Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 741–745

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of Substituted Imidazopyrazines as Ligands for the Human Somatostatin Receptor Subtype 5

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Received 29 May 2000; revised 12 January 2001; accepted 19 January 2001

Abstract—A new preparation of trisubstituted imidazopyrazines and dihydroimidazopyrazines via parallel synthesis using aminoacids and bromoketones resulted in the discovery of non-peptidic sst_5 selective agonists.

Somatostatin (SRIF) is a cyclic tetradecapeptide that inhibits the release of various peptide hormones including growth hormone (GH),[1] insulin, glucagon[2] and gastrin.[3] SRIF has been shown to inhibit cell proliferation[4] and can also act as a neurotransmitter.[5] The biological effects of SRIF are mediated through five distinct G protein-coupled receptor subtypes (sst_{1-5})[6] for which the respective physiological role remains to be fully determined. The therapeutic applications of somatostatin are limited by its low metabolic stability in vivo, and this has led to the development of more stable peptidic analogues such as the hexapeptide seglitide[7] (MK 678, **I**, Fig. 1) and the cyclic octapeptides octreotide[8] (Sandostatin®, **II**, Fig. 1) and lanreotide[9] (Somatuline®, **III**, Fig. 1). It can be argued that the clinical use of this class of therapeutics is limited by their lack of oral bioavailability.[14] Thus, the discovery of orally active compounds which interact with somatostatin receptors could be potentially beneficial.

Extensive investigations of structure–activity relationships of peptide based SRIF analogues have shown that the tetrapeptide sequence Phe[7]-D-Trp[8]-Lys[9]-Thr[10] is essential for biological activity.[10,11] From the results of NMR and crystallographic studies,[12] several analogues have been shown to adopt a β II′ turn around the Trp[8]-Lys[9] dipeptide, and non-peptide mimics involving the use of a sugar[13–15] or a benzodiazepinone[16] core to

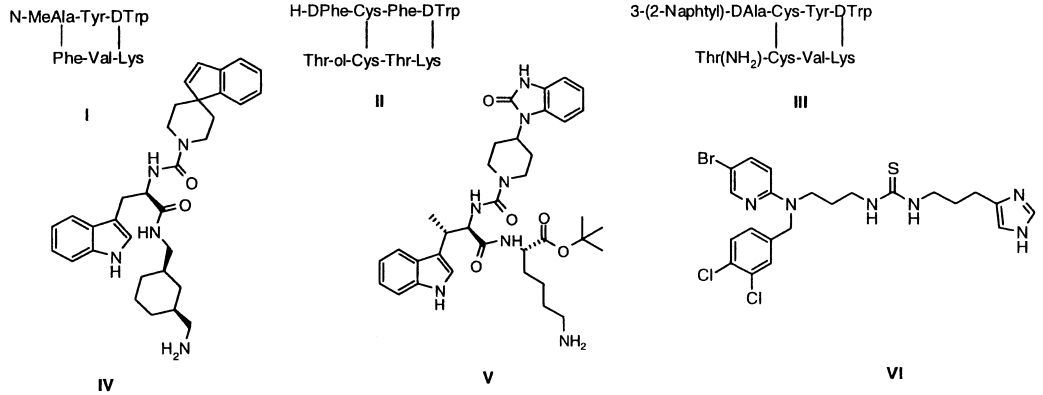

Figure 1. Structures of MK 678 (**I**), octreotide (**II**), lanreotide (**III**), L-054,264 (**IV**), L-054,522 (**V**), and NNC 26-9100 (**VI**).

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0960-894X/01/$ - see front matter
PII: S0960-894X(01)00051-8

mimic the β-turn have been described as ligands for somatostatin receptors. Recently, tryptophan derivatives (L-054,264 (**IV**), L-054,522 (**V**), Fig. 1) were reported to be potent and selective sst_2 agonists with inhibition constants (K_i) of 1.6 and 0.01 nM, respectively.[17,18] 2-Pyridylthioureas have also been identified as potent sst_4 agonists. Among them, NNC 26-9100 (**VI**, Fig. 1) exhibited a K_i value of 6 nM at the sst_4 receptor and shows good selectivity with respect to other SRIF receptor subtypes.[19]

