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Benzoxazole piperidines as selective and potent somatostatin receptor subtype 5 antagonists

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ABSTRACT

SAR studies of a recently described SST5R selective benzoxazole piperidine lead series are described with particular focus on the substitution pattern on the benzyl and benzoxazole side-chains. Introduction of a second *meta* substituent at the benzyl unit significantly lowers residual hH1 activity and insertion of substituents onto the benzoxazole periphery entirely removes remaining h5-HT_{2B} activity. Compounds with single digit nM activity, functional antagonism and favorable physicochemical properties endowed with a good pharmacokinetic profile in rats are described which should become valuable tools for exploring the pharmacological role of the SST5 receptor in vivo.

Somatostatin (SST) or somatotropin release-inhibiting factor (SRIF) was first described in 1973. It is a mammalian peptide hormone existing in two isoforms with 14 or 28 amino acids (SST-14 and SST-28, respectively) that is widely distributed throughout the body.[1,2] It exhibits multiple biological functions including antiproliferative, hormonal, and neuron-transmitter activity and was demonstrated to reduce splanchnic perfusion.[3] Peripheral SST-28 is predominantly produced in the mucosa of ileum and colon and in δ-cells of the endocrine pancreas, whereas SST-14 can primarily be found in the foregut and enteric nervous system.[4] As a hormone SST is most commonly inhibitory in nature and impedes for instance the release of growth hormone (GH), pancreatic insulin, glucagon, and gastrin.[2] SST acts via five distinct G-protein-coupled receptors (GPCR) SST1-5 that have been cloned and characterized.[2] Particularly, SST acting via SST5 receptors has been found to activate and up-regulate NMDA receptor function[5] and to control hormonal secretions (e.g., insulin, GLP-1, growth hormone).[4,6] In the pancreas, SSTR5 is prominently expressed on 87% of all insulin secreting cells.[7–10] Due to the numerous physiological functions of SST, selective receptor specific ligands and particularly antagonists would be highly interesting tool compounds to study and elucidate the diverse pharmacology of SST in more detail.[11]

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.[12] This lead series was identified following a chemogenomics approach. Biogenic amine receptors such as opioid, histamine, dopamine, and serotonine receptors were identified as closest neighbors of the SST5 receptor based on an analysis of the homology of amino acids delineating the putative consensus drug binding site in the transmembrane region of GPCRs.[13] After a focused screen of the Sigma LOPAC[14] and the Cerep BioPrint[15] compound databases the well-known hH1R antagonist Astemizole (**1**) was selected as a seed structure for lead generation activities and successfully transformed into the first small molecule selective hSST5 receptor antagonist series (Fig. 1). For example, structure **2** provided high affinity toward hSST5 (13 nM) and displayed a 200-fold selectivity versus hH1 (K_i = 2.6 μM). Assessing cross-selectivity against the other four SST subtype receptors revealed some weak affinity toward hSST1 (K_i = 1.75 μM), but essentially no interaction with hSST2, hSST3, and hSST4 (K_i > 10 μM). A second and structurally very closely related nicotinamide series with nanomolar binding was identified by a similarity analysis of GPCR affinity profiles of

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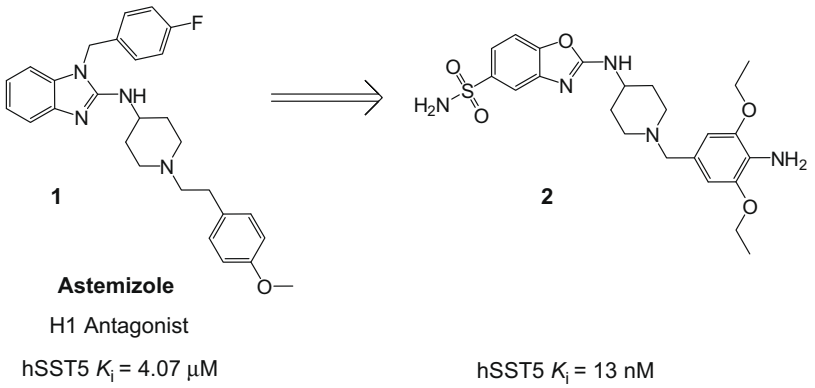


Figure 1. Astemizole (**1**) serving as a seed structure for lead generation activities resulted in SST5R antagonist **2** displaying a more than 200-fold selectivity against hH1.

