Investigation of Novel Cyclic Peptides for Preferential Activation of Melanocortin 4 Receptors

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Introduction

The melanocortin system is comprised of five complex integral membrane protein isoforms (MC1R-MC5R), which mediate many key physiological functions and are a subclass of the GPCR superfamily. Activation of the MCRs is facilitated via binding of the endogenous melanotropin agonists (α-MSH, β-MSH, γ-MSH, and ACTH) in order to stimulate the cAMP signal transduction pathway.

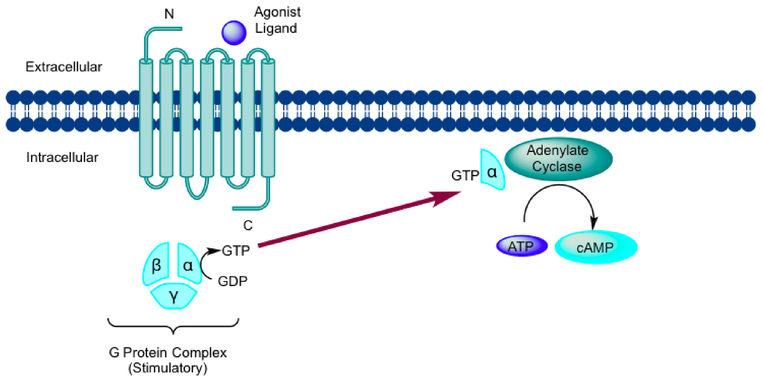

Figure 1.1—Activation of the heterotrimeric G_s protein and secondary messenger (cAMP) through agonist binding.

MC3R and MC4R have been shown to play an important role in energy metabolism and feeding behavior. They are also mediators of anti-inflammatory signaling in the brain. However, MC4R expression in the CNS is more abundant than MC3R; making this receptor an ideal target for suppressing brain inflammation. Guarini et al. showed that MC4R stimulation by NDP-α-MSH increases neuroprotection in a mouse model. Agonist analogs dubbed Anti-Inflammatory Melanotropins (AIMs) were synthesized in order to enhance ligand potency and selectivity at MC4R.

Design of AIM Drugs

Molecular recognition of agonists by the MCRs is based upon the conserved His-Phe-Arg-Trp (HFRW) pharmacophore sequence. AIMs contain multiple alterations to their structure to increase selectivity. Global stereochemical constraint is introduced via lactam bond formation, in addition β-amino acid substitutions allow for enhanced pharmacophore mobility. In order to increase stability in the binding pocket a constrained amino acid is introduced in the sequence. Halogenation of specific amino acids allows for ion induced dipole interactions in the binding pocket.

Table 1.1—Structural Sequence of Naturally-Occurring Melanotropin Agonists

Agonists	*Amino Acid Sequence*
α-MSH	Ac-SYSMEHFRWGKPV-NH_2
β-MSH	H_2N-AEKKDEGPYRMEHFRWDRFG-OH
γ-MSH	H_2N-YVMGHFRWDRFG-OH
ACTH	H_2N-SYSMEHFRWGKPVGKKRRPVKVYPNGAEDESAEAFPLEF-OH

Table 1.2—Structural Sequence of Nonselective Exogenous Melanotropins

Drugs	*Amino Acid Sequence*
NDP-α-MSH	Ac-Ser-Tyr-Ser-Nle-Glu-His-D-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2
MT-II	Ac-Nle-cyclo[Asp-His-D-Phe-Arg-Trp-Lys]-NH_2
SHU-9119	Ac-Nle-cyclo[Asp-His-D-Nal(2')-Arg-Trp-Lys]-NH2
SHU-9005	Ac-Ser-Tyr-Ser-Nle-Glu-His-(*pI*)D-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH2

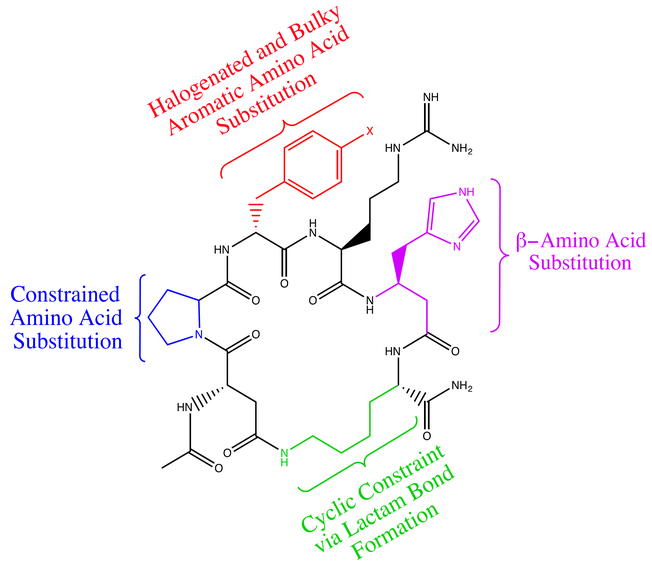

Figure 1.2— Principal features involved in the AIM design strategy.

Methods

The peptides were synthesized on low-loading Rink Amide resin using Nᵅ –FMOC chemistry and an orthogonal side chain protection strategy, which was executed through solid phase peptide synthesis. The linear chain was then acetylated at the N-terminus and cyclized via lactam bond formation. Each peptide was confirmed with Mass Spectroscopy and purified by HPLC.

