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POTENT, ORALLY BIOAVAILABLE SOMATOSTATIN AGONISTS: GOOD ABSORPTION ACHIEVED BY UREA BACKBONE CYCLIZATION

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Abstract: Backbone cyclization of urea-based somatostatin agonists resulted in novel, orally bioavailable agonists. Binding assays confirmed that the resulting conformationally constrained cyclic ureas retained the potency of their acyclic counterparts. SAR studies subsequently led to highly potent analogs, selective for receptor subtype 2, and having good oral bioavailability.

Introduction: Somatostatin is a cyclic peptide existing in two physiologically active forms, SRIF-14 and SRIF-28, which inhibits the release of a variety of bioactive molecules, including growth hormone, glucagon, insulin, and gastrin.[1] Five somatostatin receptors (SSTR1-5), all members of the G-protein linked family, have been identified and cloned.[2] The exact functional activities of each receptor subtype remain to be clearly delineated and studies aimed at identifying subtype selective ligands are currently the focus of several research efforts.[3] Moreover, the low metabolic stability of somatostatin in vivo has prompted the development of more stable peptide-based analogs such as Octreotide and MK-678, however these have poor oral bioavailability.[3e] An earlier report has detailed our work aimed towards the discovery of nonpeptide somatostatin receptor subtype-2 (SSTR-2) agonists, leading to the highly potent and SSTR-2 selective agonist L-054,522 (Figure 1).[4]

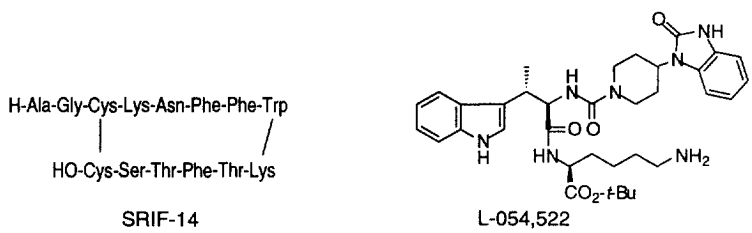

Figure 1. Structures of SRIF-14 and L-054,522

Agonist L-054,522 is comprised of a *t*-butyl capped dipeptide (β-methylTrpLys-O-*t*-Bu) connected to a 4-substituted piperidine by means of a urea linker. A major limitation of L-054,522 and most of its analogs is its low oral bioavailabilty. Based on the hypothesis that elimination of hydrogen bond donating and accepting

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groups should favor improved transport,[5] we set out to investigate modification of the urea moiety in an effort to minimize potential H-bonding interactions. Early studies in which the urea was N-methylated (**2**) or replaced by a carbamate functionality (**4**) resulted in complete loss of potency relative to the comparable compounds **1** and **3**, respectively (Figure 2[6]). Two conclusions could be drawn from these results: (1) the urea NH is involved in a key binding interaction, and/or (2) both modifications cause a change in conformation which is detrimental to binding. Since reports by Freidinger and Hirschmann[7] suggest that the peptide backbone is probably not involved in crucial interactions with the SSTR receptor, we suspected that loss of an optimal low energy conformation might be the overriding factor leading to potency loss by **2** and **4**. To block the NH group while retaining the desired backbone conformation we envisioned cyclization between both urea nitrogen atoms via a two carbon tether, resulting in the five-membered cyclic urea system depicted in hypothetical molecule **5** (Figure 2). Molecular modeling indicated that the cyclic urea motif overlaps well with the presumed low energy S-*trans* orientation of the acyclic urea NH-CO system and that an analog based on this scaffold overlaps as well or better than the acyclic urea type analogs with the model of a potent cyclic hexapeptide SSTR agonist closely related to MK-678.[8] Herein we report the successful application of the proposed cyclic urea scaffold to generate potent SSTR-2 agonists[9] with improved pharmacokinetic properties over their acyclic counterparts.

