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Synthesis and biological activity of novel peptide mimetics as melanocortin receptor agonists

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Abstract—A series of novel peptidomimetic analogs was prepared containing cyclohexyl, phenyl, or heterocyclic groups to ostensibly orient the guanidine or mimic of an arginine in a putative melanocortin receptor ligand pharmacophore. Some binding affinity at the melanocortin receptors MC_3 and MC_4 was noted. In silico docking also indicated that the relative positions of the hydrogen-bonding sites and hydrophobic regions of the compounds are reasonably well matched to the receptor-binding site. This may present a lead entry into a selective series of MC_4R agonists.

Melanocortin receptors (MCR) are members of the G-protein coupled receptor subfamily consisting of $MC_{1,2,3,4,5}$.[1,2] The MC_1 receptor has been found in melanocytes where it controls skin pigmentation while recent studies indicate that MC_4R is involved in the control of feeding behavior. As a result, the MC_4 receptor has received increased attention as a receptor target from both academia and industry. Since this subtype is localized in the central nervous system, transport of exogenous ligands across the blood-brain barrier remains one of the challenges in this field.[3,4]

Melanocyte stimulating hormone, α-MSH is a natural ligand for four of the melanocortin receptor subtypes, namely, the MC_1, MC_3, MC_4, and MC_5 receptors. This peptide hormone is 13 amino acids in length and binds with high affinity to the MC_1, MC_3, MC_4, and MC_5 receptors. Other ligands designed earlier shared a common core sequence motif: His-Phe-Arg-Trp.[5] A number of linear and cyclic peptide and peptidomimetics containing this sequence have now been reported. Most show a similar selectivity profile to the natural MSH.[6–8] Interestingly, replacement of L-Phe with D-Phe was reported to increase the affinities for the MC receptors.[9,10] Also, replacement of the His residue was shown to increase selectivity for the MC4 receptor.[11,12] It is only recently that subtype selective, orally active, small (MW < 500) ligands were reported. Workers at Merck synthesized a new class of cyclohexyl substituted piperidines that presumably mimicked this HFRW sequence (Fig. 1).[13] Compound A is a potent (EC_{50} = 2.1 nM), selective (1184-fold vs. MC_3R, 350-fold vs. MC_5R), small-molecule full agonist of the human MC_4 receptor. In an attempt to better understand this finding and related theories on the minimum pharmacophore required for MC_4 activity, we designed a related series of cyclohexyl-, phenyl-, and heterocyclic-containing linear compounds. These compounds representing the piperidine ring-opened analogs of compound A were thus designed to test the criticality of the conformational restriction aspects of this drug lead. In this letter, we report the synthesis and biological activity of novel analogs based on this concept.

The novel cyclohexyl-directed triazole analog was synthesized according to the route presented in Scheme 1. The commercially available cyclohexyl methyl ketone **1** was brominated with benzyltrimethylammonium tribromide yielding α-bromo ketone **2** in high yield (98%). Overnight reaction of **2** with sodium triazole in DMF at 50 °C yielded the α-triazole ketone **3** (91%) which,

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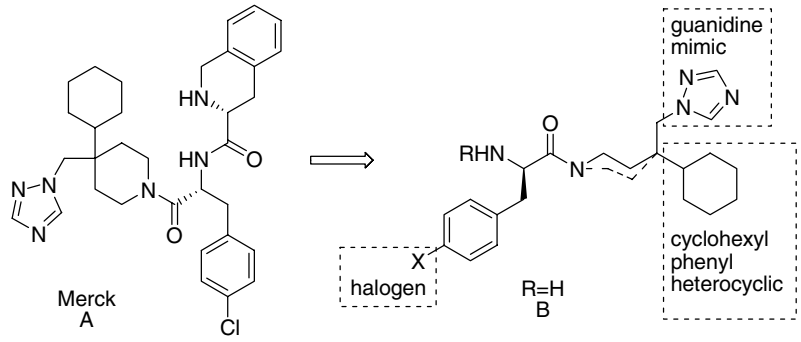
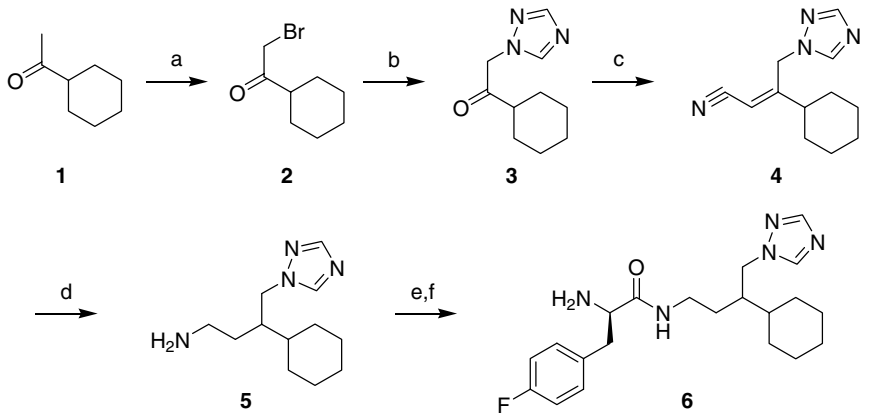