Astemizole (**1**) versus a set of in-house GPCR-biased combinatorial libraries.[16]

In this Letter we would like to present an in depth analysis of the SAR against hSST5 including the selectivity against three off-target receptors hH1, h5-HT_{2B}, and hSST1 and to discuss some physicochemical and pharmacokinetic properties of this benzoxazole series.

Having selected Astemizole (**1**) as a starting point our efforts focused on generating the minimal critical chemotype essential for hSST5R activity. These studies revealed that the *p*-fluorobenzyl side-chain can be omitted and that the phenethyl group may advantageously be replaced by appropriately substituted benzyl moieties such as 3-ethoxy-4-methoxy-benzyl or 3-ethoxy-4-chloro-benzyl side-chains. Screening for an optimal replacement of the *p*-fluorobenzyl-benzimidazole moiety soon resulted in the identification of benzothiazole as suitable surrogate of the benzimidazole group. With these two substituents on either side of the 4-amino-piperidine core, the benzothiazole and the 3-ethoxy-4-methoxy- benzyl moiety, kept constant we set out for the search of an optimal spacer replacement.

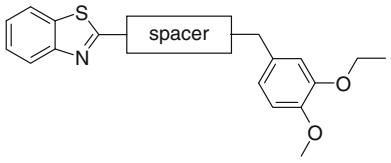
However, screening various diamino spacer units such as piperidines (**3**–**6**), diaminocyclohexanes (**7** and **8**; mixture of *cis*/*trans*) or piperazine (**9**), and phenyl moieties (**10**) did not result in any improvement of binding affinity toward hSST5R (Table 1). The benzothiazoles **3–5**, **7**–**8**, and **10** were prepared in analogy to compound **3** whose synthesis is exemplified in Scheme 1.

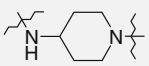
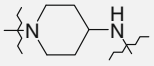
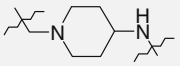
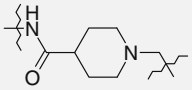
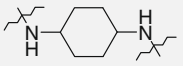
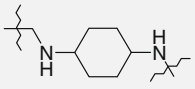
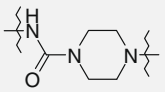
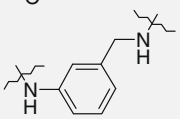
Nucleophilic aromatic substitution of 2-chloro-benzothiazole (**11**) with 4-amino-piperidine-1-carboxylic acid ethyl ester under microwave-assisted heating provided coupling product **12** in 75% yield. Deprotection of the ethylcarbamate with HBr afforded the dihydrobromide salt **13** which under reductive amination conditions was coupled with 3-ethoxy-4-methoxy-benzaldehyde furnishing the benzothiazole **3**.

Other target compounds were synthesized following a similar reaction scheme, but varying the diamino spacer unit appropriately. In the case of amide-linked target **6** the first reaction step involved an amide formation between 2-amino-benzothiazole and the corresponding BOC-protected piperidine acid under HOBT (1-hydroxy-1*H*-benzotriazole) activation. Cleavage of the protecting group under acidic conditions followed by reductive alkylation furnished the desired product **6**. Urea compound **9** was prepared by reaction of mono-BOC-protected piperazine and 2-isocyanato-benzothiazole in THF using diisopropyl-ethylamine as base.

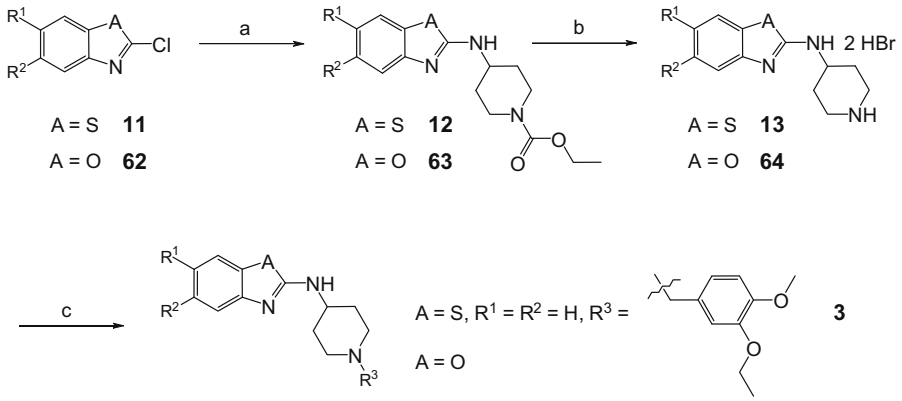
Since none of the central spacer variations improved binding, we turned our attention back to hit structure **3** focusing on further optimization of the benzothiazole moiety. However, it soon became apparent that modifications of the benzothiazole core did not significantly improve binding affinity toward hSST5R as demonstrated by 6-chloro-benzothiazole **14** or thiazolo[5,4-*b*]pyridine **15** (Table 2). In contrast, a profound jump in binding affinity was observed when going from benzothiazole to benzoxazole. Interestingly, the unsubstituted benzoxazole derivative **16** was found with hSST5 K_i = 121 nM to be the most active one, followed

