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Synthesis and SAR of 1,3-disubstituted cyclohexylmethyl urea and amide derivatives as non-peptidic motilin receptor antagonists

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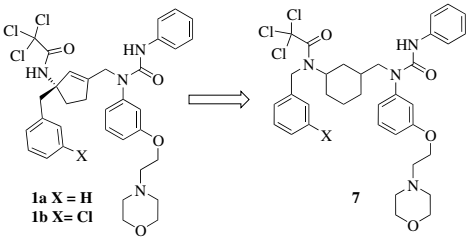
Abstract—A series of 1,3-disubstituted cyclohexylmethyl urea and amide derivatives were synthesized as motilin receptor antagonists. Starting from known motilin antagonists, **1a** and **1b**, the cyclopentene scaffold was replaced and the four recognition elements optimized to arrive at a potent novel series.

Motilin belongs to the ghrelin-motilin G-protein coupled receptor family of ligands that as a whole regulate appetite and gastrointestinal motility.[1,2] Motilin, as a gastrointestinal paracrine hormone, stimulates smooth muscle contractions affecting the motility and emptying of both the antral stomach[3] and the antroduodenal region of the small intestine.[3–5] Disruption of this coordinated contractile activity may lead to a variety of gastric motility disorders of GI tract origin. As a significant subset among these, functional disorders, such as irritable bowel syndrome (IBS) and dyspepsia, are ill-characterized multifactorial diseases with the hallmark of abnormal gastric function.[6] Discordant motilin levels may play a central role in some patients presenting with these illnesses.[7] Unfortunately, clinical studies with motilin agonists have thus far not demonstrably improved symptoms in patients.[8,9] It has recently been shown that motilin levels are elevated in IBS patients relative to healthy volunteers during all phases of motile activity.[10] This evidence would suggest that a motilin antagonist might be an appropriate course of treatment for IBS.

The discovery of compounds **1a** and **1b** represented a fundamental shift away from peptidic and macrocyclic-based motilin antagonists.[11] This small molecule series originated from a pharmacophore-model-directed database search that ultimately focused on mimicking four key binding amino acids (F^1, I^4, F^5, Y^7) of the motilin peptide. Not surprisingly, when overlaid onto this model, the peripheral functionality on compounds **1a** and **1b** overlap with these same four putative binding amino acid side-chains of motilin. In the further exploration of this series, we focused on changes to the series that conceptually would maintain the spatial arrangement of the peripheral functionality.



On the basis of the model overlay and the structure–activity relationship (SAR) of the lead structure we concluded that the core cyclopentene ring serves largely as a scaffold for spatially displaying recognition elements.[12] Thus, it seemed plausible that the cyclopentene ring could be replaced without compromising activity. In considering possible changes to the scaffold we also wanted to address the constraints imposed by the existing synthesis route.[13] The existing approach was best suited for SAR development of the urea portion of the molecule. Benzylic groups were introduced early in the

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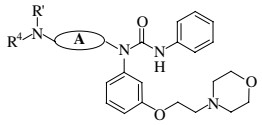
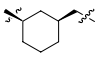
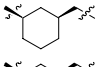
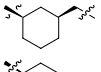
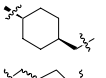
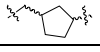
synthesis through Grignard reagent addition to 1-ethoxycyclohexen-3-one. The stereogenic center was subsequently set by an aza-Claisen rearrangement. This rearrangement step was particularly limiting in scope as it necessitated the inclusion of the stereogenic center in all analogs. Consequently, the significance of the quaternary center was unknown, though the clear binding preference for one enantiomer over the other was informative. In addition to conforming to the spatial requirement then, a new scaffold would ideally lend itself to a facile synthetic route for substitution at all four recognition elements sites and provide some insight as to the role of the stereogenic center. We reasoned all of these objectives might be achieved by moving the benzylic group in a peptoid-like fashion from the quaternary stereogenic center in compounds **1a** and **1b** to the nitrogen of the trichloroacetamide, initially giving derivatives such as **7**.

