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Ergoline derivatives as highly potent and selective antagonists at the somatostatin sst_1 receptor

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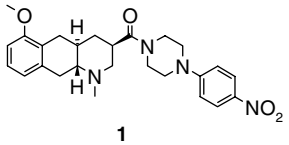
Abstract—Non-peptidic compounds containing the octahydro-indolo[4,3-*fg*]quinoline (ergoline) structural element have been optimized into derivatives with high affinity (pK_d r $sst_1 > 9$) and selectivity (>1000-fold for h sst_1 over h sst_2–h sst_5) for the somatostatin sst_1 receptor. In functional assays, these ergolines act as antagonists at human recombinant sst_1 receptors. Pharmacokinetic studies in rodents reveal good oral bioavailability and brain penetration for some of these compounds.

Somatostatin (somatotropin-release-inhibiting factor, SRIF) is a widely distributed peptide hormone/neurotransmitter[1] that occurs in two biologically active forms, a tetradecapeptide $SRIF_{14}$ and a 28-amino acid peptide $SRIF_{28}$. To date, five somatostatin receptor subtypes (sst_1 to sst_5) have been cloned and characterized, all belonging to the G-protein-coupled receptor superfamily.[2–4] In order to elucidate the function of the different SRIF receptor subtypes and to evaluate the potential of somatostatin receptor ligands as therapeutic agents, there is a need for non-peptidic, metabolically stable, potent and subtype selective SRIF receptor agonists and antagonists.[5] In two recent publications[6,7] we have described the identification of potent and selective non-peptidic somatostatin sst_1 receptor antagonists based on the octahydrobenzo[*g*]quinoline (obeline) core structure (e.g., **1**). Herein, we present the optimization of a second class of sst_1 receptor antagonists which are based on the octahydro-indolo[4,3-*fg*]quinoline (ergoline)[8] structural moiety (e.g., **2**). The main goal of this effort was to identify structurally diverse sst_1 antagonists which retain the excellent potency, selectivity and PK properties of the obelines, but are synthetically more easily accessible. Ergolines are bioisosteres of obelines,[9] and their core moiety is known to be readily available from the natural products, lysergic acid or paspalic acid.

A set of compounds from our corporate compound collection containing the ergoline substructure was screened in a radioligand binding assay for the rat sst_1 and sst_2 receptors. Indeed, several of these ergolines showed appreciable affinity for the sst_1 receptor, the most potent being the pyridin-2-yl-piperazine derivative **2** with a pK_d of 7.85 for r sst_1 and good selectivity over r sst_2 ($pK_d = 4.75$).



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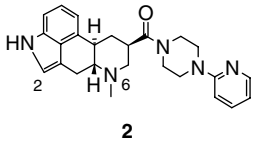

2

In this paper, we present the structure–activity relationship that was explored for lead compound **2**, focusing on positions 2 and 6 of the ergoline ring system, as well as the aryl piperazine moiety. Affinities to the sst_1 and sst_2 receptors were determined in a radioligand binding assay performed in rat cortex membranes using [^{125}I]SRIF-14 in the presence of 120 mM NaCl (sst_1)[10] or [^{125}I][Tyr^3]octreotide (sst_2).[11]

For derivatives with halogen substituents in position 2, sst_1 affinity was retained (**3** and **5**, Table 1) or even increased in the case of bromine (**4**), while no major effect on selectivity over sst_2 was observed. Of four other variations in this position (**6**–**9**), only the thiomethyl moiety of **7** led to an improvement in sst_1 affinity. Due to con-

Keywords: Somatostatin; GPCR; Somatostatin sst_1 receptor; Selective sst_1 receptor antagonists; Ergolines; Lysergic acid derivatives.
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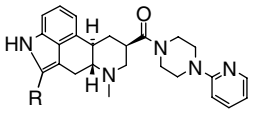
Table 1. Binding affinities of ergoline derivatives to rat sst_1 and sst_2 receptors (variations at position 2)



R	Compound	pK_d r sst_1[a]	pK_d r sst_2[a]
–H	**2**	7.85 ± 0.08	4.75 ± 0.16
–Cl	**3**	7.89 ± 0.12	n.d.
–Br	**4**	8.35 ± 0.18	4.78 ± 0.06
–I	**5**	7.78 ± 0.08	5.25 ± 0.02
–OH	**6**	6.62 ± 0.05	5.07 ± 0.06
–SMe	**7**	8.62 ± 0.28	5.07 ± 0.21
–SOMe	**8**	5.58 ± 0.07	5.03 ± 0.07
$–SO_2Me$	**9**	6.51 ± 0.06	5.11 ± 0.07

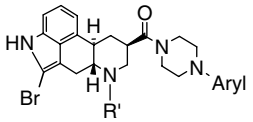
[a] Mean ± SEM. Number of experiments: $n = 3–6$.

cerns with the metabolic stability of **7**, the 2-bromo-substituted ergoline core of **4** was chosen for further optimization.