Figure 1. Constrained (A) and linear (B) conformations of the ligand.



Scheme 1. Reagents and conditions: (a) $PhCH_2N(CH_3)_3Br_3$, CH_2Cl_2, 5 °C–rt, 4 h, 98%; (b) Sodium triazole, DMF, 5 °C, 91%; (c) diethyl phosphonoacetonitrile, LiCl, DBU, CH_3CN, rt, 90%; (d) H_2, Ni, NH_4OH, MeOH, 40 psi, 4 h, 98%; (e) Boc-D-Phe(*p*-F)-OH, EDC, HOBt, NMM, DMF, rt, overnight, 84%; (f) TFA, CH_2Cl_2, 97%.

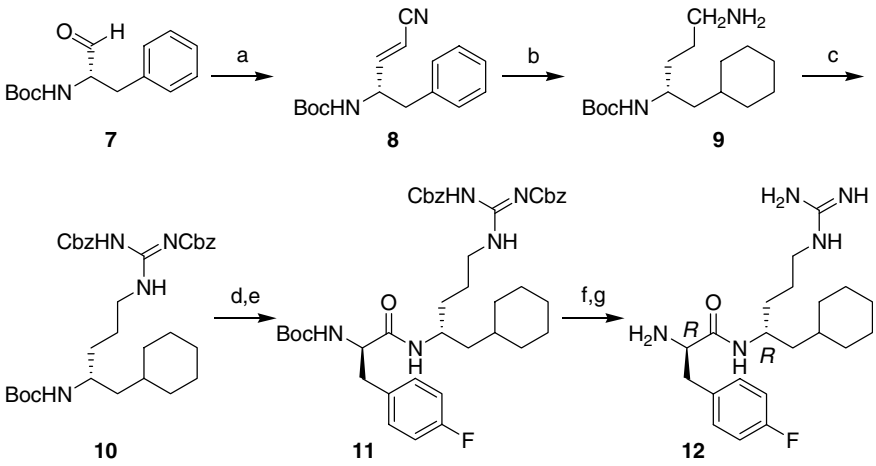
via a facile Horner–Wadsworth–Emmons reaction with diethyl phosphonoacetonitrile in the presence of LiCl and DBU, provided α-, β-unsaturated nitrile **4** in 90% yield. The primary amine **5** was obtained in almost quantitative yield by hydrogenation of **4** on a Parr shaker at 40 psi. This amine was coupled with Boc-D-Phe(*p*-F)-OH in the presence of HOBt, EDC, and NMM. The crude product after reaction workup was treated with trifluoroacetic acid and purified by reverse-phase HPLC to generate the final compound **6** as trifluoroacetate salt in a yield of 81% for two steps.

Scheme 2 illustrates the synthesis of dipeptidomimetics **12** from the commercially available N-*t*-Boc-L-phenylalanial **7**. The aldehyde **7** was coupled to diethyl phosphonoacetonitrile via a Horner–Wadsworth–Emmons reaction in 89% yield. The resulting α-, β-unsaturated nitrile **8** was reduced to the amine **9** (92%). Rh/alumina was an optimal catalyst for this step to hydrogenate the double bond nitrile and the phenyl ring in one step. The free primary amine **9** was converted to the di-Cbz protected guanidine derivative **10** by guanylation with *N*,*N*-di-Cbz-*S*-methylpseudothiourea in the presence of $HgCl_2$ and Et_3N. After deprotection with 50% TFA in CH_2Cl_2, **10** was coupled to Boc-D-Phe(*p*-F)-OH to generate **11**, followed by removal of Boc with 50% TFA in CH_2Cl_2 and catalytic hydrogenation to remove the Cbz group. The dipeptidomimetics **12** was obtained after HPLC purification.