Table 1
Modification of the central 4-amino-piperidine spacer



Compound		hSST5 K_i[a]
3		330
4		>1000
5		>1000
6		>1000
7		>1000
8		>1000
9		>1000
10		>1000

[a] K_i in nM, radioligand binding assay.[17]



Scheme 1. Reagents and conditions: (a) **12**: 4-amino-piperidine-1-carboxylic acid ethyl ester, TEA, MWH 180 °C, 5 min, 75%. **63**: 4-amino-piperidine-1-carboxylic acid ethyl ester, DMF, rt, 18 h, 45%; (b) **13** and **64**: HBr (48%) in water, reflux, 2 h, quant; (c) **3**: 3-ethoxy-4-methoxy-benzaldehyde, HOAc, DIEA, $NaCNBH_3$, EtOH, 40 °C, 18 h, 62%.[18] A = 0: corresponding benzaldehyde, HOAc, DIEA, $NaCNBH_3$, EtOH, 40 °C, 18 h; purification by prep. HPLC.[18]

by 5-chloro-benzoxazole **17** with 194 nM and 5-ethylsulfone-benzoxazole **18** with 196 nM, respectively.

Other analogs such as 5-nitro- (**19**), 5-trifluoromethoxy- (**20**), and 5-acetamide- (**21**) substituted benzoxazoles or benzoxazole sulfonamides (**22–24**) exhibited K_i values clearly above 200 nM. Furthermore, the oxazolo-pyridines **25** and **26** showed considerably lower activity compared with benzoxazole **16**, and aza-isomer **27** turned out to be devoid of any activity. Remarkably, the unsubstituted benzimidazole **28** lacking the *p*-fluorobenzyl group present in seed structure **1** was also found to be entirely inactive.

Earlier exploration of the SAR around the benzyl side-chain had revealed that compounds containing an ethoxy group in *meta* position are of particular interest as exemplified by benzothiazole **3** and benzoxazole **16**, respectively.[12] Exchange of the *meta* ethoxy in **16** with an ethylamino group (**29**) resulted in a reduction of affinity by a factor of 3, whereas introduction of an N atom providing pyrido derivative **30** gave completely inactive compounds (Table 3).

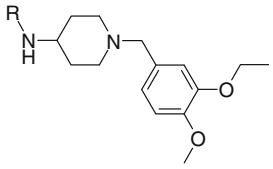
Interestingly, larger alkyl groups such as isobutyl (**31**) or cyclopentyl (**32**) afforded with a K_i of 23 nM and 83 nM, respectively, higher binding affinity toward hSST5R, but at the expense of increased MW, higher lipophilicity and lower microsomal stability. Other variations of the *meta* ethoxy group in **16** such as *n*-propoxy (**33**), methoxy (**34**), or fluoroethoxy (**35**) were noticeably less active. Screening of the *para* position revealed that small substituents like methyl (**36**), hydroxy (**37**), chlorine (**38**), or fluorine (**39**) are clearly preferred, whereby methyl derivative **36** with a K_i of 37 nM was the most active one in this series. Less favorable moieties are sterically more demanding groups such as trifluoromethyl (**40**), cyclopropoxy (**41**), or isopropoxy (**42**). Interestingly, introduction of an additional ethoxy group into the second *meta* position was equally well tolerated providing very active compounds like **43** with K_i = 33 nM. Also ethoxycarbonyl (**44**) and fluorine (**45**) in *para* position as well as the sterically rather demanding tetrahydropyranyl-oxy moiety in *meta* position (**46**) produced quite active compounds with K_i = 156 nM, 178 nM, and 199 nM, respectively. Interestingly, moving the *p*-F atom in **45** to the ortho position of the phenyl ring, as in compounds **47** or **48**, was not tolerated and resulted in complete loss of affinity. However, the bis-isopropoxy derivative **49** was again surprisingly active with a K_i of 31 nM.