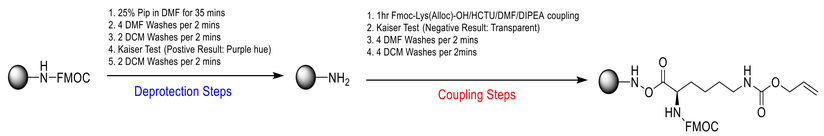

Figure 1.3—Solid phase peptide synthesis scheme.

Pharmacodynamics of AIMs were assessed by cAMP assays. This assay compares cAMP levels produced by receptor stimulation via binding of AIMs with a specific radiolabeled [^{3}H]-cAMP concentration. The assay was ran on MCR transfected HEK293 cells and was evaluated on a MicroBeta^2 counter.

Biological Results

	hMC1R		hMC3R		hMC4R		hMC5R	
Drug	**EC_{50} (nM)**	**Act%**	**EC_{50} (nM)**	**Act%**	**EC_{50} (nM)**	**Act%**	**EC_{50} (nM)**	**Act%**
AIM 1	120 ± 24	88	680 ± 25	88	>1000	63	580 ± 340	95
AIM 2	220 ± 70	120	>1000	89	47±24	67	>1000	90
AIM 3*	190 ± 90	82	120 ± 10	87	16±4	84	290 ± 50	82
AIM 4*	64 ± 10	90	450 ± 830	76	630±175	46	530 ± 65	72
AIM 5	340 ± 0	100	>1000	59	>1000	84	>1000	62
AIM 6	>1000	61	>1000	49	>1000	120	>1000	51
AIM 7	99 ± 0	67	>1000	24	240±10	57	>1000	21
AIM 8*	130 ± 0	89	360 ± 25	46	110±90	66	>1000	38
AIM 9**	240 ± 11	90	>1000	63	4±2	87	295 ± 165	76
MT-II	32	100	3	100	10	100	2	100

Table 1.3—cAMP Assay Data for AIMs.

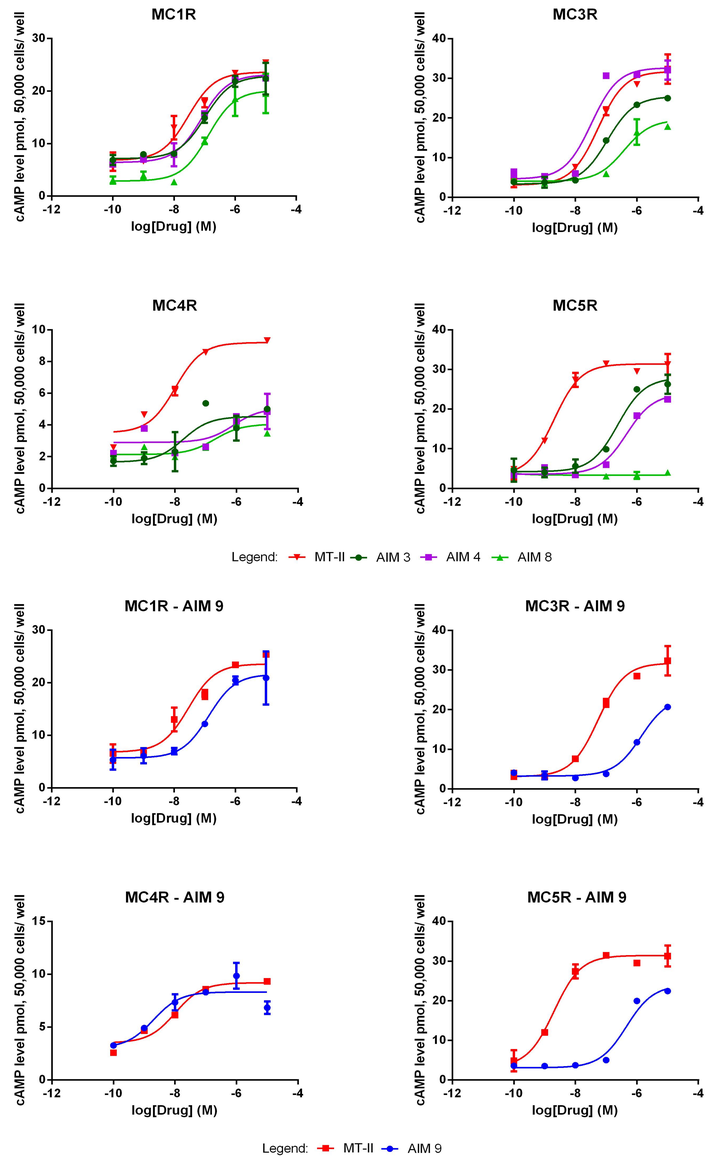

Figure 1.4—cAMP Assay Data for AIMs 3, 4, 5, 9, and the control MT-II on MCR's.

Conclusions

Current biological data suggests that all AIM molecules act as agonists on MCRs. Global constraint, β-amino acid substitutions, halogenation of the pharmacophore and a constrained amino acid addition all point towards selectivity for MC4R. This is best exhibited in AIM 9 – a peptide that contains all of the aforementioned modifications. Our future directions are to conduct detailed binding studies for AIMs on the MC4R and use the results to elucidate the structure of this receptor.

References

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[2] Cai M, Hruby VJ (2016) Design of Cyclized Selective Melanotropins. *Pep. Sci.* 106: 876-883
[3] Giuliani D, Guarini S (2013) Melanocortins protects against progression of Alzheimer's diseases in triple transgenic mice by targeting multiple pathophysiology pathways. *Neurobio. of Aging.* 35: 537-547

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