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Design, synthesis, and biological evaluation of a new class of small molecule peptide mimetics targeting the melanocortin receptors

James P. Cain, **Alexander V. Mayorov**, **Minying Cai**, **Hui Wang**, **Bahar Tan**, **Kevin Chandler**, **YeonSun Lee**, **Ravil R. Petrov**, **Dev Trivedi**, and **Victor J. Hruby***
Department of Chemistry, University of Arizona, Tucson, AZ 85721, USA

Abstract

A new bicyclic template has been developed for the synthesis of peptide mimetics. Straightforward synthetic steps, starting from amino acids, allow the facile construction of a wide range of analogs. This system was designed to target the melanocortin receptors (MCRs), with functional group selection based on a known pharmacophore and guidance from molecular modeling to rationally identify positional and stereochemical isomers likely to be active. The functions of hMCRs are critical to myriad biological activities, including pigmentation, steroidogenesis, energy homeostasis, erectile activity, and inflammation. These G-protein-coupled receptors (GPCRs) are targets for drug discovery in a number of areas, including cancer, pain, and obesity therapeutics. All compounds from this series tested to date are antagonists which bind with high affinity. Importantly, many are highly selective for a particular MCR subtype, including some of the first completely hMC5R-selective antagonists reported.

Keywords

Melanocortins; Peptide mimetics; GPCRs

The human melanocortin receptors (hMCRs) comprise a family of five Type I, or rhodopsin-like, G-protein-coupled receptors (GPCRs) to which a wide array of biological functions has been ascribed.[1] Some examples include nociception, inflammation, energy balance, and sexual function. From the early understanding of the role MCRs play in pigmentation to recent revelations concerning their relevance to pain, new studies have continually uncovered crucial but previously unknown actions of this receptor system. Beyond advancing our knowledge of basic biology, the understanding and modulation of MCR function also has clinical relevance, with potential therapeutic value for addressing obesity,[2] cachexia,[3] pain,[4] inflammatory diseases,[5] and sexual dysfunction,[6] as well as the diagnosis and treatment of certain cancers.[7]

Much research to date has relied on natural and synthetic peptide ligands for these receptors. The MCRs are unique in that both endogenous agonists (α-, β-, γ-MSH, ACTH) and antagonists (agouti, AGRP) for the system have been discovered.[8] Each of the agonists contains the His-Phe-Arg-Trp tetrad, the minimum sequence necessary for activation of all melanocortin receptors.[9] Both endogenous antagonists contain an Arg-Phe-Phe sequence. Extensive melanotropin peptide structure–activity relationship (SAR) studies by our group and others have identified modifications which enhance potency, stability, or selectivity.[10] The value of

*Corresponding author. Tel.: +1 520 621 6332; fax: +1 520 621 8407; e-mail: hruby@u.arizona.edu

the ligands generated—particularly the standard agonists NDP-α-MSH and MT-II, and the antagonist, SHU9119, is hard to overestimate.

Nevertheless, with respect to certain applications in biology and medicine, the possession of small molecules with activity at the MCRs and properties complementary to those of peptides would be advantageous. Considerable effort in both academic and industrial laboratories has been directed toward this end.[11] Successful examples have come from screening libraries,[12] 'privileged structure' design strategies,[13] and ligand-based rational design using computational chemistry.[14]

We decided to employ this latter modeling approach to guide our design of small molecule peptide mimetics. Careful consideration of potential molecular scaffolds for β-turn mimetics led us to the bicyclic structure in Figure 1. From a synthetic point of view, compounds of this type are easily constructed from amino acids, allowing us to take advantage both of the naturally available chiral pool and our group's extensive repertoire of unnatural amino acid syntheses.[15]

Interestingly, none of our initial target molecules had been previously reported. Despite the prevalence of piperazine-based structures in medicinal chemistry,[16,17] the pyrrolopiperazine moiety remains surprisingly underutilized.[18] On the other hand, diketopiperazines, including the cyclodipeptides that are key precursors to our final structures, are found in a wide variety of natural products and synthetic ligands.[19-21] As a consequence, a significant body of methodology for the synthesis of these molecules has been developed.[22]