The compounds found in Tables 1–3 are representative of the many analogs synthesized and were primarily chosen to illustrate the major SAR advancements. All of the compounds, with the exception of **13**, were synthesized according to Scheme 1.[14] Starting from *cis*-3-aminocyclohexanecarboxylic acid, **2**, the amine was protected with trityl chloride to give **3**.[15] The protected amino acid was subsequently coupled with a variety of substituted anilines, the first recognition element, to arrive at amide intermediates **4** in good yield. LAH reduction of the amide group then provided a secondary amine that was capped with phenyl isocyanate or 4-fluorobenzoyl chloride to give compounds **5**, incorporating the second recognition element. After trityl deprotection, Lewis acid-mediated reductive amination proved to be a general method for introducing benzaldehydes, the third recognition element.[16] The resulting secondary amines, **6**, were then capped with an acid chloride, the fourth recognition element, to provide compounds **7a**–**s**. The enantiomers **7n** and **7o** were separated from a racemic mixture by chiral chromatography. Compound **8** was prepared by an analogous route starting from cis 4-aminocyclohexylcarboxylic acid. The des-benzylic analog **7t** was synthesized directly from intermediate **5** by deprotection of the amine and capping with 3-methoxybenzoyl chloride.

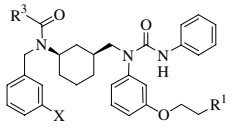
Table 1. Motilin inhibitory activity of alternative scaffolds[a]



Compound[18] (stereo)	A	R′	R^4	Binding assay[19] IC_{50} (μM)
6a (*rac*)		H	3-ClBn	28%
7a (*rac*)		3-MeOPhCO	3-ClBn	0.650
7t (*rac*)		3-MeOPhCO	H	26%
8 (*rac*)		3-MeOPhCO	3-ClBn	6%
13 (dia)		Cl_3CCO	3-ClBn	4%

[a] IC_{50} values are means of at least two determinations; CV was ±24%. % inhibition of ^{125}I-motilin binding measured at 1 μM.

Table 2. Motilin inhibitory activity of 1,3-cyclohexylmethyl ureas[a]



Compound[18] (racemic)	R^1	R^3	X	Binding assay[19] IC_{50} (μM)
7b	Morpholino	CCl_3	H	0.957
7c	Morpholino	CCl_3	Cl	23%
7d	Morpholino	CCl_3	CF_3	58%
7e	Morpholino	CCl_3	NO_2	0.035
7f	Morpholino	3-NO_2Ph	H	1.16
7g	Morpholino	2-Furyl	H	0.656
7h	Morpholino	2-Ph(ethyl)	H	0.730
7i	Morpholino	4-Biphenyl	H	3%
7j	Morpholino	*tert*-Butyl	H	50%
7k	Pyrrolidin-1-yl	*tert*-Butyl	H	0.021
1a				0.020
1b				0.010

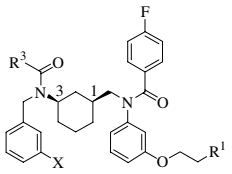
[a] IC_{50} values are means of at least two determinations; CV was ±24%. % inhibition of ^{125}I-motilin binding measured at 1 μM.

The methylene spacer and the carbocyclic ring in the scaffold were transposed according to Scheme 2. Reductive amination of 3-cyanopentanone with the substituted aniline **9** gave the 1,3-substituted cyclopentane ring, **10**. This aniline was then reacted with phenyl isocyanate to produce the highly congested trisubstituted urea **11** in low yield. Reduction of the nitrile provided the primary amine **12** for further substitution. A second reductive amination and acylation gave the desired compound **13** as a 1:1 mixture of diastereomers.