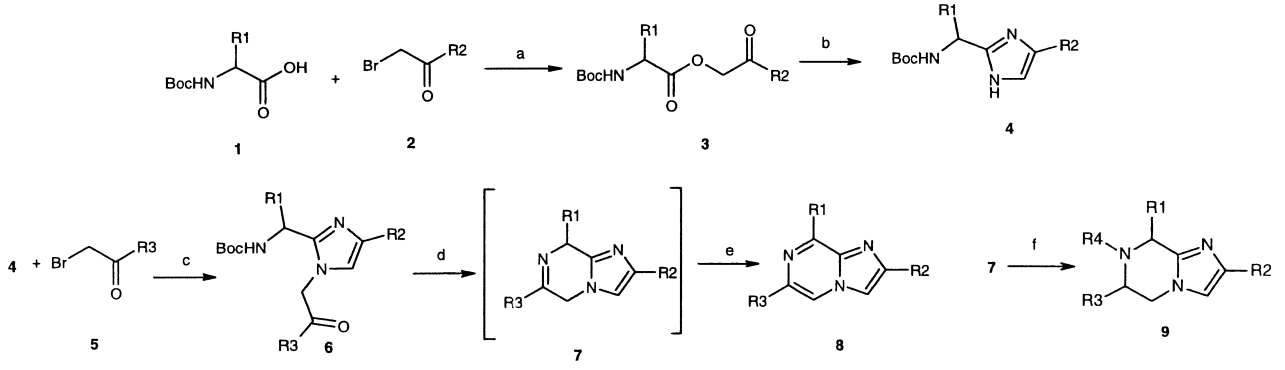
Knowing that dipeptides can be mimicked by five-membered heterocycles such as thiazoles, oxazoles or imidazoles[20] and also that the imidazole ring is an excellent amide isostere,[21] we focused our synthetic efforts on the development of an imidazole chemistry leading to a series of imidazopyrazine derivatives. One of our goals was to access this scaffold through parallel synthesis in order to produce rapidly a large number of structurally diverse compounds. As an example, we wish to report the synthesis of trisubstituted imidazopyrazines and their binding profiles at somatostatin receptors, and particularly the sst_5 subtype.

Chemistry

The synthesis of imidazo[1,2-*a*]pyrazine has been the subject of a recent review.[22] Several approaches, including the condensation of an α-halocarbonyl compound with a 2-aminopyrazine derivative,[23] and the reaction of an α-aminoalcohol with a chloropyrazine, followed by oxidation of the alcohol to a ketone and dehydrative ring closure[24] have been described.

We developed an original strategy (Scheme 1) where the imidazole core is first created from an *N*-protected α-aminoacid,[20] and alkylated with an α-bromoketone. Subsequent cleavage of the *N*-protecting group cyclizes to the desired bicyclic species. As described in Scheme 1, the *N*-protected α-aminoacid (**1**) was transformed into its cesium salt and condensed with an appropriate α-bromoketone (**2**). Cyclization of the resulting keto-ester (**3**) using ammonium acetate in refluxing xylene afforded the imidazole derivative (**4**), after work up and purification by flash chromatography. Following this procedure, more than 15 imidazole derivatives (**4**) were prepared. *N*-alkylation of the imidazole ring was then carried out using a solution-phase parallel strategy. The nucleophilic character of the free nitrogen atom of imidazole was enhanced by polymer supported *N*-methyl morpholine,[25] which permitted substitution with α-bromoketone (**5**). The resulting *N*-alkylated compound (**6**) was isolated after addition of aminomethyl polystyrene resin to the reaction mixture to trap any excess of bromoketone. The acidic cleavage of the *tert*-butoxy carbonyl group allowed spontaneous cyclization to yield the dihydroimidazopyrazine (**7**). When R1 was a 3-methyl-indole group, the imidazopyrazine (**7**) could be purified and characterized. In all other cases, the intermediate (**7**) was not stable and was oxidized to the corresponding fully aromatized imidazopyrazine (**8**) either by air oxidation in methanolic solution or by addition of an oxidation agent such as manganese dioxide or chromic acid supported on resin (Magtrieve®).[26] The dihydro intermediate (**7**) could be reduced to the tetrahydroimidazopyrazine by treatment with borohydride supported on resin in MeOH.[27] The secondary amino group was acylated in chloroform in the presence of morpholinomethylpolystyrene resin to afford *N*-substituted tetrahydroimidazopyrazines (**9**).

