug binding site of SST5R. With this strategy, opioid, histamine, dopamine, and serotonine receptors were identified as the closest neighbors of SST5R. The H1 antagonist astemizole was chosen as a seed structure and subsequently transformed into a SST5 receptor antagonist with nanomolar binding affinity devoid of the original target activity.

Somatostatin (SST[a]) or somatotropin release-inhibiting factor (SRIF) is a cyclic tetradecapeptide hormone originally isolated from sheep hypothalamus and sequenced by Brazeau et al. in 1973.[1] It is widely distributed throughout the body and exhibits multiple biological functions that are most commonly inhibitory in nature, such as the release of growth hormone (GH), pancreatic insulin, glucagon, and gastrin.[2] In addition, it was demonstrated that SST also shows antiproliferative and neurotransmitter activities and reduces splanchnic perfusion.[3] SST acts via five distinct G-protein-coupled receptors (GPCRs), SST1–5, which all have been cloned and characterized.[2] Detailed structure–activity relationship (SAR) studies have revealed that the tetrapeptide motif Phe[7]-Trp[8]-Lys[9]-Thr[10] is essential for biological activity and adopts a β-II-turn conformation according to NMR and crystallographic investigations. Several research groups have capitalized on this structural motif, developing β-strand mimetics.[4] Because of the numerous physiological functions of SST, selective ligands are of high interest and might play an important role in the treatment of various human diseases. For instance, SST acting via SST5 receptors has been found to activate and up-regulate NMDA receptor function[5] or to control hormonal secretions (e.g., insulin, growth hormone).[6] SST has a very short half-life in the circulation, and therefore, numerous research groups have focused on the design of nonpeptidic SST ligands exhibiting improved metabolic stability. Although a number of peptidic and nonpeptidic hSST5 receptor–ligands have been published,[6–13] no small-molecule, selective hSST5 receptor antagonists have been described so far. We report here the discovery of compounds of the general structure **1**, which are receptor subtype 5 selective somatostatin antagonists.



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To quickly identify selective and low molecular weight hSST5R ligands suitable for lead optimization without having to commit substantial resources for high-throughput screening (HTS), we opted for a focused screening approach. Because of a lack of small-molecule hSST5 receptor antagonists, classical approaches such as 2D/3D similarity searches or pharmacophore modeling could not be utilized.[14–16] Therefore, we implemented a chemogenomics strategy based on an analysis of the homology of amino acids delineating the putative consensus drug binding site in the transmembrane region of GPCRs. Class A GPCRs with known small-molecule ligands were ranked with respect to the similarity of the binding pocket of the hSST5 receptor.[17] Biogenic amine receptors such as opioid, histamine, dopamine,

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[a] Abbreviations: SST, somatostatin; SRIF, somatotropin release-inhibiting factor; GH, growth hormone; GPCRs, G-protein-coupled receptors; SAR, structure–activity relationship; NMDA, *N*-methyl-D-aspartate; SST5R, somatostatin subtype 5 receptor; R, receptor; h, human; HTS, high-throughput screening; H1, histamine subtype 1 receptor; D2, dopaminergic subtype 2 receptor; DMF, *N*,*N*-dimethylformamide; MWH, microwave-assisted heating; DCE, dichloroethane; DIEA, diisopropylethylamine; TEA, triethylamine.

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Figure 1. Analysis of amino acid homology of the putative consensus drug binding site in the transmembrane region of biogenic amine receptors closely related to hSST5R. The amino acids are colored according to physicochemical properties. The highly conserved aspartic acid (D) is highlighted in magenta. The selected 35 helix and EC2 loop positions are taken from Table 1 in ref 17.

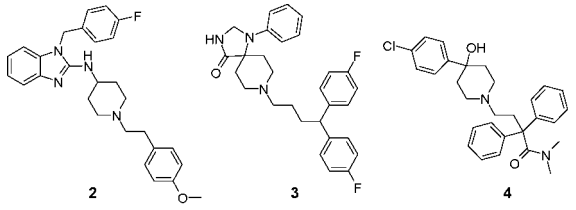


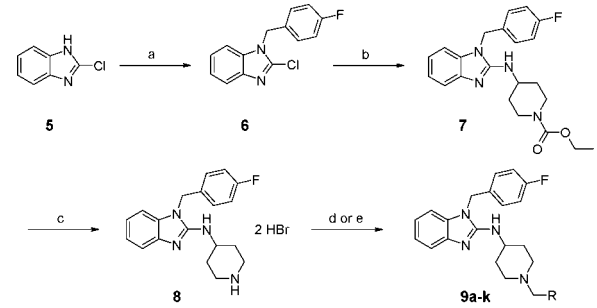
Figure 2. Identification of hSST5 receptor antagonist seed structures by screening of the Sigma LOPAC and Cerep BioPrint databases.

and serotonin receptors were identified as closest neighbors of SST5R (Figure 1).