The observation that the presence of an ortho (cf. **45** with **47**) as well as the increasing size (cf. series **36**–**42**) of a *para* substituent exhibits a detrimental influence on the activity of these compounds might be explained by an appropriate preorganization of the adjacent *m*-ethoxy side-chain. Indeed, a search in the Cambridge structural database (CSD) revealed that alkoxy groups such as methoxy and ethoxy substituents adopt an in-plane conformation with respect to the phenyl ring.[19] In addition, a *para* substituent between the two *meta* alkoxy groups enforces an anti orientation of the alkyl chains in order to minimize steric repulsion. This result is confirmed by in-house single crystal X-ray analysis studies of the *p*-hydroxyethoxy derivative **48**.[20,21] Although this compound was found to be inactive on the hSST5 receptor due to the *o*-F and the *p*-hydroxyethoxy motif, it serves as a nice example for illustrating the coplanar arrangement of the *m*-ethoxy group with the aromatic system. The deviation from planarity of the exocyclic O(3)–C(28) bond and the aromatic system was measured to be only 2.3° (Fig. 2). The two planes defined by the benzoxazole unit and the coplanar three atoms of the piperidine chair conformation bisect at an angle of 53.2°. Moreover, the structure also corroborates the staggered conformation typically observed for a hydroxyethoxy motif due to the gauche effect (φ [O(4)–C(31)–C(30)–O(2)] = 75.8°).[19]

It subsequently became apparent that the second *meta* vector of the benzyl needle was of particular significance as structures without a substituent at this position still carry significant hH1R activity. For instance, **16** with an hSST5R activity of 121 nM revealed only a 5.2-fold selectivity over hH1 (K_i = 0.624 μM) and similar observations were made with analogues bearing a *p*-Cl (**38**, hH1 K_i = 0.266 μM, 2.4-fold) or *p*-F group (**39**, hH1 K_i = 0.826 μM, 4.3-fold; Table 4). In addition, substituents at the rim of the benzoxazole moiety did not resolve this issue as shown by examples **22** (hH1 K_i = 0.520 μM, 1.0-fold) carrying a sulfonamide, **50** (hH1 K_i = 0.923 μM, 5.1-fold) containing a cyclopropylamide group, **24** (hH1 K_i = 1.013 μM, 3.0-fold) and **51** (hH1 K_i = 1.374 μM, 5.5-fold) with a chloro/sulfonamide substitution pattern, or **52** (hH1 K_i = 0.199 μM, 1.0-fold) bearing an inverse phenyl-sulfonamide group.

However, this off-target activity was found to be significantly reduced, in some cases almost eliminated, by introducing a second vector onto position R^4 of the benzyl moiety. Compound **2** (hH1 K_i = 2.6 μM, 200-fold) and **53** (hH1 K_i = 5.0 μM, 96-fold) containing an ethoxy moiety or **54** (hH1 K_i = 38.4 μM, 698-fold) with a more bulky tetrahydropyranyl-oxy group nicely illustrate this. In general, the sterically more demanding the R^4 substituent, the more efficiently hH1 activity was found to be suppressed, whereas a direct comparison between **46** and **54** indicated for selected side-chain combinations a synergistic effect of substituents on R^2 and R^4 with respect to reduction of hH1R activity. Whereas for **46** (hH1 K_i = 3.6 μM) only an 18-fold lowering in hH1R binding was observed due to the reduced activity on the hSST5 receptor, the

Table 2
Refinement of the benzothiazole side-chain keeping the 3-ethoxy-4-methoxy benzyl motif connected to the 4-amino piperidine spacer constant