The structural diversity of imidazopyrazines obtained by this strategy was inhibited by the limited molecular diversity of commercially available bromoketones, which are used in two occasions in our strategy. Therefore, efforts were focused on the synthesis of original and diverse bromoketones. Two different methods were applied allowing the rapid preparation of 15 additional bromoketones. In the first case, a carboxylic acid was activated as an acyl chloride or a mixed anhydride[28] and then transformed into a diazoketone using trimethylsilyldiazomethane. Bromination was then carried out using HBr in dichloromethane. In the second case, a methylketone was converted to the corresponding bromoketone using $CuBr_2$[29] or poly(vinylpyridinium hydrobromide perbromide) resin[30] as brominating agent. We were particularly attracted to the preparation of methyl-4-bromo-2,2-dimethyl-3-oxobutanoate (**10**)

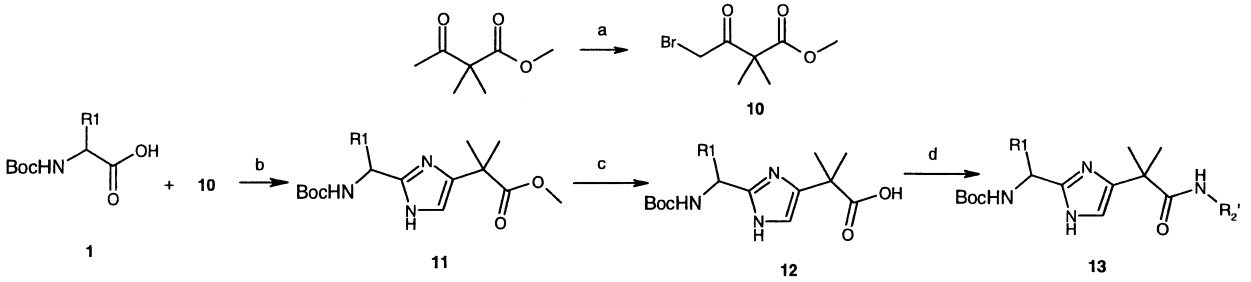


Scheme 1. (a) (i) Cs_2CO_3 (0.5 equiv), EtOH/H_2O (1:1); (ii) bromoketone (**2**) (1 equiv), DMF; (b) NH_4OAc (20 equiv), xylene, 150 °C, 1 h, Dean–Stark; (c) polymer supported *N*-methyl-morpholine (2 equiv), DMF; (d) 10% TFA in DCM; (e) DMSO or MeOH, 24 h, 20 °C or Magtrieve®, MeOH, 3 h, 40 °C; (f) (i) polymer supported borohydride (4 equiv), MeOH, pH 5; (ii) R^4COCl (1.3 equiv), $CHCl_3$, polymer supported *N*-methyl-morpholine (2 equiv).

(Scheme 2) and conversion to the imidazole intermediate (**11**). The acid derivative (**12**) could be easily coupled to various primary or secondary amines, giving 4-substituted imidazoles (**13**). This procedure dramatically increased the diversity at the R2 position.

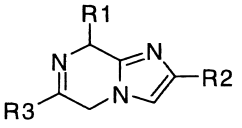
Results and Discussion

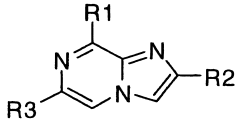
We have prepared libraries of dihydroimidazopyrazines (**7**) and imidazopyrazines (**8**) incorporating side chains of the somatostatin β-turn. More than 300 individual

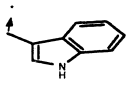
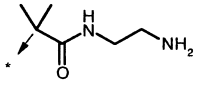
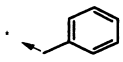
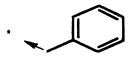
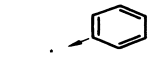
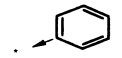
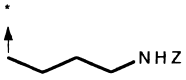
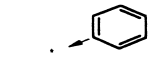
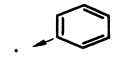
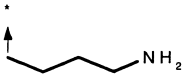
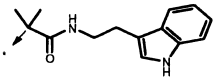
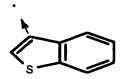
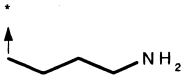
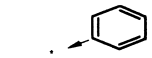
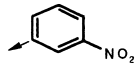
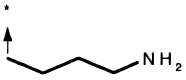
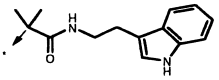
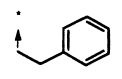
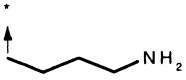
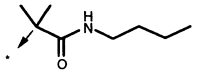
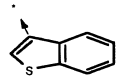
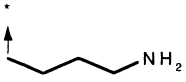
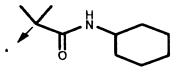
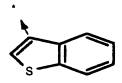
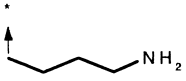
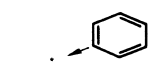
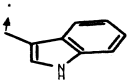
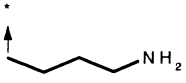
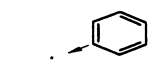
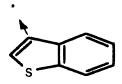


Scheme 2. (a) $CuBr_2$ (2.5 equiv), AcOEt, 80 °C, 5 h; (b) (i) Cs_2CO_3 (0.5 equiv), $EtOH/H_2O$ (1:1); (ii) bromoketone (**9**) (1 equiv), DMF; (iii) NH_4OAc (20 equiv), xylene, 150 °C, 1 h, Dean–Stark; (c) $LiOH{\cdot}H_2O$ (6.6 equiv), THF, 3 h, 80 °C; (d) $R_2'NH_2$ (1.1 equiv), CDI (1.2 equiv), THF, 1 h.