The functional groups appended to our template were chosen based on SAR both for peptide ligands and previous MCR-targeted small molecules. As noted above, peptides active at all five MCRs contain the His-Phe-Arg-Trp sequence, while the minimum chemical features seemingly common to all active small molecules are the presence of two hydrophobic aromatic groups and a basic nitrogen. In the design of our first set of compounds, we intended to explore the effect of variations in the type and orientation of the hydrophobic groups and of the presence or absence of arginine.

All four stereoisomeric variants of a number of such compounds were modeled and compared to the solution conformations[23] of MT-II, a superpotent, nonselective agonist for the MCRs.[24] One relatively simple example is shown in Figure 2. Spectroscopy and molecular modeling have suggested that the receptors recognize a β-turn structure in MT-II, probably centered at the histidine and phenylalanine residues.[23] The new structures with the best overlap against MT-II were predicted to have the best biological activity. We also hoped to identify structurally distinct features of molecules within this subset which demonstrated greater receptor subtype selectivity. Thus, the synthesis and biological evaluation of multiple analogs, some with better overlap than others, would allow us to simultaneously probe the structural requirements of MCRs and to test the validity of our model.

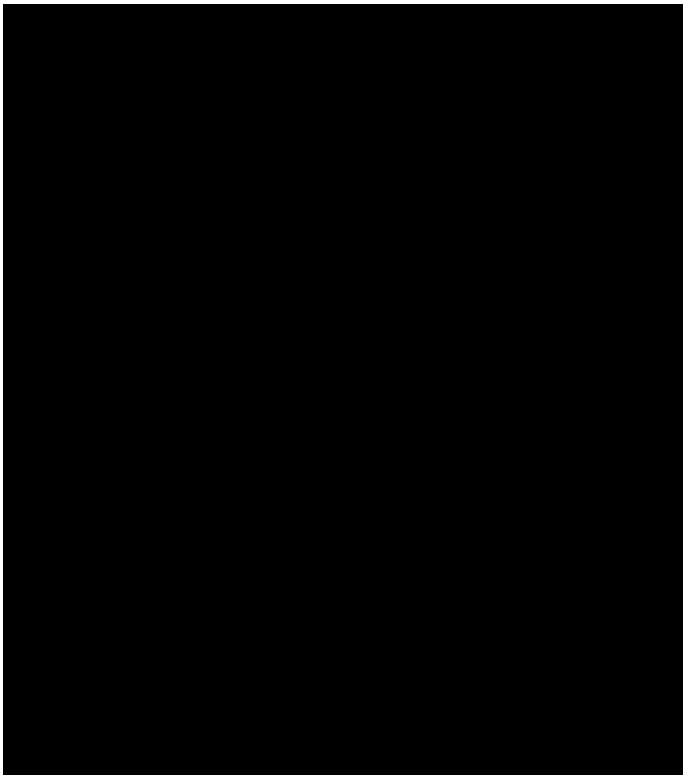
The synthesis of our first set of analogs began with the construction of the core template from L-proline and D-or L-phenylalanine (Scheme 1, L-phenylalanine shown). The dipeptide of these amino acids was easily obtained by BOP-mediated coupling. Deprotection of the Boc-protected amine using TFA was followed by cyclization in triethylamine and methanol to yield the diketopiperazine. Reduction with LAH provided the diamine core structure.

Functionalization of this core structure could be achieved with a variety of acylation chemistries (Scheme 2). Reaction with phenylacetyl chloride provided **HMC001** (3S,6S) and **HMC002** (3*S*,6*R*). A convenient means of introducing a spacer was the reaction with succinic anhydride, followed by amide bond coupling, to generate **HMC013** (3S, 6S) and **HMC014** (3*S*,6*R*). This coupling reaction itself, using BOP, was convenient and effective for

functionalizing the core secondary amine, and was used with indolepropionic acid to access **HMC009** (3*S*, 6*S*) and **HMC010** (3*S*,6*R*). The same coupling chemistry was used to produce **HMC021** and **HMC025**. All compounds were purified using RP-HPLC.