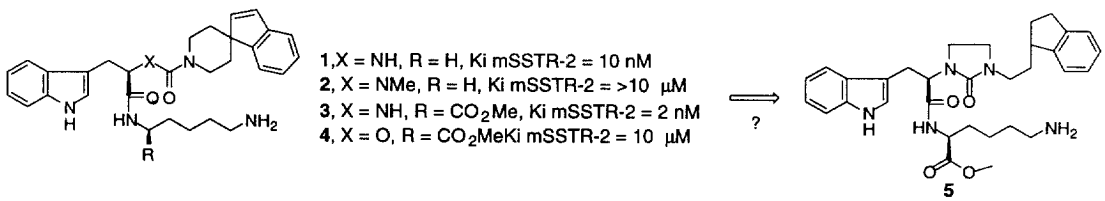
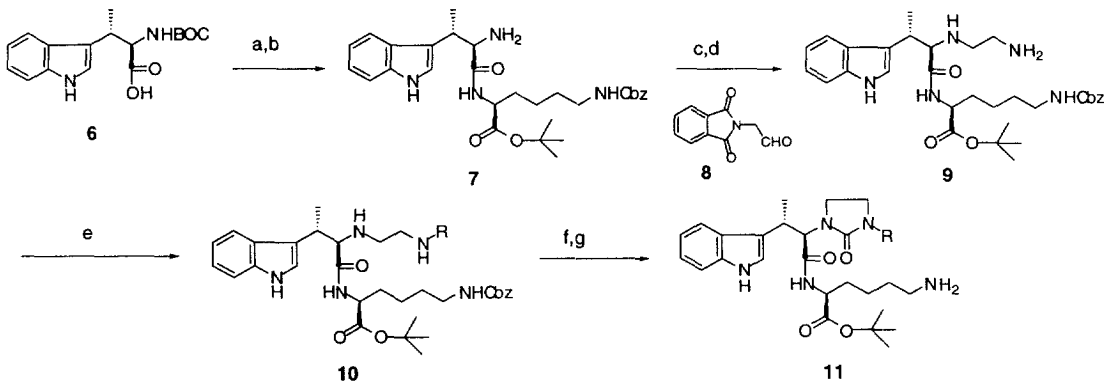


Figure 2. Effect on potency of urea methylation, carbamate substitution, and cyclization of backbone.

Chemistry: Cyclic urea-based compounds were prepared according to Scheme 1 or to Scheme 2, depending on the availability of starting materials. In Scheme 1 BOC-2*R*,3*S*-β-Me-Trp-OH[10](**6**) was coupled to H-Lys(Z)-O-*t*-Bu, and the BOC group was selectively removed in the presence of the *t*-butyl ester. Reductive alkylation of **7** with aldehyde **8**, hydrazinolysis of the phthalimide group, and a second reductive alkylation step employing a variety of aldehydes or ketones furnished diamines **10**. Urea cyclization was accomplished using phosgene and final deprotection (H_2, Pd/C) afforded the target analogs. In Scheme 2, acid **6** was converted to its methyl ester **12** and subsequently reductively alkylated with aldehyde **13** or aldehydes of the general type **15** (both prepared as shown). In the former case, deprotection was followed by a second reductive alkylation step employing a variety of aldehydes, then urea cyclization and LiOH mediated hydrolysis of the methyl ester functionality. Coupling of the resultant acids **17** with several monoprotected diamines and appropriate deprotection provided analogs **18**. Alternatively, intermediates of the type **16** were