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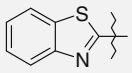
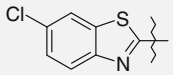
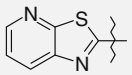
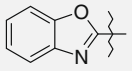
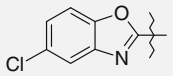
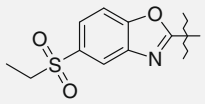
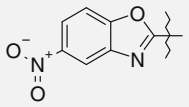
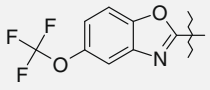
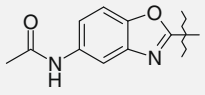
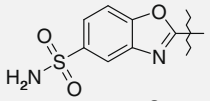
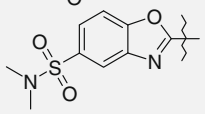
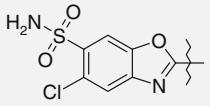
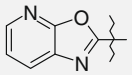
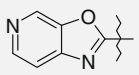
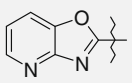
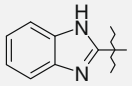
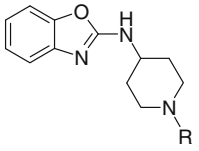
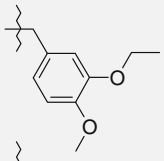
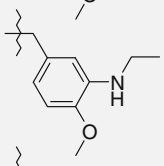
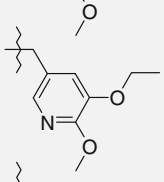
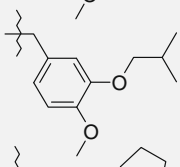
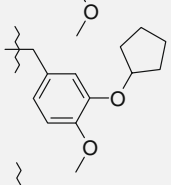
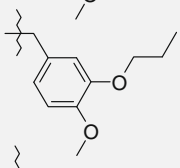
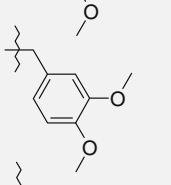
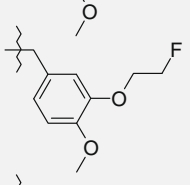
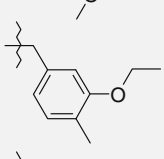
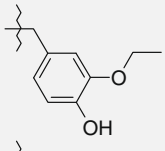
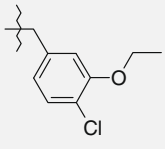
3		330
14		361
15		309
16		121
17		194
18		196
19		252
20		354
21		357
22		529
23		226
24		343
25		633
26		720
27		>1000
28		>1000

[a] K_i in nM, radioligand binding assay.[17]

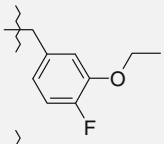
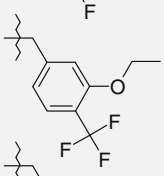
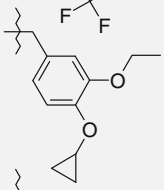
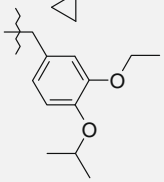
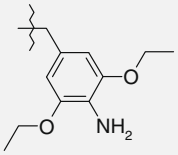
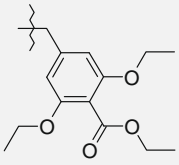
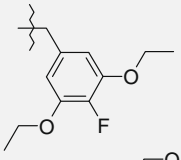
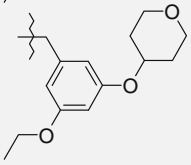
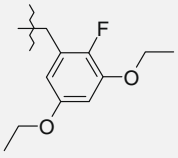
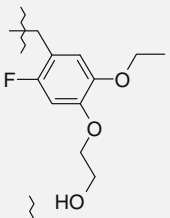
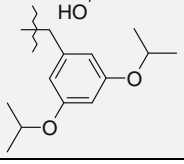
Table 3
Variation of the substitution pattern of the benzyl side-chain



Compound		hSST5 K_i[a]
16		121
29		371
30		>1000
31		23
32		83
33		293
34		472
35		487
36		37
37		66
38		113

(*continued on next page*)

Table 3 (*continued*)

Compound		hSST5 K_i[a]
39		194
40		524
41		537
42		>1000
43		33
44		156
45		178
46		199
47		>1000
48		>1000
49		31

[a] K_i in nM, radioligand binding assay.[17]

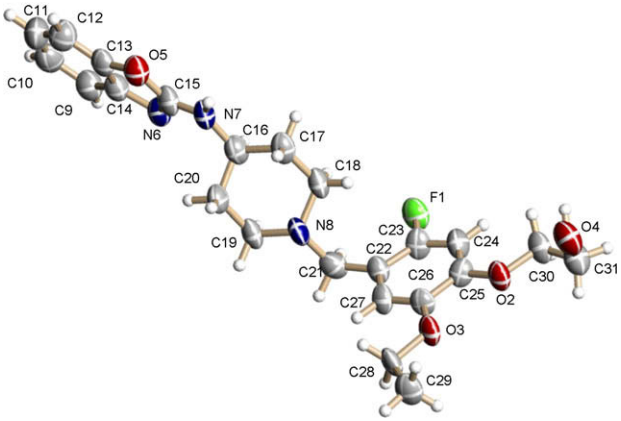


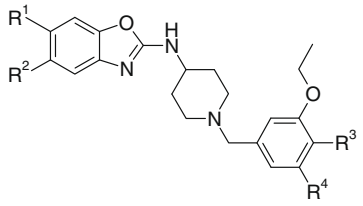
Figure 2. Single crystal X-ray diffraction analysis of benzoxazole **48** illustrating the anti in-plane conformation of the *m*-ethoxy group and the significant twist of the benzoxazole unit with respect to the piperidine core. The ORTEP drawing depicts thermal ellipsoids at a 30% probability level.