Recently, a related method to classify and characterize GPCRs according to their ligand recognition properties has been published by Högberg et al.[18]

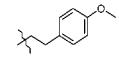
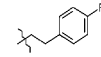
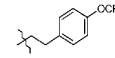
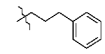
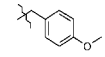
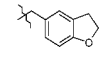
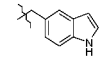
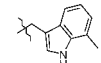
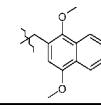
The Sigma LOPAC[19] and the Cerep BioPrint[20] compound databases, which contain pharmacologically active reference compounds and marketed/withdrawn drugs targeting biogenic amine receptors, were selected for a focused screen. As predicted by the above sequence comparison of the consensus drug binding site, three different, albeit structurally closely related, biogenic amine receptor–ligands with a micromolar K_i against hSST5R were identified: astemizole (**2**),[21] a second-generation hH1R antihistamine; fluspirilene (**3**),[22] a hD2R antagonist used for the treatment of psychotic disorders; and loperamide (**4**),[23] a peripherally acting opioid receptor agonist used for the management of chronic diarrhea. All had affinities in the low micromolar range of 4.07, 4.51, and 4.52 μM against hSST5R, respectively (Figure 2). On the basis of hSST5R potency and structural simplicity, we selected astemizole (**2**) as our seed structure to initiate lead generation activities. Primary SAR studies on **2** focused on the modification of the *p*-methoxyphenethyl group as outlined in Scheme 1. The synthetic strategy was mainly adopted from the published synthesis of astemizole (**2**), although several steps were slightly modified, providing increased yields of intermediates.[24] Deprotonation of benzimidazole **5** with NaH in DMF and subsequent reaction with *p*-fluorobenzyl bromide provided alkylated intermediate **6**. Neat reaction of benzimidazole **6** with 4-aminopiperidine-1-carboxylic acid ethyl ester under microwave-assisted heating to 180 °C provided piperidine **7** in excellent yield. Removal of the ethyl carbamate protection group by heating to reflux with hydrobromic acid in water furnished dihydrobromide salt **8**, which was used directly in the consecutive reaction step without further purification. Finally, preparation of target structures **9a–d** was achieved by in situ oxidation of the corresponding alcohols with Dess−Martin periodinane in dichloroethane followed by reductive amination with piperidine building block **8** and sodium cyanoborohydride. Similarily, compounds **9e–k** were synthesized by reductive amination from piperidine **8** and the corresponding aldehydes.

Scheme 1[a]



[a] Reagents and conditions: (a) NaH, *p*-fluorobenzyl bromide, DMF, 60 °C, 18 h, 81%; (b) 4-aminopiperidine-1-carboxylic acid ethyl ester, MW, 180 °C, 1 h, 90%; (c) HBr (48%) in water, reflux, 18 h, quantitative. (d) **9a–d**: corresponding alcohol, Dess−Martin periodinane, DCE, 40 °C, 2 h, then addition of **8**, HOAc, DIEA, $NaCNBH_3$, DCE, 40 °C, 18 h. (e) **9e–k**: corresponding aldehyde, HOAc, DIEA, $NaCNBH_3$, EtOH, 40 °C, 18 h.

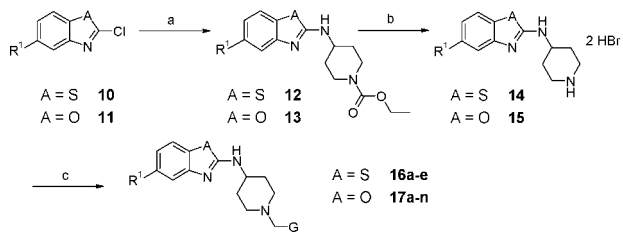
Table 1. hSST5 Receptor Radioligand Binding Data of Compounds **2** and **9a–k**

Entry	Compound	R	hSST5R Ki (μM)
1	**2**		4.07
2	**9a**		> 10
3	**9b**		> 10
4	**9c**		5.00
5	**9d**		> 10
6	**9e**		> 10
7	**9f**		6.12
8	**9g**		3.95
9	**9h**		1.63
10	**9i**		3.95
11	**9j**		1.44
12	**9k**		1.23

Interestingly, replacement of the *p*-methoxy group in astemizole (**2**) with relatively small substituents such as fluorine (**9a**) or trifluoromethoxy (**9b**) leads to hSST5R inactive compounds, whereas attachment of a simple unsubstituted phenethyl group (**9c**) mainly restored activity in the low micromolar range (Table 1).