Table 1. Inhibition constant (K_i) values of imidazopyrazines on human SRIF receptors





Compound	R1	R2	R3	K_i (nM)				
				sst_1[a]	sst_2[a]	sst_3[a]	sst_4[a]	sst_5[b]
7a				>10,000	>10,000	>10,000	>10,000	>1000
8a				>5000	>5000	>5000	>5000	>5000
8b				>5000	>5000	>5000	>5000	>5000
8c				4000	3200	10,000	>10,000	2300±740
8d				3400	7900	2500	>10,000	430±69
8e				4600	8600	1200	7200	1200±300
8f				>10,000	>10,000	8900	>10,000	540±20
8g				9700	9500	3700	>10,000	360±21
8h				3300	6000	3100	>10,000	790±130
8i				2900	2100	1700	7200	600±53

[a]Data represent single values from one binding experiment.
[b]Data represent the mean±SEM of 2–3 experiments.

compounds were rapidly prepared by parallel synthesis methods. The purity of the compounds was determined by LC/MS.[31] UV purity of the compounds presented here is above 80%. Competitive inhibition of [^{125}I-Tyr^{11}]SRIF-14 (NEN Life Science Products) binding to membranes isolated from CHO-K1 cells stably expressing each human SRIF receptor subtype, was measured in 96-well plates by an adaptation of the method of Shimon and al.[32] Compounds were tested at 10 μM. Inhibition constants (K_i) were determined for compounds eliciting more than 70% inhibition at 10 μM.

The highest affinity was obtained with an aminobutyl group at R1 (**8c–i**, Table 1), a functionality that could be considered to mimic the Lys^9 side chain extending off the β-turn backbone. K_i measurements showed that these compounds bind preferentially to sst_5 receptors. The use of an indole containing fragment at the R2 position in an attempt to mimic the Trp^8 side chain of the β-turn did not lead to potent ligands (**8c**, **8e**), but substitution at this position with phenyl, cyclohexyl or *n*-butyl containing moieties gave analogues with submicromolar inhibition constants (**8d**, **8f–i**). Moreover, when R3 was a 3-nitrophenyl or a benzothiophene, we obtained potent sst_5 ligands with K_i values of 430 nM and 360 nM, respectively (**8d**, **8g**, Table 1). The most potent compound (**8g**)[33] was 10- to 30-fold less active at the other SRIF receptor subtypes. Reduction of dihydroimidazopyrazine (**7**) and substitution of the amino group to obtain a series of *N*-substituted tetrahydroimidazopyrazines resulted in a loss of activity (data not shown). In a functional assay based on inhibition of forskolin-induced accumulation of adenosine cyclic 3′-5′-monophosphate (cAMP) in CHO-K1 cells expressing human sst_5 receptors,[34] derivative **8g** displayed the characteristics of an agonist with EC_{50} value of 1600 ± 610 nM ($n = 3$). In this series of experiments, SRIF-14 ($K_i = 120 \pm 4$ pM on sst_5 receptors) elicited an EC_{50} of 65 ± 13 pM.

In conclusion, a series of diverse imidazopyrazines and dihydroimidazopyrazines has been synthesized, and their binding affinity to each of the human somatostatin receptor subtypes have been determined. Higher affinities were obtained with a basic group in the R1 position, and we hypothesize that this might be mimicking the side chain of the Lys^9 residue of the β-turn. These novel preferential non-peptidic sst_5 ligands were shown to be agonists. Sst_5 receptors have a predominant role in inhibiting the release of insulin from pancreatic islets[35] and GH from pituitary cells.[32] It was suggested that sst_5 agonists may be effective in the treatment of GH and/or prolactin secreting adenomas.[36,37] Our results validate the hypothesis that small heterocyclic scaffolds can provide SRIF receptor ligands with agonist characteristics.

Acknowledgements

We wish to thank the Analytical Department for performing the LC/MS analyses.

References and Notes

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34. CHO-K1 confluent cells expressing the human sst_5 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 sst_5 agonist. 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).
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