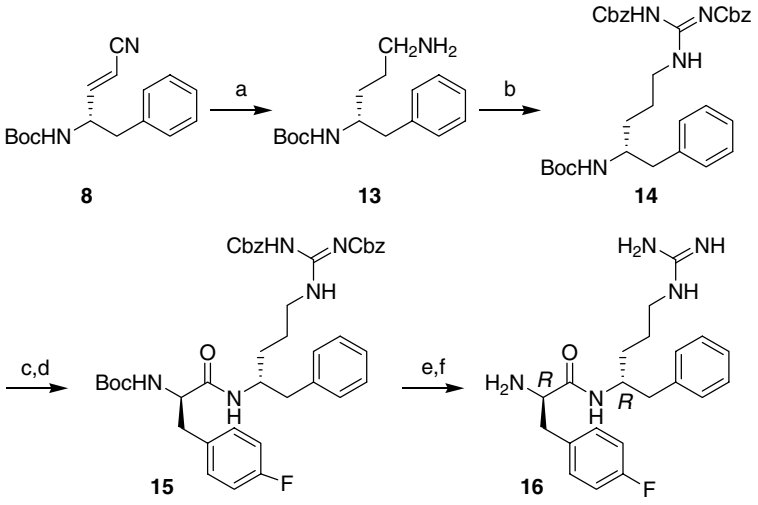
Phenyl analog **16** of cyclohexyl compound **12** was prepared as shown in Scheme 3 starting from α-, β-unsaturated nitrile **8**. Catalyzed by Raney nickel, it was reduced to saturated amine **13** with the phenyl ring intact. The final target **16** was obtained from primary amine **13** via **14**, and **15**, following the procedures used to prepare **12**.

Schemes 4 and 5 outline the methodology for preparing dipeptides D-Phe(*p*-F)-Arg with terminal modifications. Boc-D-Phe(*p*-F)-OH **17** was coupled with the methyl ester of Arg **18** under typical EDC/HOBt coupling conditions, resulting in dipeptide **19**. The terminal methyl ester group was converted to the corresponding acid **20** with LiOH in THF and water (3:1) followed by acidification. EDC/HOBt coupling of the acid **20** and piperidine gave **21**, which was transformed to dipeptide **22** after removal of the Boc group with TFA in CH_2CH_2 and hydrogenolytic removal of the nitro group in structure **21**.

As shown in Scheme 5, methyl ester **19** was reduced to alcohol **23** with $LiBH_4$ in THF at room temperature in a reasonable yield (77%). In the presence of DEAD and Ph_3P, this alcohol reacted with piperidine in a Mitsunobu reaction in a moderate yield (41%), generating dipeptidomimetics **24**. Treatment of **24** with TFA in CH_2CH_2 and hydrogenolysis of the nitro group, followed by HPLC purification, provided the tertiary amine analog **25**.



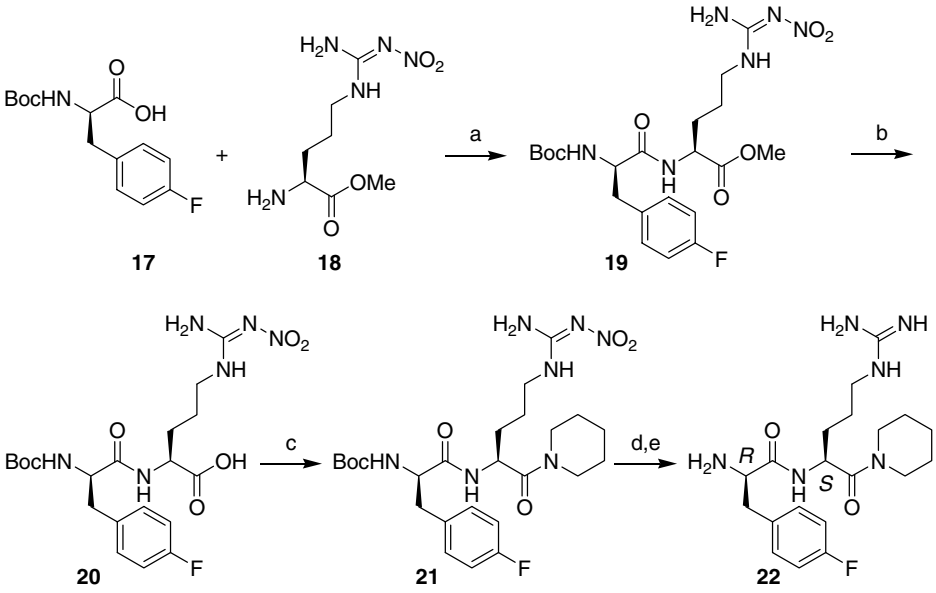
Scheme 2. Reagents and conditions: (a) diethyl phosphonoacetonitrile, LiCl, DBU, CH_3CN, rt, 89%; (b) H_2, Rh/alumina, NH_4OH, MeOH, 45 psi, 22 h, 92%; (c) 1,3-bis(benzyloxycarbonyl)-2-methyl-2-thiopseudourea, $HgCl_2$, Et_3N, DMF, 74%; (d) TFA, CH_2Cl_2, 98%; (e) Boc-D-Phe(*p*-F)-OH, EDC, HOBt, NMM, DMF, rt, overnight, 80%; (f) TFA, CH_2Cl_2, 97%; (g) H_2, Pd/C, MeOH; 99%.