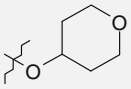
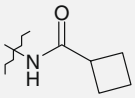
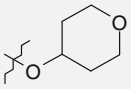
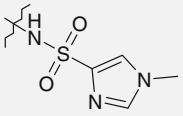
additional ethylsulfone moiety in **54** notably helped to enhance this ratio to 698.

In addition to the expected hH1 liability given the origin of the hit series, a selectivity screen on 8 different serotonine receptors showed that unsubstituted benzoxazoles such as **16** or **38** are rather potent ligands for h5-HT_{2B} (K_i = 0.041 μM and 0.074 μM, respectively), whereas no affinity was found for all other serotonine receptor subtypes. In contrast to the hH1 liability, attachments onto the benzoxazole rim were able to efficiently resolve this issue (see cyclopropylamide **50** or free sulfonamide **51**, both compounds h5-HT_{2B} K_i >10 μM). Interestingly, this effect seems to be rather independent on both the steric and electronic nature of the additional substituents. In contrast to the SAR observed for hH1R binding, substituents on R4 seemed to have little influence on h5-HT_{2B} activity as demonstrated for instance by **46** (h5-HT_{2B} K_i = 0.094 μM).

With respect to the other four SST subtype receptors, compounds with an unsubstituted benzoxazole moiety such as **38** or **39** were devoid of any hSST1 activity (K_i >10 μM). For compounds bearing slightly polar R2 groups, some minor or even no affinity for hSST1 was observed (*e.g.*, **2**: K_i = 1.75 μM, 134-fold; **50**: K_i = 4.95 μM, 27-fold; **59**: K_i = 4.50 μM, 888-fold; **60:** K_i > 10 μM; **61**: K_i > 10 μM), whereas substituents at R4 seem to have little influence on binding potency. However, essentially no interaction was observed for all compounds investigated against the other three subtype SST receptors hSST2, hSST3, and hSST4 (K_i >10 μM). Having successfully addressed the known selectivity issues we turned our attention to the hERG potassium channel liability, a well known issue of compounds containing basic amines flanked by lipophilic side-chains.[22–25] Indeed, several revealed rather high hERG binding exemplified by compounds **16** (IC_{50} <0.1 μM), **18** (IC_{50} = 0.18 μM), or **58** (IC_{50} = 0.43 μM).[26] As expected, attachment of polar groups such as a free sulfonamide moiety to the periphery of the benzoxazole unit like in compound **22** were able to significantly reduce, albeit not completely eliminate, the hERG binding potential (IC_{50} = 3.5 μM). Extensive QSAR studies showed that more hydrophilic compounds bearing carboxylate groups are required in order to entirely eliminate hERG channel affinity (**59** and **60** both IC_{50} >10 μM). Similar observations have been made by other research groups and were explained by unfavorable electrostatic interactions of the carboxylic acid group within the hERG channel binding cavity.[27] Interestingly, the distance between the positively charged piperidine nitrogen and the carboxylic acid group turned out to be crucial. Connection of the carboxylate moiety via alkyoxy-spacers to the benzoxazole group (e.g.; 3-carboxy-propoxy side-chain) resulted in a return to single digit μM hERG binding affinity (data not shown).

Table 4
Physicochemical properties of selected benzoxazoles **2**, **16**, **18**, **22**, **24**, **38**, **39**, **46**, and **50–61**



