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Novel Non-Peptide Ligands for the Somatostatin sst_3 Receptor

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Abstract—A series of imidazole derivatives has been prepared using high throughput parallel synthesis. Several compounds showed high affinity (K_i in 10^{-6}–10^{-8} M range) and selectivity at recombinant human somatostatin receptor subtype 3 ($hsst_3$).

Somatostatin (SRIF) is a widely distributed peptide that exhibits multiple biological functions such as inhibition of the release of growth hormone, insulin, glucagon and gastrin.[1,2] SRIF also possesses antiproliferative and neurotransmitter activities.[3] Five somatostatin receptors (sst_{1-5}), all members of the G-protein coupled family, have been cloned,[4–6] however, the precise functional activities of these receptors remain to be determined.[7] Peptide analogues of SRIF, such as SMS 201-995 (Octreotide)[8] and BIM 23014 (Lanreotide), have been synthesized and developed for clinical use. Further studies on cyclic or linear peptide analogues[9,10] are still being reported but poor oral bioavailability, rapid proteolysis and the relatively high cost of synthesis can be considered as limitations for their clinical exploitation. Consequently, considerable efforts have been reported on the development of peptidomimetics[11,12] of SRIF which have the potential for oral bioavailability. Pioneering work on non-peptide structures designed to mimic the peptide backbone of small cyclic peptides with β-D-glucose,[13] xylofuranose,[14] mannitol,[15] or benzodiazepinone[16] scaffolds have yielded compounds with affinities for SRIF receptors in the micromolar range. More recently, non-peptide structures with high affinity and selectivity for the human somatostatin receptor subtypes have been identified, namely on sst_2[17] and sst_4.[18,19] In the present article, we report the design, synthesis and biological evaluation of new potent ligands for the sst_3 receptor.

Imidazole dipeptide mimetics or 'dipeptide azoles' were first described in the context of substance P antagonists,[20] and more recently have been used to develop endothelin antagonists,[21] HIV protease inhibitors[22] and matrix metalloproteinase inhibitors.[23] In the current application, however, we were not interested in inserting the dipeptide azole in a peptide sequence, but rather to use it as an organic scaffold for rapid parallel synthesis methods.

The derivatives **1** were prepared from commercially available *N*-protected (Boc and Z) amino acids. Condensation of the cesium salts with α-bromoketones gave access to ketoester intermediates that were then cyclized with excess of ammonium acetate in refluxing xylene with azeotropic removal of water. Removal of the urethane *N*-protecting group (PG) was carried out either by hydrogenolysis (palladium on charcoal in ethanol) for the benzyloxycarbonyl group (Z) or by acidic treatment (hydrochloric acid in ethyl acetate) for the *tert*-butyloxycarbonyl group (Boc) yielding compounds **2** (Scheme 1) which were isolated with yields ranged from 40 to 80% depending on the nature of R2 group. A variety of aromatic, aliphatic and basic amino acids were used in the synthesis of **2**: those present in the SRIF β turn (Phe7, Trp8, Lys9) were especially targeted.

Among the enantiopure or racemic imidazole derivatives **1** and **2** synthesized, indolyl derivatives exhibited significant inhibition percentages (at 10^{-5} M) of radioligand ([^{125}I]Tyr^{11}-SRIF) binding to the sst_3 receptor expressed in isolated membranes from CHO-K1 cells.[24] A chiral preference for a D-Trp starting material was discovered comparing the K_i values of **1a** and **1c** at the