We synthesized a variety of carbocyclic ring structures, **A**, as cyclopentene ring replacements (Table 1). Among these analogs only the 1,3-disubstituted carbocyclic ring derivatives had appreciable activity. As an example, the 1,3-disubstituted cyclohexyl ring systems were uniformly more potent than their 1,4-disubstituted counterparts (**7a** vs **8**).[17] It was also found that the location of the methylene spacer was critical as transposing it with the carbocyclic ring, as in **13**, led to a series devoid of activity. Each of the four recognition elements on the carbocyclic core contributed to optimal potency. For instance, removal of the benzyl group from the amide diminished the little activity we had seen, **7t** versus **7a**. Conversely, removal of the acyl group had a similar effect, **6a** versus **7a**.

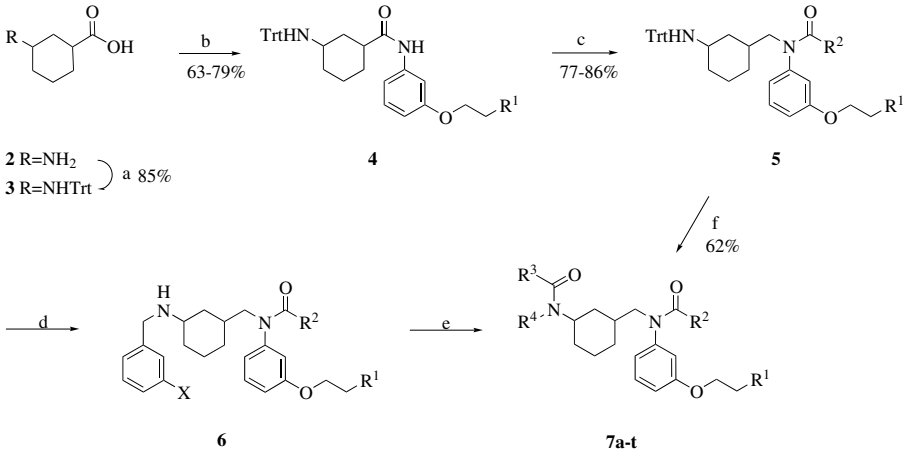
After exploring more than a dozen alternative scaffolds, we focused our SAR development on 1,3-disubstituted cyclohexyl ring systems (Table 2). When directly compared with the original series, these derivatives showed relatively poor inhibition, **7b**–**c** versus **1a** and **1b**. Nevertheless, we did learn some important SAR from these

Table 3. Inhibitory and functional activity of select motilin antagonists



Compound[18] (racemic)	R^1	R^3	X	Binding assay[19] IC_{50}[a] (μM)	Tissue assay[20] IC_{50}[b] (μM)	Human[21] recombinant cellular assay IC_{50}[c] (μM)
7l	Pyrrolidin-1-yl	*tert*-Butyl	H	0.029	0.024	0.990 ± 0.264
7m	Morpholino	*tert*-Butyl	H	0.772	—	—
7n (1*S*,3*R*)	Pyrrolidin-1-yl	*tert*-Butyl	H	0.010	0.008	0.338 ± 0.059
7o (1*R*,3*S*)	Pyrrolidin-1-yl	*tert*-Butyl	H	0.072	0.638	2.48 ± 0.737
7p	Pyrrolidin-1-yl	CCl_3	H	0.058	0.027	—
7q	Pyrrolidin-1-yl	CCl_3	3-Cl	0.058	0.036	0.267 ± 0.170
7r	Piperidin-1-yl	CCl_3	3-Cl	0.063	0.021	0.228 ± 0.043
7s	Pyrrolidin-1-yl	CCl_3	3-NO_2	0.014	0.072	0.052

