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Potent macrocyclic antagonists to the motilin receptor presenting novel unnatural amino acids

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Abstract—Novel, potent small molecule motilin receptor antagonists are described. These peptidomimetic macrocycles are composed of a tripeptide cyclized backbone-to-backbone with a nonpeptidic tether and bear new unnatural amino acids containing basic side chains.
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Motilin is a peptide hormone released from entero-chromaffin cells in the gut wall, that acts locally on the motilin receptor (*h*MOT-R) to stimulate contraction of the gut smooth muscle.[1,2] Activation of *h*MOT-R provokes peristaltic contractions of the gut in a pattern analogous to phase III of the migrating motor complex (MMC).[3] *h*MOT-R is a G-protein coupled receptor (GPCR) predominantly found in the gut wall of the stomach and upper intestine. Molecules interacting with *h*MOT-R have potential for treatment of GI disorders associated with altered gut motility, particularly irritable bowel syndrome or functional dyspepsia.[4,5]

To expand on a previous report of a new class of macrocyclic peptidomimetics antagonists to *h*MOT-R,[6] we herein describe a subclass of macrocycles with increased potency as *h*MOT-R antagonists. These macrocycles contain new unnatural amino acids bearing basic side chains. SAR as well as the synthesis of the unnatural amino acids and the corresponding macrocycles are presented.

High throughput screening of ca 10,000 macrocyclic peptidomimetics led to the identification of macrocycle **1** (K_i 137 nM, Fig. 1, functional IC_{50} 74 nM, Fig. 4) bearing a Nva residue as the AA_3 amino acid as the first *h*MOT-R antagonist of this class.[6] Initial SAR studies

led to the synthesis of analogous macrocycles bearing polar residues at the AA_3 position, including **2** bearing an Asp residue, clearly indicating that a carboxylate was unsuitable in this position (K_i 2.5 μM). On the other hand, macrocycles **3** and **4**, bearing Lys and Orn residues, were associated with 5- to 7-fold decrease in potency (K_i 664 and 889 nM). Despite lower potency, the different polarity profile of these molecules opened an avenue to broaden the class and create compounds with modified physicochemical and pharmaceutical properties. This led to the synthesis of several potent *h*MOT-R antagonists bearing basic or heterocyclic residues, as described below.

As an initial effort, the length of the exocyclic arm bearing the polar residue at AA_3 was shortened (Fig. 2).[7] Compared to macrocycles **3** and **4**, macrocycles bearing an L-diaminobutyrate (Dab, **5**) or diamino-propionate residue (Dap, a basic isostere of Nva, see **6**) were significantly more potent, the latter essentially equipotent to initial lead **1** (**6** vs **1**, K_i 171 vs 137 nM). Alkylation of the Dap residue with an isopropyl group led to a moderate decrease in potency (**7**, K_i 376 nM), whereas acetylation had a more dramatic impact (**8**, K_i 2 μM). While these two analogues are isosteric, they are not isoelectronic and results clearly indicate the importance of basicity at that position.

Within the newly discovered Dap series, SAR was narrowed by finer modifications to the aromatic ring substitution pattern (Fig. 2). Replacement of the 4-OH residue of (D)Tyr by less polar substituents such as 3-Cl, 4-Cl and
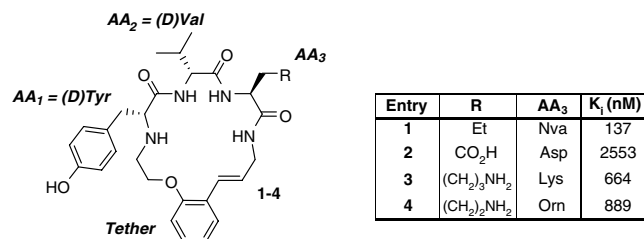
Figure 1. Initial hit from HTS and early analogues.