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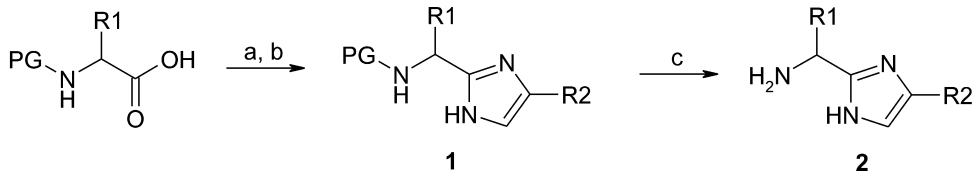
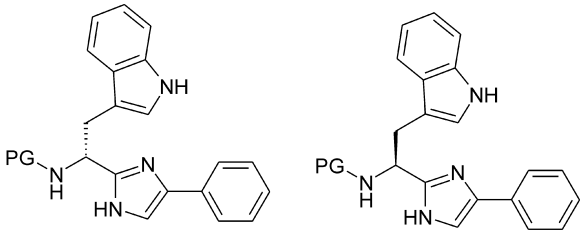


Scheme 1. Reagents and conditions: (a) (i) Cs_2CO_3 (0.5 equiv), DMF/H_2O or EtOH/H_2O; (ii) $BrCH_2COR2$ (1 equiv), DMF; (b) ammonium acetate (20 equiv), xylene, reflux; (c) for PG = Z: Pd/C, EtOH, H_2, and for PG = Boc: AcOEt, HCl 3 N.

sst_3 receptor (210 and 3200 nM, respectively). The selectivity of **1a** was calculated to be superior to 47 over the other somatostatin receptors. Then we have observed that compound **1b** (K_i = 1400 nM) was about 7-fold less potent than **1a** and that the removal of the protecting group induced a decrease of potency at sst_3 receptor (K_i of compound **2a** superior to 5000 nM) suggesting a great influence of substitution on primary amine present in **2a** for binding to sst_3 receptor. In addition, a parallel investigation on the nature of the R2 group (*tert*-Bu or substituted aromatic), starting from Boc-D-Trp, allowed us to conclude that an unsubstituted phenyl moiety was a key element for affinity at sst_3 (data not shown).



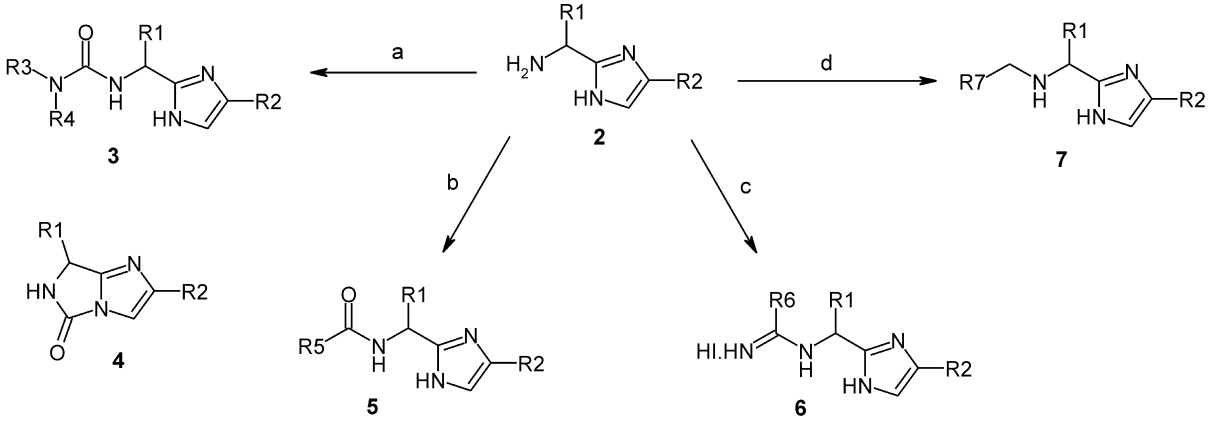
PG = Boc **1a**
PG = Z **1b**
PG = H **2a**

PG = Boc **1c**

These initial encouraging results prompted us to develop procedures for rapid parallel acylations of imidazole derivatives **2** and particularly from **2a**. These chemistries were carried out in solution using resin scavenger methodologies (Scheme 2).[25–27]