Biological assays of these compounds were performed using human melanocortin receptors (hMCRs) expressed in whole HEK293 cells.[25] Binding was determined by measuring competition with ^{125}I-labeled NDP-α-MSH.[26] Binding efficiency refers to the degree of displacement of the radiolabeled ligand by the compound being assayed, relative to the maximum displacement caused by excess amounts of MT-II. Functional activity (activation of adenylate cyclase) was determined by measuring the cAMP produced by the cells after its incubation with ligand.[26]

Scheme 1.
Reagents: (a) BOP, HOBt, DIEA, DMF; (b) TFA; (c) TEA, MeOH; (d) $LiAlH_4$, THF.



Scheme 2.
Reagents: (a) Phenylacetyl chloride, DIEA, DMAP, DCM; (b) succinic anhydride, DMAP; (c) tryptamine, BOP, HOBt, DIEA, DMF; (d) indolepropioinic acid, BOP, HOBt, DIEA, DMF; (e) Boc-6-aminohexanoic acid, BOP, HOBt, DIEA, DMF; (f) TFA; (g) indolepropionic acid, BOP, HOBt, DIEA, DMF; (h) Boc-L-arginine, BOP, HOBt, DIEA, DMF; (i) TFA; (j) indolepropionic acid, BOP, HOBt, DIEA, DMF.

The assay results for our first set of compounds (Table 1) are very encouraging. Each of the analogs tested thus far binds to one or more of the receptor subtypes, and most with high selectivity. **HMC001**, for instance, binds strongly to MC1, with greater than 150-fold selectivity over MC3, and no measurable binding at other subtypes. **HMC002**, with the opposite stereochemistry at C6, also binds strongly to MC1. The effect of switching the chirality of C6 in the analogs containing an indolepropionyl group is dramatic. **HMC009** binds only to MC5, while **HMC010** binds to MC1 and MC3. For both **HMC009** and **HMC010**, only 50% displacement of radioligand is observed at each relevant receptor subtype.

Each of the remaining members of this test set contains an indole ring separated from the bicyclic core by a relatively long linker group. The biological results for all of these compounds are remarkably similar, exhibiting complete MC5 selectivity, with subnanomolar IC_{50} values and 50–65% binding efficiency. The presence of arginine in **HMC025** does not appear to have a significant effect on binding affinity or cAMP accumulation.

The discovery of five new MC5-selective antagonists (**HMC009**, **HMC013**, **HMC014**, **HMC021**, and **HMC025**) may prove useful for the study of this relatively unexplored receptor subtype. The most widely expressed of the MCRs, the MC5R has been linked to energy homeostasis[27] and thermoregulation.[28] Interesting recent work has identified a putative behavioral role in the regulation of aggression related to pheromone signaling in mice.[29] Chemical biology using a small molecule may offer advantages over the use of MC5R knockout mice in future studies.

Most importantly, we have developed a new small molecule template which provides compounds recognized by the target receptors. The inability of these ligands to completely displace ^{125}I-NDP-α-MSH, however, suggests the possibility of an allosteric binding mode. This interaction may occur at a site distinct from or partially overlapping the residues which bind standard ligands, with the induction of an alternative receptor conformation having different reactivity toward intracellular components. Additional studies will be needed to clarify this issue, and allow a more clear assessment of the role that β-turn mimicry plays in molecular recognition for this series.

In addition, new analogs are being produced with the goals of achieving selectivity at each receptor subtype and engendering functional agonism through rational substitutions on the core structure. A full paper describing the results of these studies will follow shortly.

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Figure 1.
General structure of peptide mimetics.