Entry	R	AA$_3$	K$_i$ (nM)
1	Et	Nva	137
2	CO$_2$H	Asp	2553
3	(CH$_2$)$_3$NH$_2$	Lys	664
4	(CH$_2$)$_2$NH$_2$	Orn	889

Entry	R	X	K$_i$ (nM)
5	CH$_2$NH$_2$	4-OH	342
6	NH$_2$	4-OH	171
7	NH-CH(CH$_3$)$_2$	4-OH	376
8	NH-COCH$_3$	4-OH	1946
9		3-Cl	64
10	NH$_2$	4-Cl	60
11		3-CF$_3$	15
12	NHCH$_3$	4-Cl	31
13		3-CF$_3$	6
14	N(CH$_3$)$_2$	3-Cl	27
15		4-Cl	16
16	NH(C=NH)NH$_2$	4-Cl	6
17		3-CF$_3$	6
18	NH(C=O)NH$_2$	4-Cl	543
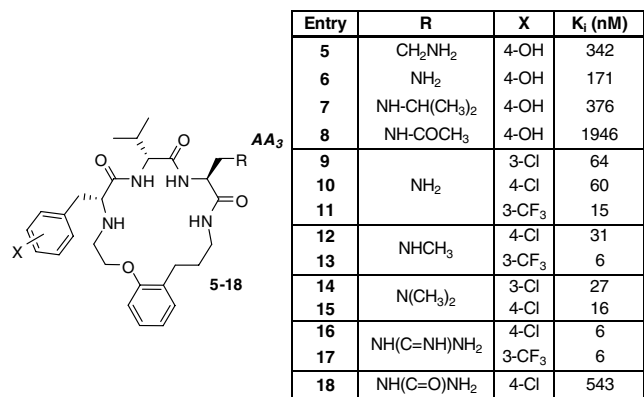
Figure 2. Macrocycles bearing shorter AA$_3$ exocyclic substituents.

3-CF$_3$ led to significant increase in potency (respectively, **9**, **10** and **11**, K_i 64, 60 and 15 nM). Monomethylation and dimethylation of the amine proved beneficial for potency (**12**, **13**, K_i 31 and 6 nM and **14**, **15**, K_i 27 and 16 nM, respectively). To be noted, the Dap(Me$_2$) residue constitutes a polar isosteric replacement for Leu. Replacement of Dap with an Ala(guanidinyl) residue was found to also generate very potent macrocycles (**16**, **17**, K_i 6 nM). Compounds **11** and **13** proved to be robust antagonists to *h*MOT-R functionally (IC$_{50}$ 8 and 3 nM, Fig. 4). It is interesting to note that replacement of the guanidine functionality by the neutral yet isosteric urea dropped potency by two orders of magnitude (**18**, K_i 543 nM), in agreement with the result obtained with Dap(Ac) **8**. Altogether these results confirm that basicity in this subclass is a key element to potency.

The next part of the effort investigated the impact of decreased basicity of the guanidine moiety on the one hand, and evaluating heterocyclic appendages on the other hand. A variety of approaches to maintain the guanidine's unique hydrogen bonding capability while decreasing its basicity have been reported,[8] several of which were applied to this system in the form of guanidine mimetics (Fig. 3). Heteroaryl-substituted Dap analogues gave interesting results, albeit with a moderate decrease in potency (**19** and **20**, K_i 20 and 41 nM). Cyclization of the guanidine further reduced potency (**21** and **22**, K_i 51 and 87 nM), as did modification to a cyanoguanidine (**23**, K_i 100 nM), the latter result being in agreement with results of **8** and **18**. Replacement of the guanidine by an amidine also had a negative impact (**24** and **25**, K_i 57 and 27 nM). Finally, lengthening of the distance between backbone and guanidine chain by one carbon atom also resulted in a decrease in potency (**26**,

Entry	X	R	K$_i$ (nM)		Entry	X	R	K$_i$ (nM)
19	CF$_3$		20		27	CF$_3$		4.6
20	Cl		41		28	CF$_3$		17
21	Cl		51		29	CF$_3$		23
22	Cl		87		30	CF$_3$		21
23	Cl		100		31	Cl		18
24	Cl		57		32	Cl		62
25	Cl		27		33	F		116
26	Cl		33					

Figure 3. Guanidine mimetics and heterocycles at AA$_3$ position.

