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Piperidinyl-nicotinamides as potent and selective somatostatin receptor subtype 5 antagonists

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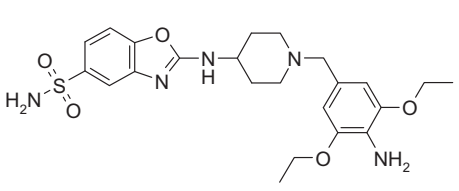
ABSTRACT

Nicotinamides of benzyl-substituted 4-aminopiperidines and their seven-membered analogs of generic structure **2** and **2′** have been discovered as potent and selective SST5 antagonists. The activity (K_i) ranges from 2.4 to 436 nM. Most compounds exhibit decent physicochemical properties and follow a clear SAR pattern. Interestingly enough, the receptor is strongly enantiodiscriminating and binds in the amino-azepane-series only the (*R*)-enantiomer.

Somatostatin (SST) or somatotropin release-inhibiting factor (SRIF), discovered more than 30 years ago,[1] is a mammalian peptide hormone comprising 14 or 28 amino acids (SST-14 and SST-28, respectively) that is widely distributed throughout the body where it exhibits a plethora of biological functions, including antiproliferative, hormonal, and neuron-transmitter activity.[2] Peripheral SST-28 is predominantly expressed in the mucosa of ileum and colon and in β-cells of the endocrine pancreas, whereas SST-14 can be found in the foregut and enteric nervous system.[3] As a hormone SST is mostly inhibitory and, for instance, impedes the release of growth hormone (GH), pancreatic insulin, glucagon, and gastrin.[4] SST acts via five distinct G-protein coupled receptors (GPCR)



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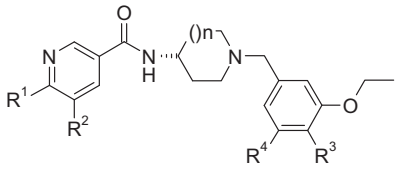
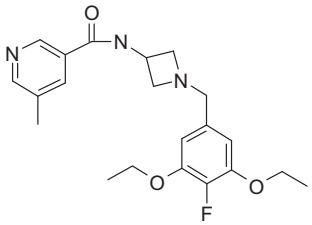