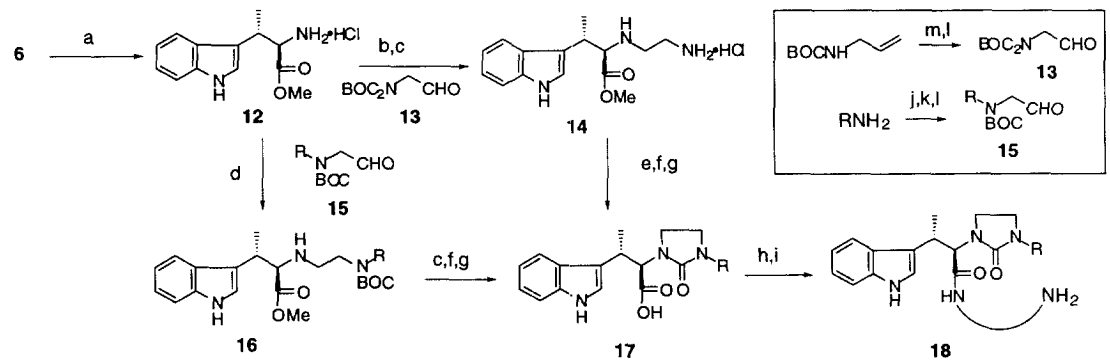
manipulated using similar chemistry to give **18**, the difference between the two approaches being only in the source of the R group (amine or aldehyde/ketone).

Scheme 1



Reagents and conditions: (a) HLys(Z)-O-*t*-Bu•HCl, DIEA, EDC, HOBt, DCM; (b) MsOH, MeOH; (c) **8**, $NaBH_3CN$, cat AcOH, MeOH; (d) H_2NNH_2, EtOH, reflux; (e) RCHO, $NaBH_3CN$, MeOH, cat. AcOH; (f) 1.9 M $COCl_2$/toluene, DIEA, DCM, 0 °C; (g) H_2, Pd/C, MeOH.

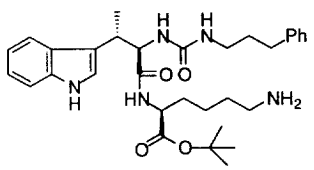
Scheme 2



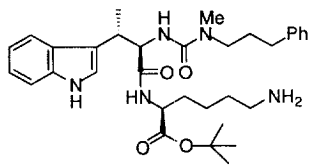
Reagents and conditions: (a) $SOCl_2$, MeOH, reflux, 3 h. (b) **13**, $NaBH_3CN$, NaOAc, MeOH; (c) HCl, EtOAc; (d) **15**, $NaBH_3CN$, NaOAc, MeOH (e) RCHO, $NaBH_3CN$, NaOAc, MeOH; (f) 1.9 M $COCl_2$/toluene, DIEA, DCM, 0 °C; (g) LiOH•H_2O, THF/MeOH/H_2O (1:1:1); (h) HLys(Z)-O-*t*-Bu•HCl (or other mono-protected diamines), DIEA, EDC, HOBt, DCM; (i) HCl, EtOAc or H_2, Pd/C, MeOH; (j) BOC_2O, DCM; (k) KHMDS, THF, 0 °C; allyl bromide; (l) O_3, DCM, -78 °C; DMS; (m) BOC_2O, DMAP, DCM.

Results and Discussion: As an initial proof of concept, compounds **19**, **20**, and **21** (Figure 3) were prepared[6] and their in vitro binding affinities as well as their pharmacokinetic characteristics (beagles) were evaluated.[11] Importantly, the binding data indicated that backbone urea cyclization resulted in little or no loss in binding affinity, suggesting that the rigid conformation imposed by cyclization closely approximates the binding conformation of the acyclic urea system, that a urea NH is probably not required for binding, and that the two

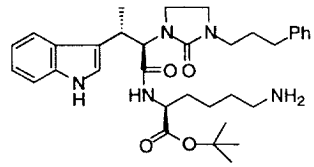
carbon tether does not interfere with the ligand-receptor fit. Moreover, we were encouraged by the observation of a significant level of bioavailability (5%) for cyclic urea **21**, which was superior to that observed for its acyclic counterpart **19**, assuming linear pharmacokinetics. As a result, we set out to explore the SAR of **21**