K_i 33 nM). Altogether, even though the preferred group was the guanidine, isosteric analogues gave interesting results in terms of potency.

Other heteroaromatic groups were also studied, such as pyrrolidine (**27**, K_i 4.6 nM). The latter proved to be the most potent antagonist in this class, with an IC$_{50}$ of 2 nM (Fig. 4). It was found that both triazole, tetrazole and pyrazole were reasonably well tolerated at this position ($K_i \sim 20$ nM) although functional IC$_{50}$ suffered compared to the pyrrolidine moiety (Fig. 4). Finally, imidazole and thiazole analogues both showed a decrease in potency.

Essentially, these results confirmed that the presence of a basic appendage on the macrocycle is beneficial for compound affinity to *h*MOT-R, with a preference for alkylated or cyclic variants of the Dap moiety (**13**, **16**, **17**, **27**), and Ala(guanidinyl) or guanidine mimetics (**16**, **17**, **19**). It was found also that several heteroaromatic substitutions could be tolerated, albeit less preferred.

Entry	K$_i$ (nM)	IC$_{50}$ (nM)
1	137	74
11	15	8
12	31	33
13	6	3
14	27	95
17	6	28
27	4.6	2
31	18	56

Figure 4. Functional assay results for best hits.

Binding studies were performed in duplicate on CHO cells transfected with *h*MOT-R using [^{125}I]-motilin (test concentration, 0.36 nM) as the radioligand. Antagonist activity was measured on selected compounds using the AequoScreen™ (Euroscreen, Belgium) cell line in an aequorin assay measuring the inhibition of motilin-induced (EC$_{80}$ 0.14 nM) calcium release (Fig. 4).

From a synthetic point of view, macrocycles **1**–**6**, **9**–**11**, **20**, **31**–**33**, **48**, **49**, **51** and **52** were made using solid phase parallel synthesis as described previously,[6] other macrocycles were synthesized in solution (Scheme 2). All macrocycles were purified by reverse phase HPLC, and testing was performed on pure materials exclusively.[9]

Unnatural amino acids β-(2-aminothiazolyl) alanine **36**, β-(imidazol-1-yl)alanine **38** and β-(pyrazol-1-yl)alanine **39** (Scheme 1) were obtained by Mitsunobu reaction on serine amide **34**[10] or using Vederas' β-lactone (Scheme 1)[11] and used as such for macrocycle synthesis.[6]
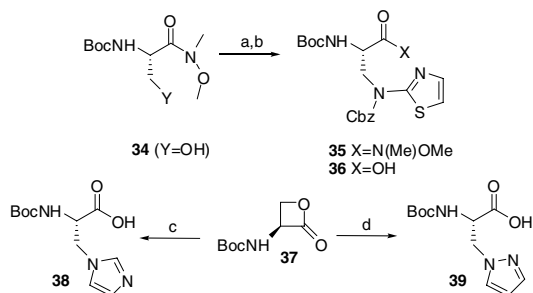
Azidoalanine-containing macrocycles **44**–**47** were prepared in solution (Scheme 2). β-Azidoalanine amide **41** was synthesized and the peptide assembled using Boc chemistry through the attachment of betsylated (Bts)[12] aromatic AA$_1$ to give **42**. The tether moiety was introduced by Fukuyama–Mitsunobu reaction.[13] Subsequent deprotections and macrolactamization in the presence of DEPBT[14] gave macrocycles **44**–**46**.