Removal of the *N*-methyl substituent of the 2-bromo-ergoline core (**10**), as well as its replacement with longer or functionalized alkyl groups (**11**–**14**), led to a dramatic loss of sst_1 affinity (Table 2). Also, an ethyl group did not confer an improvement regarding potency and selectivity, as demonstrated with the pair **15/16**. The methyl group as it is found in ergot natural products was therefore retained in this position for all further derivatives.

Investigation of the structure–sst_1 affinity relationship for the aryl piperazine moiety revealed that independent of the nature of the substituent in position 2 (R = H, Cl or Br), the 1-methyl-1H-pyridin-2-one (**17**–**19**), benzo[1,2,5]oxadiazole (**29** and **30**) and [1,2,5]thiadiazolo-[3,4-*b*]pyridine (**31**–**33**) moieties conferred the most promising sst_1 affinity and sst_2 selectivity values (Table 3). These findings are well in line with the SAR data generated in the obeline series,[6,7] with the notable exception

Table 2. Binding affinities of ergoline derivatives to rat sst_1 and sst_2 receptors (variations at position 6)



R′	Aryl	Compound	pK_d r sst_1[a]	pK_d r sst_2[a]
–H	2-Pyridyl	**10**	6.77 ± 0.07	5.10 ± 0.07
–Me	2-Pyridyl	**2**	8.35 ± 0.18	4.78 ± 0.06
–*n*Bu	2-Pyridyl	**11**	5.94 ± 0.05	n.d.
	2-Pyridyl	**12**	5.76 ± 0.05	n.d.
	2-Pyridyl	**13**	5.83 ± 0.04	n.d.
	2-Pyridyl	**14**	5.63 ± 0.07	n.d.
–Me	3,4-Di-F-phenyl	**15**	9.31 ± 0.06	5.09 ± 0.05
–Et	3,4-Di-F-phenyl	**16**	9.01 ± 0.05	4.88 ± 0.09

[a] Mean ± SEM. Number of experiments: $n = 3–6$.

of the 4-nitrophenyl moiety which led to derivatives with very high affinities in the obeline class, but much less so in the ergoline series (**25**–**27**). Based on their impressive rat receptor binding profile, as well as structural considerations (maximally diverse aryl piperazine moiety), the two 2-bromo-ergoline derivatives **19** and **30** were chosen for further profiling.

All ergoline derivatives described herein were prepared starting from biotechnologically available, enantiomerically pure paspalic acid **34**. The syntheses of somatostatin ligands **19** and **30** are outlined in Scheme 1 as representative examples. Hydrogenation of **34**, followed by esterification, regioselective bromination in position 2 and saponification of the ester group afforded acid **36**. Amide formation with the two respective aryl piperazines[7] was affected using either carbonyl-diimidazole or propyl-phosphonic anhydride to afford the final products in overall yields of 44% and 43%, respectively.

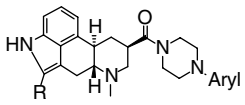
The enantiomers of **19** and **30** were prepared starting from the methyl ester of natural (−)-lysergic acid **37** (Scheme 2). Racemization of **37** to acid *rac*-**38** was achieved by heating with hydrazine,[12,13] followed by hydrolysis of the intermediate racemic hydrazide. *rac*-**38** was converted to *rac*-**19** and *rac*-**30**, respectively, in a sequence analogous to the one described for **19** and **30**. Separation by HPLC on chiral stationary phase afforded the desired (+)-enantiomers *ent*-**19** and *ent*-**30**, respectively, along with the corresponding (−)-enantiomers.