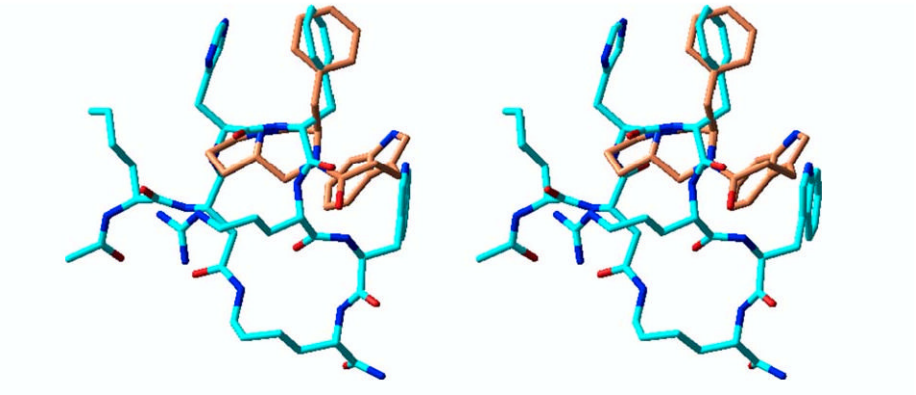

Figure 2.
Superimposition of computational model of **HMC001** (orange) and NMR-derived structure of **MT-II** (cyan).[23]

Table 1

Binding and second messenger bioassay results

	hMC1R				hMC3R				hMC4R				hMC5R			
	IC_{50} [a] (nM)	B.E. (%) [b]	EC_{50} [c] (nM)	Act. %	IC_{50} (nM)	B.E. (%)	EC_{50} (nM)	Act. %	IC_{50} (nM)	B.E. (%)	EC_{50} (nM)	Act. %	IC_{50} (nM)	B.E. (%)	EC_{50} (nM)	Act. %
HMC001	2.2 (±0.4)	80	>10,000	0	350 (±60)	70	>10000	0	>10,000	—	2.7	30	>10,000	—	>10000	0
HMC002	1.3 (±0.3)	90	>10,000	0	5.0 (±0.5)	30	>10000	0	1.2 (±0.5)	30	>10000	0	>10,000	—	>10000	0
HMC009	>10,000	—	>10,000	0	>10,000	—	>10000	0	>10,000	—	>10000	0	0.8 (±0.2)	50	>10000	0
HMC010	4.0 (±1.0)	50	>10,000	0	1.2 (±0.3)	50	>10000	0	>10,000	—	>10000	0	>10,000	—	>10000	0
HMC013	>10,000	—	>10,000	0	>10,000	—	>10000	0	>10,000	—	>10000	0	0.5 (±0.1)	50	>10000	0
HMC014	>10,000	—	>10,000	0	>10,000	—	>10000	0	>10,000	—	>10000	0	0.1 (±0.01)	60	>10000	0
HMC021	>10,000	—	>10,000	0	>10,000	—	>10000	0	>10,000	—	>10000	0	0.4 (±0.1)	65	>10000	0
HMC025	>10,000	—	>10,000	20	>10,000	—	>10000	0	>10,000	—	>10000	0	0.1 (±0.01)	60	>10000	0
MT-II	0.10 (±0.01)	100	1.0 (±0.2)	100	1.9 (±0.3)	100	2.4 (±0.4)	100	1.8 (±0.4)	100	2.3 (±0.5)	100	7.0(±2.2)	100	8.0 (±2.4)	100

[a] IC_{50}, concentration of compound at 50% specific binding. Values are means of three experiments done in duplicates; standard deviation is given in parentheses.

[b] Binding Efficiency (maximum radioligand displacement by cmpd/maximum radioligand displacement by MT-II).

[c] EC_{50}, effective concentration of compound that was able to generate 50% maximal intracellular cAMP accumulation. Compounds were tested at a range of concentrations from 10^{-10} to 10^{-5} M.