N-monosubstituted amines (Scheme 3) were obtained from macrocycles **44**–**47** by Staudinger azide reduction followed by reductive alkylation with acetone to give **7** or by Bts protection followed by Mitsunobu alkylation and Bts removal to give **12** and **13**.
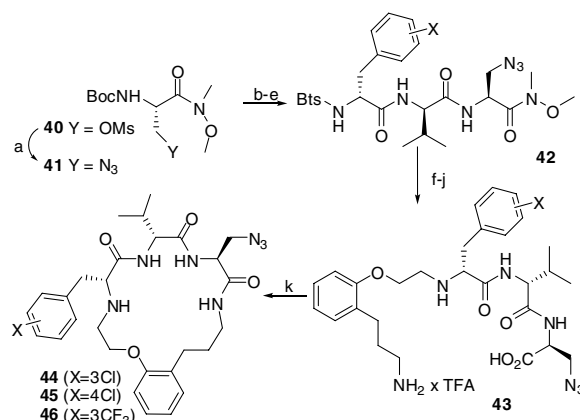
On the other hand, *N,N*-dimethylation required Boc protection of the secondary amine, followed by reductive alkylation of the exocyclic primary amine and Boc removal (**14** and **15**).

Guanidino alanine macrocycles **16** and **17** were obtained by reaction with Goodman's guanidinylation reagent[15] followed by Boc acidolysis.

N-Ac and N-urea analogues (**8** and **18**) were made from Bts-protected macrocycles **48** and **49** (Scheme 4).


Scheme 2. Reagents and conditions: (a) NaN$_3$, DMF, 50 °C (70%); (b) TFA, Et$_3$SiH, DCM (quant.); (c) Boc–(D)Val, EDCI, (6Cl)HOBt, DIPEA, DCM:THF (98%); (d) TFA, Et$_3$SiH, DCM (quant.); (e) Bts–(D)Phe(X)–OH, HATU, DIPEA, DCM–THF (95%); (f) HO(CH$_2$)$_2$O–C$_6$H$_4$–(CH$_2$)$_3$–NHBoc, DIAD, PPh$_3$, THF (90%); (g) HS(CH$_2$)$_2$CO$_2$H, Na$_2$CO$_3$, DMF (90%); (h) LiOH, THF:H$_2$O (98%); (j) TFA, Et$_3$SiH, DCM (quant.); (k) DEPBT, DIPEA, THF (80%).
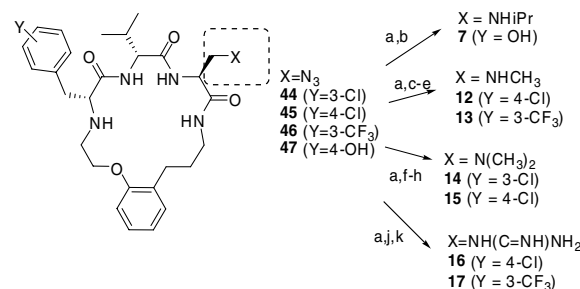
Scheme 3. Reagents: (a) PPh$_3$, H$_2$O; (b) acetone, NaBH$_3$CN, AcOH, HC(OMe)$_3$:MeCN (75%); (c) Bts–Cl, DIPEA, DCM (95%); (d) MeOH, DIAD–PPh$_3$, THF (90%); (e) HS(CH$_2$)$_2$CO$_2$H, K$_2$CO$_3$, DMF (90%); (f) Boc$_2$O, Na$_2$CO$_3$, THF–water (85%); (g) H$_2$CO, NaBH$_3$CN, AcOH, HC(OMe)$_3$, MeCN (75%); (h) HCl–dioxane (98%); (j) BocNH–C(=NTf)–NHBoc, Et$_3$N, THF; (k) TFA, Et$_3$SiH, DCM (70%, 2 steps).
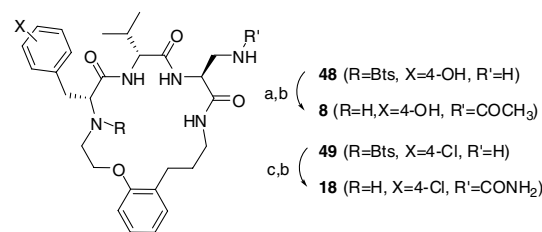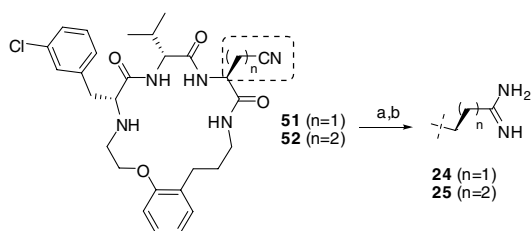