2 n=1
2' n=2



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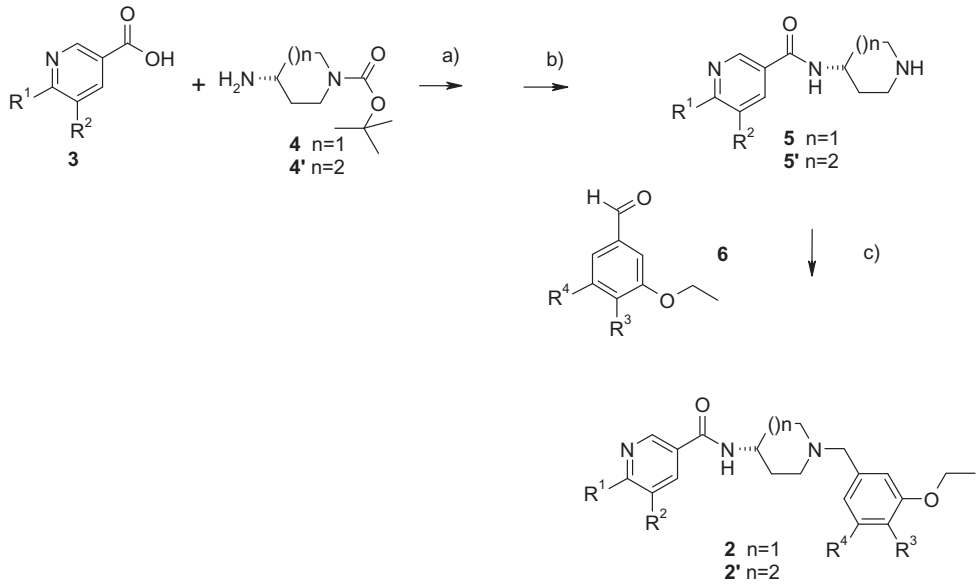
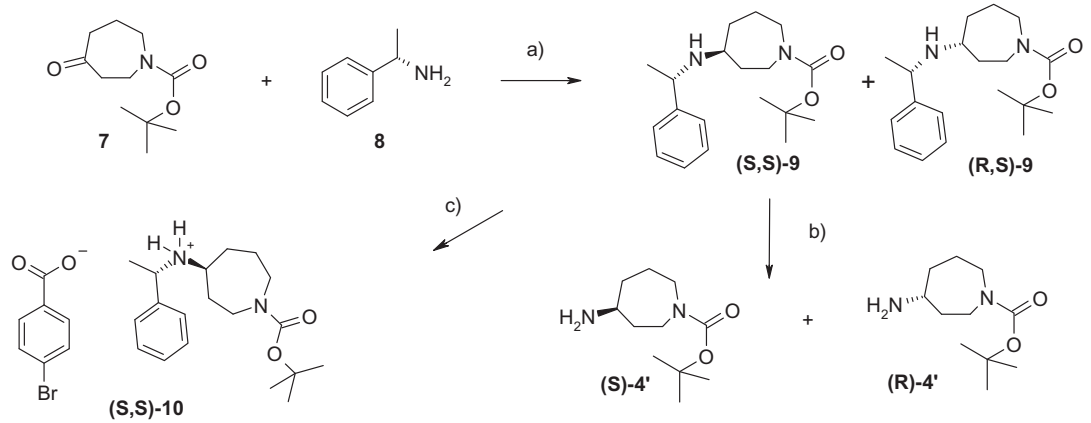
SST1–5, which all have been cloned and characterized.[4,5] Particularly, SST acting via SST5 receptors has been found to activate and up-regulate NMDA receptor function[3,6,7] and to control hormonal secretions (e.g., insulin and growth hormone).[3,7] For instance in the pancreas, the SST5 receptor is prominently expressed in up to 100% of all β-cells.[8–11] Due to the numerous, in detail not yet very well understood physiological functions of SST, selective receptor specific ligands would be highly valuable tool compounds to study and explore its rich pharmacology in more detail.[12] Whether they deserve also attention as putative drugs remains to be established. We have recently reported on the identification of receptor subtype 5 selective SST5 antagonists containing a benzoxazole headgroup connected to a 4-amino-piperidine unit,[13] represented by the prototypic compound **1**. It soon became clear that 4-amino-piperidine is a key element of the pharmacophore. In this Letter we disclose a further extension of our work aiming at optimizing potency and selectivity as well as fine tuning of physicochemical properties, and describe synthesis and in vitro properties of piperidin-4-yl-nicotinamides **2** and their homologous azepane-derivatives, respectively. The lead structure was found by screening combinatorially prepared acylated 4-aminopiperidines to identify products with more favorable pharmacokinetic behavior. This series complements the recently published SAR study around the benzoxazoles[14] and describes the identification of lead compound **2Ai** profiled in in vivo studies.[15]

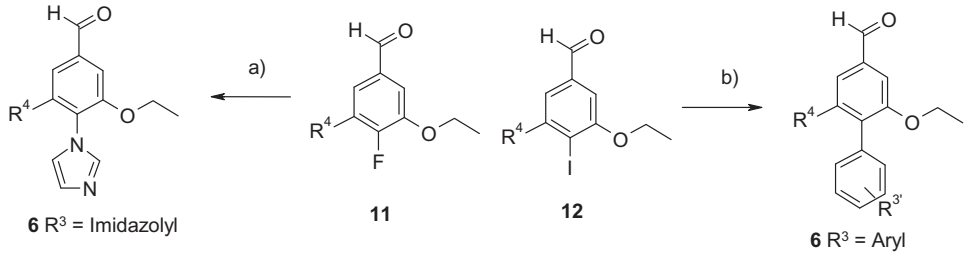
All compounds were synthesized in a straightforward manner from the appropriate nicotinic acids **3** which were either commercially available or prepared according to standard methods. Amide coupling to BOC-protected aminopiperidine **4** or its homolgue **4′** yielded after ensuing deprotection intermediates **5** or **5′** which were then transformed by reductive amination[17,18] with the corresponding *m*-ethoxy-benzaldehydes **6** into the target molecules **2** and **2′**, respectively (see Scheme 1). Intermediates **6**, if not commercially available or prepared by trivial transformations, were obtained either by an S_NAr reaction of the fluoride **11**[19] or by a modified Suzuki-reaction (see Scheme 3). (4*RS*)-Aminoazepan-1-*tert*-butylcarbamate **4′**, indispensable starting material to produce the seven-membered analogs, was prepared from 4-piperidone hydrate as described in the literature.[20,21] Resolution was achieved by performing a reductive amination of ketone **7** with (*S*)-1-phenylethylamine **8** to give a roughly 1:1 epimeric mixture of **9** which



Scheme 1. Reagents and conditions: (a) benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate, *N*-ethyldiisopropylamine, THF, rt, 14 h, 60–95%; (b) 4 M HCl in dioxane, 2 h, rt, ∼quant.; or 20% TFA in CH_2Cl_2, 2 h, rt, ∼quant.; (c) 3 equiv Ti(O*i*Pr)$_4$, 2 equiv $NaCNBH_3$, *i*PrOH, 14 h, rt, 40–70%.