19: K_i hSSTR-2 = 1 nM
1 mpk po, 2 beagles:
AUC = 314 ng h/mL
Cmax = 86 ng/mL
F = 1%



20: K_i hSSTR-2 = 2.2 nM
1 mpk po, 2 beagles:
AUC = 333 ng h/mL
Cmax = 103 ng/mL



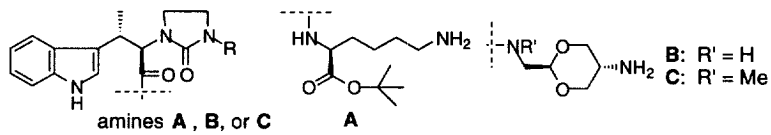
21: K_i hSSTR-2 = 3.6 nM
2 mpk po, 2 beagles:
AUC = 837 ng h/mL
Cmax = 350 ng/mL
F = 5%

Figure 3. Pharmacokinetics of comparable compounds **19**, **20**, and **21**.

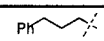
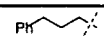
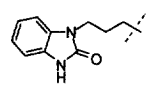
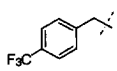
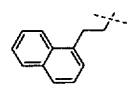
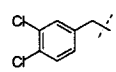
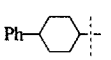
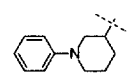
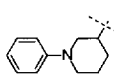
with the aim of improving potency, selectivity[12] and absorption. Our focus centered both on modification of the 3-phenylpropyl moiety and replacement of the Lys-O-*t*-Bu fragment with other diamine units expected to improve selectivity and confer improved water solubility and reduced basicity. In particular, 2-aminomethyl-5-amino-1,3-dioxane[13] was incorporated as a Lys surrogate because earlier studies in the acyclic agonist series had revealed that this diamine imparts good selectivity while sacrificing only about one order of magnitude in potency. The dioxane moiety was further expected to lead to improved aqueous solubility and reduced basicity, which we hoped would increase absorption. Table 1 outlines the in vitro results. Shortening of the phenylpropyl chain by one or two atoms (**22** and **23**) diminished the binding affinity, however incorporation of a trifluoromethyl group onto the 4-position of **23**, resulting in **24**, led to an overall improvement (K_i hSSTR-2 = 1.3 nM). Most attempts to reduce the conformational flexibility of the phenylpropyl moiety by incorporation of methyl groups (**25** and **26**) and interposing rings (**27** and **28**) resulted in similar or worsened binding affinity. An exception was the dl diastereomer of compound **29**, having an N-phenylpiperidine group attached at the 3-position, which had a K_i hSSTR-2 = 1.4 nM. The most potent compound in the series incorporating Lys, **24**, was evaluated in vivo in dogs and found to have F = 3%. Its lower bioavailability relative to **21** likely arises from its more rapid clearance (Clp = 6.9 mL/min/kg versus 2.1 mL/min/kg for **21**). Interestingly, when 2-aminomethyl-5-amino-1,3-dioxane (amine **B**) was employed as a Lys replacement (**30**), potency dropped about fivefold, however oral bioavailability improved to 19%. Earlier acyclic urea-based analogs prepared incorporating this same diamine fragment never achieved bioavailabilities in this range, suggesting a synergistic relationship between the cyclic urea and diamine groups in promoting absorption. Incorporation of a second phenyl group resulted in a twofold improvement in potency relative to **30** and a dramatic jump in oral bioavailability to 64% (**31**). Compound **31** also had excellent selectivity for hSSTR-2 (K_i hSSTR-3 = 8.4 μM, hSSTR-5 = 1.1 μM). When a benzimidazolone unit was introduced (**32**) to mimic L-