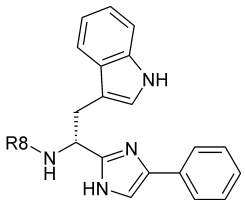
Ureas **3** were synthesized by reaction with an excess of isocyanate followed by quenching with an aminomethylated resin. Alternatively, reaction with *N,N′*-disuccinimidylcarbonate,[28,29] yielded bicyclic compounds **4** which were isolated and fully characterized. Compounds **4**, in excess, were submitted to nucleophilic attack at room temperature with primary and secondary amines, followed by quenching with aminomethylated resin to yield additional ureas **3** with increased diversity. Amides **5** were prepared by peptide coupling chemistry using excess carboxylic acid (preactivated with carbonyldiimidazole) followed by quenching with aminomethylated resin and purification through a silica gel pad. Condensation of imidazole derivatives **2** with excess thioimidates[30] at room temperature yielded amidines **6** after quenching with aminomethylated resin. We have observed that 2-propanol was the solvent of choice for this conversion to proceed in good purity and yield, compared to ethanol or dimethylformamide. Condensation of excess of imidazole derivatives **2** with aldehydes yielded imines which were reduced in the presence of Amberlite® IRA-400 borohydride. Excess primary amine **2** was removed by addition of carboxaldehyde resin giving access to pure secondary amines **7**.

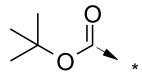
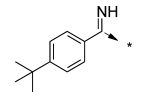
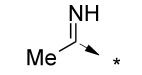
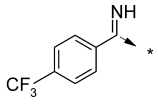
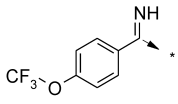
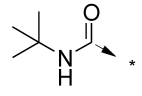
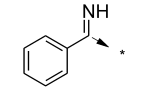
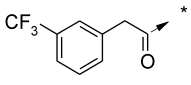
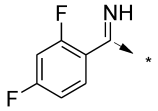
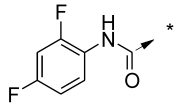
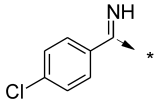
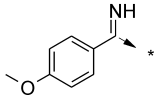
We were able to prepare rapidly greater than 1500 compounds using these solution-phase strategies with UV purity of ca. 90%.[31] According to the biological results described above, the greatest affinities for the sst_3 receptor were obtained from derivatives of 2-{(1*R*)-1-amino-2-[indol-3-yl]ethyl}-4-phenyl-1*H*-imidazole (**2a**, R8 = H, K_i values are summarized in Table 1). No compound in the series of secondary amines was more potent than **1a**. In the series of ureas, the corresponding aliphatic urea **12** was 2-fold less potent than carbamate **1a** whereas aromatic urea **16**, our best compound found in this series, exhibited a slightly better potency but a lower selectivity (15-fold). Our best amide derivative **14**



Scheme 2. Reagents and conditions: (a) R3NCO (1.2 equiv), dichloromethane, then aminomethylated resin, dichloromethane (R4 = H) or (i) *N,N′*-disuccinimidylcarbonate, acetonitrile; (ii) R3NHR4 (0.9 equiv), acetonitrile, then aminomethylated resin, tetrahydrofuran; (b) $R5CO_2H$ (1.1 equiv), carbonyldiimidazole (1.1 equiv), tetrahydrofuran, then aminomethylated resin; (c) R6NHSMe·HI (1.2 equiv), 2-propanol, then aminomethylated resin, tetrahydrofuran; (d) R7CHO (0.85 equiv), methanol, borohydride resin (Amberlite® IRA-400), then carboxaldehyde resin, dichloromethane.