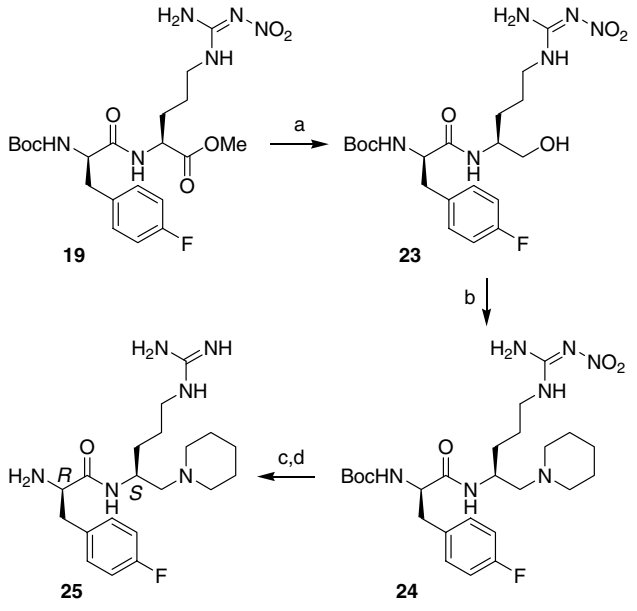
Scheme 3. Reagents and conditions: (a) H_2, Ni, NH_4OH, MeOH, 40 psi, 4 h, 95%; (b) 1,3-bis(benzyloxycarbonyl)-2-methyl-2-thiopseudourea, $HgCl_2$, Et_3N, DMF, 74%; (c) TFA, CH_2Cl_2, 98%; (d) Boc-D-Phe(*p*-F)-OH, EDC, HOBt, NMM, DMF, rt, overnight, 80%; (e) TFA, CH_2Cl_2, 97%; (f) H_2, Pd/C, MeOH; 99%.

The screening data obtained for the substances described herein and shown in Table 1 contribute to the understanding of the pharmacophoric requirements. Compound **6** is a linear analog based on the Merck scaffold A. Its weak binding affinity to MC_4R suggests that the conformational restriction of this drug lead is critical. Interestingly, when the triazole group is replaced by a guanidine moiety, compounds **12** and **16** show increased affinity to MC_4R. This guanidine function might therefore be forming stronger or more favorable interactions with the receptor than the triazole moiety. Replacement of the cyclohexyl group with a piperidine functionality for compounds **22** and **25** led to poor affinity analogs ($K_i > 25$ μM). In this case, a stereochemical change ($R \rightarrow S$) was also made at the guanidinium side chain. From this preliminary evidence, it appears that the guanidine function with an *R* configuration and a directing cyclohexyl or phenyl group are necessary in addition to conformational restriction.

In an effort to understand the differences in the binding affinity of various analogs, we constructed a homology model of the MC_4 receptor using the known human MC_4 receptor amino acid sequence[15,16] and site-directed mutagenesis studies.[17,18] The computer model was created by mapping the transmembrane sections of the human MC_4 receptor from the primary amino acid sequence onto the crystal structure of rhodopsin.[19] The extracellular loops were constructed using a protocol described previously.[20] The model was subsequently optimized with molecular dynamics simulations and refined using SAR data generated in our laboratory (data not shown) as well as results from site-directed mutagenesis experiments. Individual ligands were then docked manually and energy minimized within the putative binding site of the receptor.[21] Figure 2 shows compound **12** docked in the receptor and indicates specific interactions between residues in the transmembrane (TM) region of the receptor and the ligand. More specifically,



Scheme 4. Reagents and conditions: (a) EDC, HOBt, NMM, DMF, rt, overnight, 80%; (b) LiOH, THF, H_2O; (c) EDC, HOBt, NMM, DMF, rt, overnight, 71%; (d) H_2, Pd/C, MeOH; 99%; (e) TFA, CH_2Cl_2, 97%.