Reducing chain flexibility by fusion of the ethylene bridge into a 1,2,3,4-tetrahydronaphthalene system again resulted in a clear loss of activity (**9d**). Further elongation of the carbon chain yielding a phenpropyl side chain (**9e**) as well as attachment of

Scheme 2[a]



[a] Reagents and conditions. (a) **12**: 4-aminopiperidine-1-carboxylic acid ethyl ester, TEA, MW, 180 °C, 5 min, 75%. **13**: 4-aminopiperidine-1-carboxylic acid ethyl ester, DMF, room temp, 18 h, 45%. (b) **14** and **15**: HBr (48%) in water, reflux, 2 h, quantitative. (c) Corresponding benzaldehyde, HOAc, DIEA, $NaCNBH_3$, EtOH, 40 °C, 18 h.

short linear or branched saturated alkyl side chains (not shown) did not provide active compounds. Quickly, it became apparent that the phenethyl moiety is not mandatory for binding and substitution by a simple benzyl side chain (**9f**) is tolerated well without major loss of activity when compared with seed structure **2**.

Bringing in para substituents at the benzyl group usually showed some improvement in binding as evidenced by **9g**. A further gain in affinity was achived by a dihydrobenzofurane moiety (**9h**), but also indoles connected via positions 5 (**9i**) and 3 (**9j**) provided low micromolar compounds. Finally, the 1,4-dimethoxy-2-naphthyl derivative **9k** was the compound with the highest activity identified after this first round of optimization. With this improved hit structure **9k** in hand, we then turned our attention to the optimization of the benzimidazole part, keeping the naphthyl side chain fixed (Scheme 2).

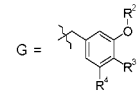
Nucleophilic aromatic substitution of benzothiazole **10** and benzoxazole **11** with 4-aminopiperidine-1-carboxylic acid ethyl ester under microwave-assisted heating (MWH) or at room temperature furnished coupling products **12** and **13**, respectively. Deprotection of the ethyl carbamate with HBr afforded the dihydrobromide salts **14** and **15**, which under reductive amination conditions were coupled with the corresponding aldehydes, providing access to target compounds **16a** and **17a**, respectively.

Exchange of the entire *p*-fluorobenzylbenzimidazole moiety in **9k** with benzothiazole (**16a**) resulted in an instant gain in affinity by a factor of 1.8 (Table 2). A similar reduction, although with a factor of 1.5 less pronounced, was obtained when switching to a benzoxazole group (**17a**). As already observed in the benzimidazole series **9**, the physicochemically rather unfavorable 1,4-dimethoxy-2-naphthyl group can also be replaced in series **16** by benzyl substituents, as demonstrated with **16b**,**c**. However, affinities tended to be in the low micromolar rather than submicromolar range. After several benzyl side chains with various substitution patterns were screened, a breakthrough was observed for compounds containing an ethoxy side chain at the meta position, which provided two promising hits with 0.149 μM (**16d**) and 0.326 μM (**16e**). A further gain in affinity of about a factor 2.5 was noted when going to the corresponding benzoxazole derivatives (**17b**,**c**).