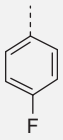
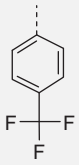
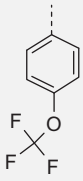
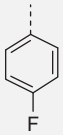
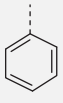
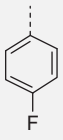
Scheme 2. Reagents and conditions: (a) 3 equiv Ti(O*i*Pr)$_4$, 2 equiv $NaCNBH_3$, *i*PrOH, 14 h, rt, 80%, followed by preparative HPLC; (b) Pd/C (10%), H_2 (1 atm), AcOH, 14 h, rt, ∼quant.; (c) crystallize **S,S-9** (which leads to inactive isomers) in presence of 1 equiv of 4-bromobenzoic acid in CH_2Cl_2.



Scheme 3. Reagents and conditions: (a) 2 equiv imidazole, K_2CO_3, DMSO, 110°, 2 h, 65–85%; (b) 2 equiv arylboronic acid, 20% tricyclohexylphosphine, 10% $Pd(OAc)_2$, K_3PO_4, toluene/water, 14 h, 110°, 80–90%.

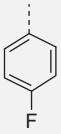
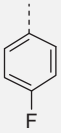
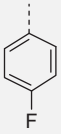
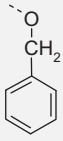
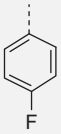
could readily be separated by preparative HPLC into the (*S*,*S*)- and (*S*,*R*)-isomer. Hydrogenolytic cleavage under acidic conditions led then to homochiral **(S)-4** and **(R)-4**, respectively. The absolute configuration was determined by X-ray analysis of compound **10**, the *p*-bromobenzoate salt of **(S,S)-9**; this intermediate led to inactive SST5 ligands (Scheme 2).[22]

Table 1
SST5-activity and physicochemical properties of nicotinamides **2** (aminopiperidines) and **2′** (aminoazepanes)

Compound	R^1	R^2	R^3	R^4	hSST5[a] (nM)	Selectivity hSST1	H1 %inhib at 3 μM	Lysa[b] (μg/mL)	Pampa *Pe*[c] (10^{-6} cm/s)	log *D*[d]	pK_a (B)[e] (piperidine nitrogen)
2Aa	H	Me	Me	H	21	>500	81	>433	2.94	3.03	8.08
2Ba	NH_2	H	Me	H	19	>500	85	375	2.40	2.24	8.27
2Ab	H	Me		H	4.9	84	89	101	1.32	4.10	7.87
2Ac	H	Me		H	11	156	48	<1	0.03	>4.0	7.96
(RS)-2′Bd	NH_2	H	F	F	436	nd	nd	nd	nd	nd	nd
2Ae	H	Me			11	213	33	473	3.17	2.61	7.34
2Af	H	Me	Me		4.0	nd	nd	106	2.36	3.61	7.92
2Ag	H	Me	Cl		4.4	nd	nd	216	3.82	3.43	7.46
2Ah	H	Me			2.4	743	12	<1.0	~0	>4.0	nd
2Bi	NH_2	H			7.1	407	4.7	123	1.39	3.15	7.86
2Cj		H	F		6.3	141	35	>520	2.41	3.39	7.84
2Dk		H			6.9	439	13	155	2.07	3.13	7.90
2Ei	Cl	H			7.9	54	7.1	76	nd	3.55	7.80

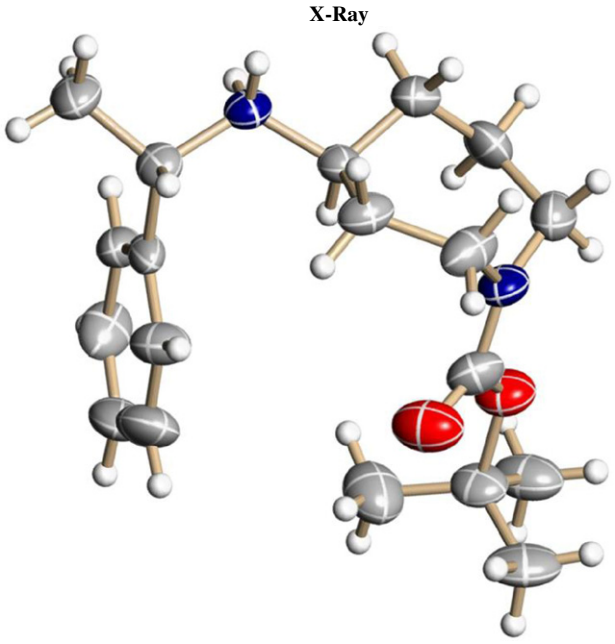
(*continued on next page*)