Scheme 5. Reagents and conditions: (a) $LiBH_4$, THF, rt, overnight, 77%; (b) piperidine, DEAD, Ph_3P, THF, −78 °C, 41%; (c) H_2, Pd/C, MeOH; 99%; (e) TFA, CH_2Cl_2, 97%.

the model suggests that the guanidinium group of compound **12** forms a salt bridge with the carboxylates of Glu100 (TM2) and Asp122 (TM3), while the terminal amine interacts with Asp126 (TM3). The hydrophobic fluoro-phenyl is anchored deeper in the transmembrane domain of the receptor and appears to reside in a hydrophobic pocket where it interacts with Trp258 (TM5), Phe261 (TM6), and Phe262 (TM6). The cyclohexyl ring occupies a hydrophobic region located at the putative extracellular interface of TM7 and possibly interacts with Met281 (TM7), Phe284 (TM7), and Leu288 (TM7). These interactions appear to be maintained in

Table 1. Binding affinity K_i (μM) and potency EC_{50} (μM) on MCR subtypes[a]

Compound	MC_1		MC_3		MC_4	
	K_i	EC_{50}	K_i	EC_{50}	K_i	EC_{50}
A	0.3	0.005	0.125	0.16	0.003	0.001
B	>10	2.0	2.3	0.23	0.023	0.016
6	>10	>25	4.5	>50	>50	>25
12	—	>25	9.1	>15	6.7	>50
16	>25	—	3.5	>100	4.2	>100
22	>100	>50	>100	>25	>25	>50
25	>100	>50	>100	>100	>100	>100

[a] The analogs were screened against the human MC1R, MC3R, and MC4R.[14] Data represent means of at least three experiments.

compound **16** where the cyclohexyl ring is substituted for a phenyl ring. The change in stereochemistry of the guanidinium side chain for compounds **22** and **25** is detrimental to their binding which is most probably due to the loss of interaction with Asp122 and Glu100. The alternate location of the putative binding site proposed by Wikberg et al.[22,23] based on work performed in the MC_1 receptor may further explain the structure activity results described here.

In summary, we have described the design, synthesis, and modeling of a series of novel peptidomimetics with low molecular weight (<500) via very practical synthetic strategies. We utilized cyclohexyl, phenyl, and piperidine rings as Trp mimics to direct the orientation of the guanidine side chain or its replacements within the Arg component of the HfRW pharmacophore. Docking experiments within a receptor binding model offer a reasonable understanding of the SAR for some members of this series, although alternate binding modes should not be excluded. It is likely that the increased conformational freedom of these compounds caused by the opening of the piperidine ring is partly responsible for the

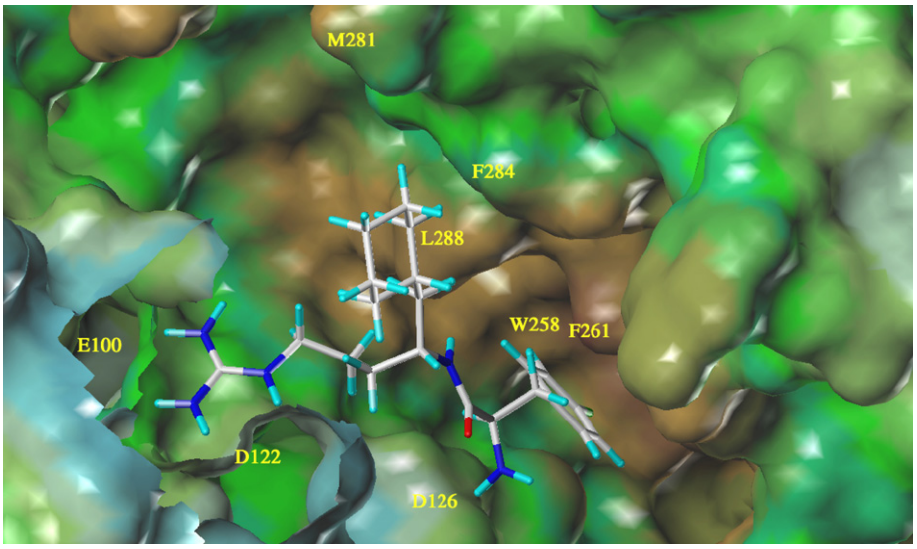


Figure 2. Hydrophobic surface of the MC_4R model with docked ligand **12**. The hydrophobic regions are shown in brown/green, the hydrophilic regions in blue/gray. The surface on portions of extracellular loop 1 has been removed for clarity.

lower binding affinity. Further optimization should be pursued, perhaps including attempts to add new contact residues within the MC_4R pharmacophore.

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