Table 1. Inhibition constants (K_i) of substituted imidazole derivatives on human sst_3 receptor



Compound	R8	Purity (%)[a]	K_i (nM)				
			sst_1	sst_2	sst_3[b]	sst_4	sst_5
1a		99	> 10,000	> 10,000	210 ± 40	> 10,000	> 10,000
8[c]		94	1880	5920	1400 ± 400	> 10,000	1870
9[c]		90	2840	> 10,000	700 ± 40	5920	1190
10[c]		76	3430	6040	500 ± 90	6660	2120
11[c]		84	2570	4220	410 ± 110	3970	1130
12		97	> 10,000	> 10,000	400 ± 50	> 10,000	> 10,000
13[c]		91	1520	8680	250 ± 50	7090	2360
14		99	9100	> 10,000	240 ± 20	> 10,000	> 10,000
15[c]		73	1550	> 10,000	200 ± 60	> 10,000	2250
16		99[d]	2600	> 10,000	170 ± 10	> 10,000	3650
17[c]		86	1730	3470	79 ± 12	3840	691
18[c]		91	2310	6180	36 ± 5	5010	1620
SRIF-14	—	—	0.36	0.049	0.14 ± 0.02	0.59	0.14

[a]UV purity of crude products was determined by reverse phase HPLC (see ref 31).
[b]Mean ± SEM. Number of experiences: $n = 2–3$.
[c]Iodohydrate salts.
[d]Compound purified on a small pad of silica gel because of insufficient initial purity (58%).

exhibited both potency and selectivity comparable to **1a** for sst_3 receptor. In the amidine series, the phenylamidine derivative **13** is slightly less potent than **1a**. Removal of the aromatic moiety of **13** induced a notable decrease of potency (compound **9** is about 3-fold less potent than **1a**). Both the nature and the position of substitution on the phenyl moiety of amidine **13** appeared to play crucial roles for biological activity. Whether 2,4-difluoro substitution gave compound **15** with comparable potency than **1a**, *para*-substitution seemed to have more impact on potency. We have thus identified an amidine derivative **18** with a *p*-methoxy substitution which is about 6-fold more potent than **1a** whereas a bulker group (*p*-^{t}Bu) present in amidine derivative **8** clearly decreased the affinity. *p*-CF_3 (**10**) and *p*-OCF_3 (**11**) amidine derivatives were, respectively, about 14- and 11-fold less potent than **18**. Then *p*-Cl substituted compound **17** was both 2-fold less potent and less selective (about 8-fold) than **18**.

Finally our best compound **18** with *p*-methoxy substitution on the phenylamidine moiety showed both good selectivity (45-fold) and an affinity ($K_i = 36$ nM) for sst_3 receptor comparable to previously published data (L-796,778, selectivity 50 and $K_i = 24$ nM).[32]

In a functional assay based on the accumulation of adenosine cyclic 3′-5′-monophosphate (cAMP) in CHO-K1 cells expressing human sst_3 receptors,[33] derivative **18** was not able to reduce the cAMP production elicited by forskolin. It blocked the inhibitory effect of SRIF-14 on forskolin-induced cAMP accumulation with a K_b value (concentration that shifts SRIF-14 dose-response 2-fold) of 420 nM, demonstrating its antagonist property.

In conclusion, we have identified a novel series of selective ligands for the human somatostatin subtype 3 receptor with affinities in the nanomolar range. These imidazole derivatives, obtained by rapid parallel synthesis from amino acids, may serve as new pharmacological tools for future investigations on this biological target. Further optimization of these leads to increase affinity while retaining selectivity will be reported in due course.

Acknowledgements

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33. CHO-K1 confluent cells expressing the human sst_3 receptor were washed twice with RPMI 1640 containing 0.2% BSA at 37 °C and incubated for 5 min with 1 μM isobutylmethylxanthine at 37 °C. Then, they were incubated for 20 min at 37 °C with 1 μM forskolin and increasing concentrations of tested compound (agonist activity) or with 1 μM forskolin and increasing concentrations of SRIF-14 with or without the tested compound (antagonist activity) at 0.1, 1 or 10 μM. Rapid aspiration and addition of 0.1 M HCl stopped the reaction (Nickols, G. A. *Eur. J. Pharmacol*. **1985**, *116*, 137). Cyclic AMP levels were determined by radioimmunoassay using Flash Plates (NEN Life Science Products).