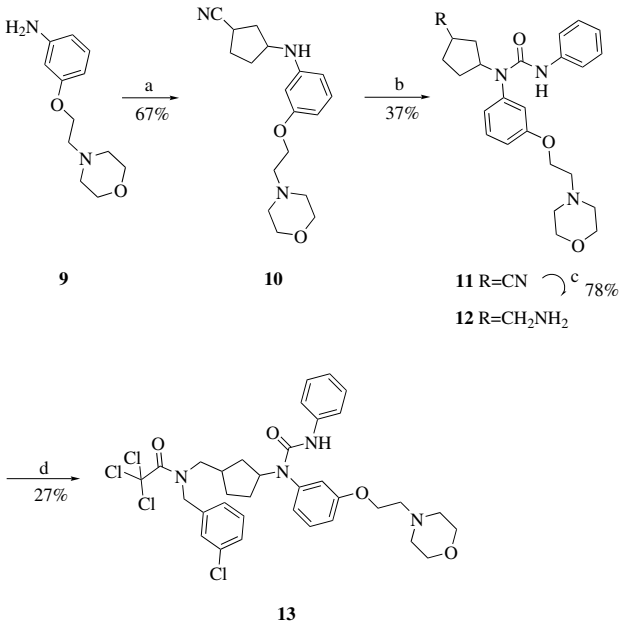
[a] IC_{50} values are means of at least two separate determinations; CV was ±24%.
[b] IC_{50} values are means of at least two separate determinations; CV was ±30%.
[c] IC_{50} values are means ± SE of 2–6 determinations.



Scheme 1. Reagents and conditions: (a) (1) TMSCl, DCM–CH_3CN; (2) TrtCl, TEA; (b) substituted anilines, PyBop™, DIEA, DCM; (c) (1) LAH, THF; (2) PhNCO, DCM or 4-FPhCOCl, DIEA, DCM; (d) (1) 10% TFA, DCM; (2) ArCHO, Ti(*i*-OPr)$_4$, DCM; (3) EtOH, $NaBH_3CN$; (e) R_3COCl, DCM; (f) (1) 10% TFA, DCM; (2) 3-MeOPhCOCl, DCM.

analogs. As already shown by example **7a** in Table 1, the trichloroacetyl group found in **7b–c** could be replaced with a variety of aromatic and heteroaromatic containing carbonyls, **7f–h**, without substantially altering potency. It was only once multiple aromatic rings, such as biphenyl **7i**, or fused rings (data not shown) were introduced that there was a marked reduction in inhibition. On the benzyl portion of the molecule, the addition of electron-withdrawing groups to the meta-position was tolerated, **7b–d**, but only the introduction of a nitro group significantly improved potency, **7e**. The most dramatic improvement in activity resulted from replacing the morpholino group with other tertiary amines, for example, compound **7j** versus **7k**. This was a surprising finding given that tertiary amines were interchangeable in the original series.[13]

Data from the original series suggested that the phenylurea group could be replaced with 4-fluorophenyl amide. The results with this substitution in our series are shown in Table 3. As a general rule this substitution produced compounds with similar activity; for instance, the amide **7l** (IC_{50} = 29 nM) versus the urea **7k** (IC_{50} = 21 nM, Table 2). The nature of the basic side-chain plays a critical role in these derivatives as well, **7l** versus **7m**. Activity was not strictly limited to pyrrolidine-containing molecules. The analogous piperidine derivatives, absent the oxygen heteroatom of the morpholino group, were nearly as active (**7q** vs **7r**). With the preferred pyrrolidine side-chain in place, the introduction of a nitro group to the benzylic ring now had a more modest 4-fold improvement in binding inhibition, **7p** versus **7s**. Once again, no similar improvement was seen from electron-withdrawing halogen substituents (**7q** vs **7p**) in the binding assay. In all cases the chirality of the compounds plays a considerable role in binding, with the (1*S*,3*R*) enantiomers consistently showing 5- to 10-fold greater potency than their antipo-



Scheme 2. Reagents and conditions: (a) (1) 3-cyanopentanone, 1% AcOH, MeOH; (2) $NaBH_3CN$; (b) phenylisocyanate, THF; (c) LAH, THF, −78 °C to rt; (d) (1) 3-chlorobenzaldehyde, 1% AcOH, MeOH; (2) $NaBH_3CN$; (3) Cl_3CCOCl, DCM.

des (**7n** vs **7o**). Analogs within the series were confirmed as antagonists by agreement between the binding, cellular, and functional tissue data.