The functional response of benzoxazole **17c** was determined in a cAMP assay, revealing $IC_{50} = 0.74$ μM. Remarkably, the overwhelming number of compounds measured were full antagonists; agonism or partial agonism was very rarely observed. The physicochemical profile of optimized hit structure **17c** turned out to be already quite promising, displaying solubility (Lysa) $S = 130$ μg/mL, a distribution coefficient log $D = 2.92$, and a passive membrane permeability (Pampa) of Pe $= 4.44 \times 10^{-6}$ cm/s.[25] The tertiary piperidine N showed a pK_a of 8.3, and a second basic transition at the benzoxazole moiety was observed at 3.3. Microsomal stability in human and mouse microsomes was in the medium range, showing clearances of CL = 9.11 and 38.28 mL min^{-1} kg^{-1}, respectively. However, **17c** inherited considerable hH1R affinity ($K_i = 0.624$ μM) from seed structure **2** and also displayed substantial potency toward the hERG potassium channel ($IC_{50} < 100$ nM), a well-known liability of astemizole (**2**).[21,26] Exploration of the chemical space of the meta position showed that larger alkyl groups such as cyclopentyl (**17d**) or isobutyl (**17e**) result in a slight further improvement in binding, whereas *n*-propyl (**17f**) was notably less active. Surprisingly, a fluoroethyl side chain (**17g**) was not well tolerated, resulting in a 4-fold decrease in binding compared with **17c**. At the para position small lipophilic groups such as fluorine (**17h**) and chlorine (**17i**) worked best, with methyl (**17j**) being the most active in the series ($K_i = 37$ nM). Interestingly, the larger lipophilic OCF_3 moiety (**17k**) reduced affinity markedly, probably because of its large electron-withdrawing potential lowering the basicity of the piperidine moiety below a certain threshold critical for receptor interaction.

With the potency of the series having been considerably improved, there only remained the outstanding issue of whether, having started with an hH1R antagonist with a hERG liability, it would be possible to move away from this undesirable polypharmacology. In keeping with current opinion,[27] attachment of the polar sulfonamide group to the oxazole in **17l** did much to improve the hERG liability, raising the IC_{50} to 3.5 μM, although at the expense of a slight decrease of hSST5R binding compared with **17c**. However, exchange of the *p*-methoxy moiety in **17l** to a chlorine group (**17m**) resulted in a significant boost in hSST5R affinity to 17 nM. Removing hH1R activity was equally facile; introduction of a second ethoxy group to the benzylamine (**17n**) not only resulted in high hSST5R affinity

Table 2. hSST5 Receptor Radioligand Binding Data of Compounds **16a–e** and **17a–n**

Entry	Compd	R^1	G			hSST5R Ki (μM)
1	**16a**	H	1,4-dimethoxy-2-naphthyl			0.683
2	**17a**	H	1,4-dimethoxy-2-naphthyl			0.794
			G = 			
		R^1	R^2	R^3	R^4	
3	**16b**	H	CH_3	OH	H	5.128
4	**16c**	H	CH_3	OCH_3	H	1.789
5	**16d**	H	C_2H_5	OH	H	0.149
6	**16e**	H	C_2H_5	OCH_3	H	0.326
7	**17b**	H	C_2H_5	OH	H	0.066
8	**17c**	H	C_2H_5	OCH_3	H	0.121
9	**17d**	H	cyclopentyl	OCH_3	H	0.083
10	**17e**	H	isobutyl	OCH_3	H	0.023
11	**17f**	H	*n*-propyl	OCH_3	H	0.293
12	**17g**	H	C_2H_4F	OCH_3	H	0.487
13	**17h**	H	C_2H_5	F	H	0.194
14	**17i**	H	C_2H_5	Cl	H	0.113
15	**17j**	H	C_2H_5	CH_3	H	0.037
16	**17k**	H	C_2H_5	OCF_3	H	0.524
17	**17l**	SO_2NH_2	C_2H_5	OCH_3	H	0.185
18	**17m**	SO_2NH_2	C_2H_5	Cl	H	0.017
19	**17n**	SO_2NH_2	C_2H_5	NH_2	OC_2H_5	0.013

(13 nM) but also provided a 200-fold selectivity versus hH1R ($K_i = 2.6\ \mu M$). Assessing cross-selectivity against the other four SSTRs revealed some minor affinity toward hSST1R $K_i = 1.75\ \mu M$ but essentially no interaction with hSST2R, hSST3R, and hSST4R ($K_i > 10\ \mu M$).

In conclusion, it has been demonstrated that by capitalizing on the similarity of GPCR transmembrane binding pockets with known small-molecule ligands, useful starting points for GPCR lead identification programs can be found without recourse to resource-demanding HTS campaigns. By careful optimization, it is possible to readily move away from the original receptor activity, as in this case where a hH1R ligand was transformed into the first known, small-molecule, selective hSST5 receptor antagonist. These ligands are endowed with promising physicochemical and metabolic properties and should serve as useful tools for the exploration of the biological role of the SST5 receptor.

Acknowledgment. We are grateful to Olivier Gavelle, Christoph Kuratli, and Tim Mamié for their excellent technical support and to Drs. Konrad Bleicher and Mark Rogers-Evans for helpful discussions.

Supporting Information Available: Experimental details and analytical data for selected compounds and details of physicochemical and biological in vitro assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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