Table 1 (*continued*)

Compound	R^1	R^2	R^3	R^4	hSST5[a] (nM)	Selectivity hSST1	H1 %inhib at 3 μM	Lysa[b] (μg/mL)	Pampa *Pe*[c] (10^{-6} cm/s)	log *D*[d]	pK_a (B)[e] (piperidine nitrogen)
2Ai	H	Me			4.4	59	5.9	94	0.46	4.01	7.69
2Ak	H	Me			5.4	224	−1.2	152	0.64	3.73	7.74
2Di		H			2.5	211	11	153	1.23	3.83	7.86
2Bl	NH_2	H			6.7	660	−1.2	5.0	0.14	3.94	7.66
2Dj		H	F		18	>500	55	330	2.98	2.66	7.83
(R)-2′Am	H	Me	Cl	H	18	175	98	nd	nd	nd	nd
(S)-2′Am	H	Me	Cl	H	637	nd	nd	nd	nd	nd	nd
2Am	H	Me	Cl	H	15	335	nd	172	1.93	3.05	7.63
(R)-2′Aj	H	Me	F		22	253	58	nd	nd	nd	nd
2Aj	H	Me	F		25	180	25	443	3.48	2.90	7.70
(R)-2′Aa	H	Me	Me	H	22	256	97	nd	nd	nd	nd
(RS)-2′Ai	H	Me			9.3	86	54	81	1.56	3.31	8.34
13 (4-ring)	(H)	(Me)	(F)		>1000	nd	nd	>499	1.93	2.44	7.19

[a] Radioligand binding assay.[16]
[b] Lyophilisation solubility assay. Solubility was measured from lyophilized DMSO stock solutions spectrophotometrically at pH 6.5 in a 50 mM phosphate buffer.
[c] Pampa (parallel artificial membrane permeation assay).
[d] log *D* values were measured spectrophotometrically at pH 7.4 in a 1-octanol/50 mM TAPSO buffer system containing 5% (v/v) DMSO.
[e] pK_a values were determined spectrophotometrically on a ProfilerSGA instrument in a SGA buffer system containing 10% (v/v) methanol at an ionic strength of 150 mM.

Table 1 summarizes the hSST5 binding affinity, selectivity against the hSST1 receptor, %inhibition at the human histamine 1 receptor at 3 μM,[23] and the physicochemical key parameters solubility, permeability, log *D* value, and pK_a of the piperidine nitrogen, respectively, of selected analogs. We knew from our former experience[13,14] that two substituents, among them a small *meta*-alkoxy-residue, is indispensable to get decent potency. Therefore, we focused on 3-ethoxy-derivatives and include in this Letter only such analogs. Close inspection of the data reveal that three exit vectors in the benzylamine part are not only necessary for achieving single digit nanomolar affinity, this additional substituent in the other *meta*-position also helps to get rid of the undesired hH1-activity as previously described (cf. **2Aa** with **2Ag**, **2Ah**, or **2Ai**). And again, the ethoxy side-chain outperformed all the few other residues tested. Fluorine is clearly inferior (**(RS)-2′Bd**)) whereas benzyloxy (**2Bl**) does not boost the affinity any further but worsens significantly the physicochemical properties. Selectivity towards SST1, on the other hand, is hardly ever an issue and always 50-fold or better.[24] In contrast to the four- and five-membered analogs which are much weaker ligands at the SST5-receptor or do not bind at all,[25] the seven-membered analogs exhibit almost identical activity to the achiral piperidine derivatives (cf. **2Aa** with **2′Aa**, **2Ai** with **2′Ai**, **2Am** with **2′Am**, and **2Aj** with **2′Aj**). Interestingly enough, the receptor is highly enantiodiscriminating, almost all activity resides in the (*R*)-series (cf. **(R)-2′Am** being 35 times more potent than the (*S*)-isomer). Solubility is found to be typically in a favorable range, with the exception of the very lipophilic analogs containing a trifluoromethylphenyl- or a trifluoromethoxyphenyl-residue. And the PAMPA values parallel this trend; again, all but very few compounds are blessed with decent to excellent permeability. SAR around the nicotinamide moiety is less straightforward; obviously, and in full agreement with our earlier observations, the receptor is quite tolerant with respect to structural changes in this region. Even significantly modulating polarity, shape, or steric encumbrance exerts only a marginal effect on the binding affinity, cf., for example, **2Aa** and **2Ba**, or **2Ai**, **2Bi**, and **2Ei**. This is in perfect agreement with our earlier observation that benzoxazoles, if coupled to appropriate aminopiperidines, deliver excellent SST5 antagonists as well.[14,26]



X-ray structure of (*S*)-4-((*S*)-1-phenyl-ethylamino)-azepane-1-carboxylic acid *tert*-butyl ester as *p*-Br-benzoate (**(S,S)-10**, only cation shown).