In summary we have demonstrated that the core cyclopentene ring found in compounds **1a** and **1b** could be replaced by a 1,3-disubstituted cyclohexyl ring system. Moving the benzylic group to the amide nitrogen eliminated the need for the quaternary stereogenic center. A significant improvement in activity resulted from replacing the morpholine side-chain with other tertiary amines. These key modifications, allowing for rapid synthesis and SAR exploration, led to novel and potent motilin antagonists.

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12. Cyclohexene ring systems have also given promising activity, see Chen, R. H.; Xiang, M. A. WO 0168621.
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17. The corresponding 1,3-substituted cyclopentyl derivatives gave a very similar SAR, see Ref. 14.
18. In general, reactions were run in parallel and compounds purified on semi-prep HPLC to >95% purity. Compounds in Tables 1–3 were characterized by ^1H NMR and LC/MS.
19. Motilin receptor preparations were made from the colons of New Zealand white rabbits (Covance Research Products, Inc., Denver, PA). The tissues were homogenized in Tris–HCl buffer, pH 7.5, with a Polytron (Brinkmann Instruments, Westbury, NY) and then centrifuged. The pellet was resuspended and stored at −80 °C. The binding assay was performed in HEPES buffer (pH 7.0) containing 15 mg/mL BSA, protease inhibitors, ^{125}I radiolabeled porcine motilin (50,000–70,000 cpm; specific activity 2000 Ci/mmol [Amersham Pharmacia, England]), test compound, and membrane preparation. The assay was incubated for 60 min at 30 °C with membrane-bound tracer separated by centrifugation. The assay signal-to-noise ratio was ~4:1 with 1 μM unlabeled porcine motilin (Peninsula Laboratories, Belmont, CA) used to measure nonspecific binding. IC_{50} values were determined using Kaleidograph software (Synergy Software, Reading, PA). Unlabelled porcine motilin displaced tracer from rabbit colon with an IC_{50} value (mean ± SE) of 0.9 ± 0.3 nM.
20. Tissue contractility studies were performed using the longitudinal muscle layer isolated from the first 8 cm of the duodenum of fasted New Zealand white rabbits of both sexes. Strips, 3× 30 mm, were attached to force displacement transducers (FT03, Grass Instruments, Quincy, MA) in tissue baths containing Krebs solution gassed with 95% O_2, 5% CO_2 at 37 °C. Resting tension was slowly increased to 1*g*. After equilibration, compounds diluted with dimethylsulfoxide were added followed 5–15 min later by porcine motilin (3 nM). Baseline and response tension levels were expressed as a percent of the response produced by 100 μM acetylcholine. Acetylcholine and porcine motilin had EC_{50} values of 4.2 ± 0.5 μM ($n = 3$) and 1.9 ± 1.5 nM ($n = 3$), respectively. No test compound at 10 μM generated any agonist activity.
21. Recombinant human motilin receptor preparations were made from transfected HEK 293 cells. Cells were transfected with motilin receptor DNA using DMRIE C® reagent (Life Technologies, Inc., Grand Island, NY) and grown in DMEM/F12 media supplemented with 10% fetal bovine serum, glutamine (Gibco BRL), and geneticin

(500 μg/mL, Life Technologies). Motilin-induced changes in intracellular calcium mobilization were measured using calcium-sensitive fluorescent dye as previously described in *Biotechnol. Lett.* **2001**, *23*, 2067. Transfected HEK 293 cells were loaded with fluo 3 AM® (5 mM, Molecular Probes, Inc., Eugene, OR) for 1 h shielded from light at room temperature. Intracellular fluorescence was measured using fluorometric imaging plate reader (FLIPR®; Molecular Devices, Inc., Sunnyvale, CA). In each experimental run, compound agonist activity and antagonist activity (against 2 nM porcine motilin) were determined. Porcine motilin stimulated calcium mobilization with an EC_{50} of 3.0 ± 0.4 nM ($n = 4$) producing a maximal response ~73-fold greater than baseline. No test compound showed agonist activity at concentrations up to 30 μM.