Scheme 4. Reagents: (a) Ac$_2$O, DIPEA, DCM; (b) PS–thiophenoxide, THF:EtOH (60–70%, 2 steps); (c) TMS–NCO, Et$_3$N, DMAP, DCM.
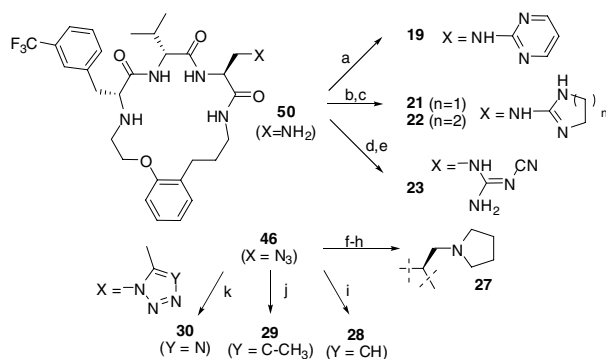
Amidine analogues **24** and **25** were obtained from nitriles **51** and **52** by Pinner synthesis (Scheme 5).[16]

Guanidine analogues **21**–**23** were synthesized by known methods,[17,18] and pyrimidine analogue **19** was obtained by SNAr reaction on 2-bromopyrimidine (Scheme 6).

Finally, heterocyclic AA$_3$ analogues were obtained by reductive alkylation of the primary amine (**27**), or by

Scheme 1. Reagents and conditions: (a) *N*-Cbz-2-aminothiazole, DIAD, PPh$_3$, THF (50%); (b) LiOH, THF–water, rt (98%); (c) TMS–imidazole, MeCN, rt (55%); (d) TMS–pyrazole, MeCN:THF, 50 °C (60%).

Scheme 5. Reagents: (a) HCl, EtOH; (b) NH$_3$, EtOH (35%, 2 steps).

Scheme 6. Reagents and conditions: (a) 2-bromopyrimidine, K$_2$CO$_3$, DMF 50 °C, (96%); (b) guanidinylation reagent, Et$_3$N, THF 50 °C; (c) TFA, Et$_3$SiH, DCM (35%, 2 steps); (d) (MeS)$_2$C=N—CN, EtOH, 50 °C; (e) NH$_3$, EtOH, 50 °C (30%, 2 steps); (f) PPh$_3$, THF–water, 40 °C (90%); (g) OHCCH$_2$CH$_2$CHO, HC(OMe)$_3$, acetic acid, THF (65%); (h) H$_2$, PtO$_2$, AcOH, EtOH (98%); (i) Propyne, CuI, DIPEA, MeCN, rt (76%); (j) 2-butyne, DMSO, 140 °C (65%); (k) CH$_3$CN, ZnBr$_2$, DMSO, 140 °C (35%).

azide [2+3] cycloaddition with a nitrile or an acetylene (**28–30**) (Scheme 6).[19]

In summary, we have identified a new series of macrocyclic antagonists to the human motilin receptor (*h*MOT-R). These compounds are characterized by the presence of a basic or heteroaromatic residue at the AA$_3$ position. In combination with halogenated residues at the AA$_1$ position, the resulting macrocycles displayed very potent antagonist activity at *h*MOT-R.

Acknowledgments

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