Compound	R^1	R^2	R^3	R^4	hSST5 K_i[a] (nM)	Lysa S[b] (μg/mL)	Pampa Pe[c] (10^{-6} cm/s)	log D[d]	KOW (clog P)	pK_a[e](B = base, A = acid)
2	H	SO_2NH_2	NH_2	OC_2H_5	13	335	0.00	1.62	2.74	8.28
16	H	H	OCH_3	H	121	130	4.43	2.92	3.92	8.30
18	H	$SO_2C_2H_5$	OCH_3	H	196	>530	1.63	2.13	3.36	8.10
22	H	SO_2NH_2	OCH_3	H	529	>614	1.64	1.51	2.80	8.05
24	SO_2NH_2	Cl	OCH_3	H	343	338	1.10	2.67	3.31	7.92
38	H	H	Cl	H	113	44	0.49	3.44	4.80	7.63
39	H	H	F	H	194	>452	4.33	3.63	4.26	7.79
46	H	H	H		199	212	2.05	3.02	3.90	7.76
50	H		OCH_3	H	182	>638	1.10	2.64	4.47	—
51	SO_2NH_2	Cl	F	H	252	32	2.45	2.78	3.65	7.61
52	H	$NHSO_2Ph$	OCH_3	H	205	145	2.20	1.93	4.83	7.72
53	H	$SO_2N(CH_3)_2$		OC_2H_5	52	22	1.31	2.89	5.22	—
54	H	$SO_2C_2H_5$	H		55	183	1.75	3.13	3.34	7.55
55	SO_2NH_2	Cl	F	OC_2H_5	252	32	2.45	2.78	3.65	7.61
56	Cl	H	Cl	H	239	44	4.81	precip	5.61	—
57	H		OCH_3	H	175	>720	0.49	1.48	3.51	7.91
58	H	$CONH_2$	F	OC_2H_5	8	148	1.61	2.96	3.64	7.72
59	H	COOH	Cl	OC_2H_5	3	14	2.09	1.82	2.94	7.59 (B) 3.89 (A)
60	COOH	H	Cl	H	23	7	3.60	1.39	2.63	7.67 (B) 4.03 (A)
61	COOH	H	F	OC_2H_5	18	6	2.89	1.33	2.43	7.70 (B) 4.01 (A)

[a] Radioligand binding assay.[17]
[b] Lyophilization 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): low: $Pe < 0.1$, medium: $0.1 < Pe < 1.0$, high: $Pe > 1.0$.
[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.

A substantial subset of compounds with high affinity to hSST5 was further profiled by functional in vitro assays. The overwhelming majority of compounds are full antagonists devoid of agonistic or inverse agonistic activity. The functional IC_{50} was, as expected, maximally up to one magnitude higher than the corresponding K_i value (e.g., **60**: IC_{50} = 98 nM vs K_i = 23 nM and **61**: IC_{50} = 72 nM vs K_i = 18 nM).

The pK_a value of the piperidine nitrogen in this series was found to be typically in the range of 7.6–8.3, rendering the compounds protoned under physiological conditions (Table 4). As expected, electron-withdrawing substituents in *para*-position of the benzyl group such as Cl (**38**), or F (**39**) lowered the pK_a value in comparison with the methoxy derivative **16** from 8.30 to 7.63 and 7.79, respectively. Interestingly, a similar reduction in basicity of the piperidine nitrogen can be achieved by introducing an additional alkoxy group in *meta* position of the benzyl moiety and without presence of a *para* substituent as a comparison of structures **39** (7.79) with **46** (7.76) or **54** (7.55) demonstrates. Remarkably, these effects are not additive, and combination of both structural elements such as a *p*-F atom and a *m*-alkoxy group in one single struc-

Table 5
Pharmacokinetic parameters in male wistar rat of representative hSST5R antagonists

Compound	CL[a] (mL min^{-1} kg^{-1})	Vss[b] (L kg^{-1})	$t_{1/2}$[c] (h)	%F[d]
16	84	4.1	0.7	6
18	126	3.8	0.4	6
22	103	8.2	1.7	4
56	37	4.6	2.6	33
57	41	2.2	1.5	2
58	28	3.3	2.5	38
59	18	1.8	4.9	12
60	27	4.3	3.1	57
61	20	1.5	2.4	39

[a] Clearance: <13 low, 13–40 medium, >40 high.
[b] Volume of distribution at steady state.
[c] Terminal half-life.
[d] Oral bioavailability.

ture does not result in a pronounced further reduction of the pK_a value as can be seen for instance from a comparison of **39** (7.79) and **46** (7.76) with **55** (7.61) or **58** (7.72).

The solubilities in phosphate buffer were in a good to excellent range being strongly influenced by the nature of the side-chains attached (Table 4). The dimethylsulfonamide analogue **53**, for instance, showed a solubility at the lower end of 22 μg/mL, whereas compound **50** containing a cyclobutylamide group exhibited excellent solubility with >638 μg/mL, respectively. Surprisingly, exchange of the *p*-Cl in compound **38** for a *p*-F atom (**39**) had a dramatic impact on the aqueous solubility increasing it from 44 μg/mL to >452 μg/mL. The permeability in the Pampa assay was found to be high for all compounds with the only exemption of sulfonamide **2** (Table 4).[28] Lipophilicity values range from 1.33 (**61**) to 3.63 (**39**) and again vary depending on the substitution pattern.