054,522, binding affinity was diminished. Compounds **33** and **34** had potencies sevenfold worse and threefold better than **30**, respectively, however they were both rapidly cleared. N-methylation of the amide in **30**, giving **35**, led to an approximately twofold improvement in potency. The advantage conferred by amide N-methylation was consistent, leading to highly potent analogs **36** and **37**. The most potent analog in this series, **37**, had an oral bioavailability of 15%. It also had excellent selectivity for SSTR-2 against two other subtypes tested (K_i hSSTR-3 >10 μM, hSSTR-5 = 1.4 μM) and was active in a functional assay which measures inhibition of growth hormone release with an IC_{50} of 70 nM. [9]

TABLE I



amines **A**, **B**, or **C** **A**

#	amine	R	K_i (nM) hSSTR-2	oral bioav.	#	amine	R	K_i (nM) hSSTR-2	oral bioav.
21	A		3.6	5%	30	B		18	19%
22	A		47	-	31	B		8.5	64%
23	A		14	-	32	B		40	-
24	A		1.3	3%	33	B		122	-
25	A		12	-	34	B		7.3	21%
26	A		5.9	-	35	C		11	-
27	A		d1: 40 d2: 6.3	-	36	C		4.6	-
28	A	3-biphenyl	80	-	37	C		d1: 2.1 d2: 14	15%
29	A		d1: 1.4						

In summary, novel SSTR-2 selective agonists possessing good oral bioavailability and high potency were discovered by modification of earlier acyclic urea-based analogs via urea backbone cyclization employing a two carbon tether. The high potency observed for cyclic urea-based analogs indicated that the rigid conformation imposed by cyclization closely approximates the binding conformation of the acyclic urea system, that a urea NH is probably not required for binding, and that the two carbon tether does not interfere

with the ligand-receptor fit. The higher oral bioavailability observed for the cyclic urea-based analogs relative to the earlier acyclic analogs is postulated to derive from capping of the urea NH group. Furthermore, a synergistic benefit in oral absorption when incorporating diamines **B** and **C**, not observed in the acyclic series, was observed in the cyclic urea series.

Acknowledgments: We would like to thank Amy Bernick for mass spectrometry support and Donald Hora, Jr. and Paul Cunningham for assistance in oral bioavailability screening.

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6. Acyclic urea based SSTR-2 agonists were prepared as described in ref 4 and in: Yang, L.; Guo, L.; Pasternak, A.; Mosley, R.; Rohrer, S.; Birzin, E.; Foor, F.; Cheng, K.; Schaeffer, J.; Patchett, A. A. *J. Med. Chem.* **1998**, *41*, 2175.
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8. For details on the modeling of acyclic agonists as well as cyclic hexapeptide agonists see ref 4. A comparison of minimized compounds **19** and **21**, overlapping the C-NCON-C urea atoms, yielded an RMS of 0.120°A. Comparisons to the cyclic hexapeptide agonist were made by visual examination.
9. Functional activities were determined either by measurement of inhibition of growth hormone release using primary cultures of rat anterior pituitary cells or by measurement of inhibition of forskolin-stimulated cAMP production in L cells stably expressing sst2 and a cAMP-responsive reporter construct, both as described in ref 4. Of the compounds reported herein, all of those evaluated in functional assays were agonists, including **21**, **30**, **35**, and **37**.
10. Huang, Z.; He, Y.-B.; Raynor, K.; Tallent, T.; Goodman, M. *J. Am. Chem. Soc.* **1992**, *114*, 9390.
11. Binding affinities were measured as described in ref 4. Except as noted, pharmacokinetics in beagles were calculated based on data obtained by po administration at 1 mpk and iv administration at 0.2 or 0.3 mpk in two dogs. Linear phamacokinetics were assumed.
12. Compound **21** already possessed good human SSTR-2 selectivity with the following K_i values: hSSTR-1 = 712 nM, hSSTR-2 = 3.6 nM, hSSTR-3 = 1290 nM, hSSTR-4 = 83 nM and hSSTR-5 = 584 nM.
13. Discovered in another program at Merck & Co., Inc. Its preparation will be described elsewhere.