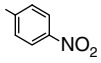
Compounds **19** and **30** were tested for their binding affinities to the human recombinant somatostatin receptors h sst_1–h sst_5.[14] Both compounds displayed affinities to the h sst_1 receptor in the low nanomolar range (pK_d = 8.76 and 8.91, respectively) and excellent selectivities (>1000-fold) over the other four somatostatin receptors (Table 4). In extensive radioligand binding studies, **19** and **30** proved to be selective over a range of 40 different neurotransmitter receptors and ligand-gated ion channels, with highest affinities found for the h dopamine D_2 receptor (pK_d = 7.25 and 6.90, respectively), the h dopamine D_4 receptor (pK_d = 6.91 and 7.11, respectively) and the h $5HT_{1A}$ receptor (pK_d = 7.30 and 7.55, respectively).[15]

Binding studies with the enantiomers of **19** and **30** showed that only derivatives with the natural (−)-configuration show appreciable sst_1 affinity: **19** is >1000-fold more potent at native rat or recombinant human sst_1 receptors than its (+)-antipode *ent*-**19** (Table 4). Similarly, **30** shows >400-fold higher affinity than *ent*-**30** at rat and human sst_1 receptors.

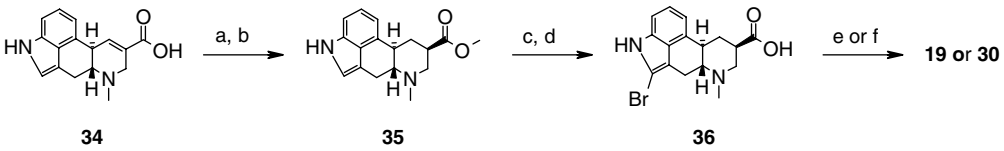
In functional assays, sst_1 ligands **19** and **30** act as antagonists at human recombinant somatostatin sst_1 receptors negatively coupled to cAMP accumulation in CCL39 cells.[16] They cause a concentration-dependent, surmountable antagonism of SRIF-14 induced inhibition of forskolin-stimulated adenylate cyclase activity with pK_b values of 7.85 ± 0.30 ($n = 5$) and 7.62 ± 0.23

Table 3. Binding affinities of ergoline derivatives to rat sst_1 and sst_2 receptors (variations of piperazine substituent)

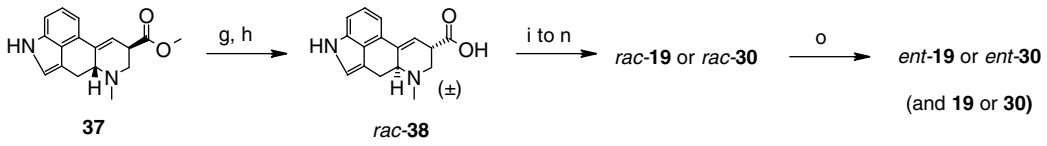


Aryl	R = H			R = Cl			R = Br		
	Compound	pK_d r sst_1[a]	pK_d r sst_2[a]	Compound	pK_d r sst_1[a]	pK_d r sst_2[a]	Compound	pK_d r sst_1[a]	pK_d r sst_2[a]
	**2**	7.85 ± 0.08	4.75 ± 0.16	**3**	7.89 ± 0.12	n.d.	**4**	8.35 ± 0.18	4.78 ± 0.06
	**17**	9.12 ± 0.08	5.11 ± 0.06	**18**	9.41 ± 0.07	4.31 ± 0.21	**19**	9.69 ± 0.05	4.71 ± 0.04
				**20**	9.42 ± 0.03	4.92 ± 0.14	**21**	9.55 ± 0.02	4.58 ± 0.03
	**22**	7.71 ± 0.07	5.66 ± 0.02	**23**	8.38 ± 0.01	5.23 ± 0.05	**24**	8.26 ± 0.06	5.70 ± 0.02
	**25**	8.41 ± 0.02	5.50 ± 0.05	**26**	8.90 ± 0.04	5.05 ± 0.13	**27**	8.87 ± 0.07	5.14 ± 0.06
				**28**	9.22 ± 0.06	4.89 ± 0.04	**15**	9.31 ± 0.06	5.09 ± 0.05
				**29**	9.41 ± 0.05	5.29 ± 0.20	**30**	9.43 ± 0.10	5.19 ± 0.10
	**31**	8.99 ± 0.03	5.52 ± 0.05	**32**	9.62 ± 0.08	5.41 ± 0.05	**33**	9.43 ± 0.09	5.34 ± 0.13

[a] Mean ± SEM. Number of experiments: $n = 3$–6.