In general, the correlation of rat in vitro microsomal and hepatocyte data to in vivo clearance values was very good (data not shown). Based on in vitro clearance data, a few compounds were selected for single dose pharmacokinetic studies in rat. The results are summarized in Table 5 and show medium to high clearance indicating in some cases extrahepatic elimination pathways (**16**, **18**, and **22**, respectively). Incorporation of a chlorine substituent on the benzoxazole moiety as well as on the *para* position of the benzyl group (**56**) led to a significant reduction of clearance and improvement of oral bioavailability. Parent benzoxazole **16**, ethylsulfone **18** as well as sulfonamides **22** and **57** showed very poor bioavailability, which might be explained by a combination of medium to high clearance and substantially increased polarity moving them into a critical range for absorption. Interestingly, in the Pampa permeation assay compounds **16**, **18**, and **22** exhibited high and compound **57** medium to high permeability (Table 4) which indicates that absorption should not be the primary limiting factor. In contrast, the less polar amide compound **58** (log D = 2.96) showed higher bioavailability and medium plasma clearance, but unfortunately also very high hERG receptor activity (IC_{50} = 0.43 μM). The attachment of a carboxylic acid moiety on the benzoxazole periphery resulted in **59** which is devoid of any hERG activity (IC_{50} >10 μM) and, due to the second *m*-ethoxy group, very selective against hH1 (>200-fold). This modification also improved clearance, but again at the expense of lower oral bioavailability. In contrast, the carboxylic acid in regioisomers **60** and **61** did not significantly affect the volume of distribution of the resulting zwitterions and allowed for a significant reduction of the clearance values. This modification had furthermore a very beneficial influence on bioavailability and half-life which for these compounds is now within a satisfactory range.

The synthesis of benzoxazole analogs followed a preparation similar to benzothiazole **3** (Scheme 1). The synthesis commences with a nucleophilic aromatic substitution of the chlorine atom in benzoxazole **62** by 4-amino-piperidine-1-carboxylic acid ethyl ester to give intermediate **63**. For some derivatives reaction rates may be enhanced significantly by conducting the reaction at elevated temperatures using either microwave irradiation or conventional heating. Deprotection of the ethylcarbamate with HBr afforded the dihydrobromide salt **64** which under reductive amination conditions was reacted with the corresponding aldehyde to yield the desired benzoxazole compound, respectively. Alternatively, target structures can also be prepared by alkylation of piperidine **64** with appropriately substituted benzyl halides.

In conclusion, the SAR of the benzoxazole series has been expanded and a number of potent and selective hSST5 antagonists were identified. The zwitterionic compounds **60** and **61** combine high activity, excellent selectivity against hH1, hSST1, h5-HT$_{2B}$, and hERG, favorable physicochemical properties and good single-dose PK properties in rat. These further improved compounds might be useful tools in the in vivo assessment of the biological role of the SST5 receptor.

Acknowledgments

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17. A CHO cells stably expressing human SSTR5 (GenBank accession number 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., IC50) was deduced from concentration-versus-cAMP graphs.

18. Compounds were purified by preparative HPLC on a Phenomenex Aqua 5 μm C18 125A 60 × 21.20 mm column equipped with a Gilson Liquid Handler 215 autosampler, two Varian Prep Star Model SD-1 pumps, a Sedex ELSD 75 lightscatter and a Dionex UVD 340S UV detector.
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20. A single crystal was mounted in a loop and cooled to 100 K in a nitrogen stream. Data were collected on a STOE Imaging Plate Diffraction System (STOE, Darmstadt) with Mo-radiation (0.71 Å) and data processed with STOE IPDS-software. The crystal structure was solved and refined with the program SHELXTL (Bruker AXS, Karlsruhe).
21. Crystallographic data for the structure in this Letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 721103. 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].
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26. The hERG current measurement was performed at automated patch clamp system PatchXpress 7000A (MDS Analytical Technologies, USA). Electrophysiologcal recordings of K^+ currents (IK_{hERG}) were conducted at room temperature (22–25 °C) using Aviva Bioscience SealChip16™ (USA). CHO cells stably expressing hERG K^+ channels (Roche, USA) were stimulated by a voltage pattern to activate hERG channels. The IK_{hERG} currents were recorded under control conditions and after compound application in the extracellular buffer (NaCl 150 mM, KCl 4 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1 mM, HEPES 10 mM, pH 7.4 with NaOH, 300–310 mOsm). Offline analysis of the peak tail current was performed using DataXpress2 software (MDS Analytical Technologies, USA). Concentration-response curves were fitted by non-linear regression analysis and the IC_{50} values were reported.
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