In conclusion, we have identified a novel series of highly potent, selective SST5-antagonists, many of them showing single digit nanomolar binding K_i-values. Compound **2Ai** had been chosen, thanks to its balanced overall profile, rather straightforward synthetic access, and its favorable pharmacokinetic properties for an exploratory in vivo study.[15] Irrespective of its mixed outcome we think that these molecules should become valuable tools for further exploring and exploiting the SST receptors in general and SST5 in particular as pharmacological target.

Acknowledgments

The authors gratefully acknowledge Stefan Bürli and Thomas Burger for chemical syntheses, Angele Costanzo and Astride Schnoebelen for in vitro testing, Dr. Christoph Ulmer for histamine screening, and Virginie Micallef, Isabelle Parrilla, Frank Senner, and Björn Wagner for physicochemical measurements.

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16. A CHO cells stably expressing human SSTR5 (GenBank Accession No. D16827, Euroscreen, Brussels, Belgium) was used for binding and functional assays; cells expressing human SSTR1, 2 and 3, and rat and mouse SSTR5 were established in-house. SST-14 was purchased from Bachem (Bubendorf, Switzerland). Membranes from cells expressing SST receptors were prepared by sonication and incubated with radiolabeled tracer (11 Tyr SST-14; Perkin-Elmer, Schwerzenbach, Switzerland, or Amersham, Dübendorf, Switzerland) and either test compound in varying concentration or, for the quantification of non-specific binding, non-labeled SST-14. The incubation was stopped by filtration through glass-fiber filters and the bound radioactivity measured to estimate the concentration of test compound required for half maximal inhibition of binding (IC_{50}) and the binding affinity (K_i). For functional experiments, transfected cells were incubated with forskolin and test compound in varying concentration. Subsequently, cellular cAMP concentration was measured using a FRET (fluorescence resonance energy transfer) based assay as previously published Roth, D.; Matile, H.; Josel, H.-P.; Enderle, T. Fast-TRF: Novel time-resolved assays for drug discovery. In *Society for Biomolecular Screening*, 11th Annual Conference and Exhibition, Geneva, 2005; p 265. The concentration of the test compound necessary to induce a half maximal effect (EC_{50}) and the efficacy compared to 0.15 nM SST-14 were determined from concentration-versus-cAMP graphs. For the determination of potential antagonism, 0.15 nM SST-14 was applied together with the test compound, and the concentration of the test compound to half maximally reverse the effect of SST-14 (i.e., IC_{50}) was deduced from concentration-versus-cAMP graphs.
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18. It proved to be advantageous to work in *i*PrOH instead of MeOH or EtOH in order to suppress the formation of the benzyl–methyl- or benzyl–ethyl ether, generated via hemiacetal formation of the corresponding benzaldehyde and ensuing reduction.
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22. Data were collected on a STOE Imaging Plate Diffraction System (STOE, Darmstadt) with Mo-radiation (0.71 Å) and data processed with STOE IPDSsoftware. The crystal structure was solved and refined with the program SHELXTL (Bruker AXS, Karlsruhe). The coordinates of the structure of compound **(S,S)-10** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 775436. These data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].
23. Since our first seeding hit turned out to be Astemizole, a potent H1-antagonist, we routinely checked all our SST5-antagonists for this putative off-target effect.
24. We spot-checked selected antagonists against SST2, SST3 as well as—to some minor extent—against SST4 and never found any significant binding.
25. Knowing from our earlier series that 3-aminopyrrolidine is a much less favorable pharmacophore than 4-aminopiperidine we did not prepare and screen the exact analogs of **2** or **2′**. To complement the picture, however, we synthesized a couple of aminoazetidines which were found to be devoid of any activity (see compound **13**).
26. Most compounds, specifically **2Ba**, **2Ab**, **2Bd**, **2Ae**, **2Ag**, **2Bh**, **2Ah**, **2Bi**, **2Cj**, **2Dk**, **2Ei**, **2Ai**, **2Ak**, **2Di**, **2Dj**, **2Aj**, **(RS)-2′Ai** were tested in a functional assay as described in Ref. 16 and were found to be devoid of any activity (EC_{50} >1 μM).