Scheme 1. Synthesis of ergoline sst_1 receptor antagonists **19** and **30**. Reagents and yields: (a) H_2, Pd/C (>90%); (b) MeOH/H_2SO_4 (95%); (c) NBS, CH_2Cl_2 (77%); (d) NaOH, H_2O/MeOH/THF (91%); (e) 1-methyl-6-piperazin-1-yl-1H-pyridin-2-one, carbonyl-diimidazole, DMF (74%); (f) 5-piperazin-1-yl-benzo[1,2,5]oxadiazole, propyl-phosphonic anhydride, pyridine/DMF (72%).

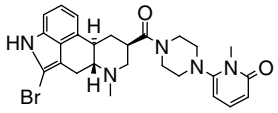


Scheme 2. Synthesis of unnatural (+)-enantiomers *ent*-**19** and *ent*-**30**. Reagents and yields: (g) hydrazine, H_2O, 20 min, 140° (50%); (h) KOH/H_2O, 2 h, 130° (100%); (i) H_2, Pd/C (79%); (j) MeOH/H_2SO_4, 2 h, 70° (75%); (k) tris-2-pyrrolidone-perbromide, THF, 15 h, rt (50%); (l) KOH, Dioxane, 15 h, rt (63%); (m) 1-methyl-6-piperazin-1-yl-1H-pyridin-2-one, propyl-phosphonic anhydride, pyridine/DMF, 15 h, rt (61%); (n) 5-piperazin-1-yl-benzo[1,2,5]oxadiazole, propyl-phosphonic anhydride, pyridine/DMF, 15 h, rt (82%); (o) HPLC on chiral stationary phase.

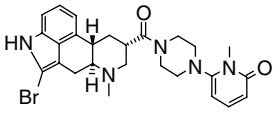
($n = 12$), respectively. Both compounds also act as antagonists at human recombinant sst_1 receptor driven luciferase activity[17]; they inhibit SRIF-28 induced luciferase activity with a pK_b value of 8.85 ± 0.19 ($n = 3$) for **19** and 9.42 ± 0.15 ($n = 3$) for **30**, and are devoid of intrinsic activity.

Pharmacokinetic studies in rats showed that both compounds are well absorbed after oral administration (absolute bioavailability of 87% and 48% for **19** and **30**, respectively). While concentrations of **19** in rat brain after oral or intravenous administration were below the limit of detection, most probably due to an efflux system

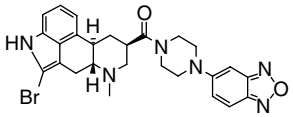
Table 4. Compounds **19**, *ent*-**19**, **30** and *ent*-**30**: physicochemical parameters and affinities for different somatostatin receptor subtypes



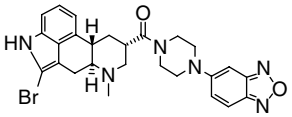
19



ent-**19**



30



ent-**30**

Compound	Mp[b]	$[\alpha]_D^{20}$[c]	pK_d[a]						
			r sst_1	r sst_2	h sst_1	h sst_2	h sst_3	h sst_4	h sst_5
19	275° (decomp.)	−96.6°	9.69 ± 0.05	4.71 ± 0.04	8.76 ± 0.01	4.91 ± 0.05	5.53 ± 0.01	5.31 ± 0.05	4.25 ± 0.10
ent-**19**	250° (decomp.)	+98.6°	6.38 ± 0.02	4.69 ± 0.02	5.71 ± 0.02	5.03 ± 0.03	5.27 ± 0.04	5.09 ± 0.01	4.16 ± 0.09
30	266° (decomp.)	−82.7°	9.43 ± 0.10	5.19 ± 0.10	8.91 ± 0.04	5.17 ± 0.05	5.63 ± 0.04	5.44 ± 0.06	5.19 ± 0.01
ent-**30**	260° (decomp.)	+84.6°	6.74 ± 0.03	4.90 ± 0.04	6.30 ± 0.01	5.41 ± 0.01	5.14 ± 0.05	5.25 ± 0.03	4.63 ± 0.02

[a] Mean ± SEM. Number of experiments: $n = 3–6$.
[b] Free base.
[c] Free base (DMF, $c = 0.5$).

at the blood–brain barrier, **30** readily entered the brain. Oral administration of 30 μmol/kg **30** to rats led to brain/plasma ratios ranging from 0.7 after 10 min to 1.7 after 24 h. Details as well as in vivo pharmacology data will be published elsewhere in due course.

In conclusion, we have identified sst_1 antagonists of a novel ergoline-type structural class with sub-nanomolar affinities to somatostatin sst_1 receptors and >1000-fold selectivity over other somatostatin receptor subtypes. They behave as full antagonists in functional assays and show promising PK properties in rodents.

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